A South African perspective investigating five nitrogen application levels for optimum sweet sorghum juice yields needed for the production of bio-ethanol

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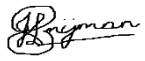
Declaration

I, Jakobus L. Snijman (0836548396), declare that the thesis entitled: *"A South African perspective investigating five nitrogen application levels for optimum sweet sorghum juice needed for the production of bio-ethanol",* submitted in the fulfilment of the requirements for the degree Philosophiae Doctor in Chemical Engineering, is my own work, except where acknowledged in the text, and has not been submitted in whole or in part to any other tertiary institution.

Signed at the North West University, Potchefstroom campus.



Signed

20 February 2020

Date

Acknowledgement

My life's journey took me to various places. The work environment privileged as I am to enjoy, covers various disciplines, as well as the cultivation of sweet stem sorghum. Many people crossed my path. I am thankful to all who helped build my character and skills to bring me to the point where I am fulfilling a long time goal.

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Summary

The rasionale behind this study was merely to determine whether the tested sweet sorghum genotypes can be utilised as a renewable bio-ethanol resourse and whether different nitrogen (N) application levels have an effect on production (biomass yield, Brix% and juice yield). It was not to quantify and qualify sweet sorghum production and not to quantify and qualify the effect of different N application levels on the production of sweet sorghum. However, the results obtained during the study did indicate a performance profile of the genotypes that was discussed in Chapter 4.

A shortage of scientific information exists in South Africa regarding the propagation of the best sweet sorghum genotypes and the application of optimum levels of nitrogen (N) fertilisers in the cultivation of the feedstock to produce bio-ethanol (EtOH) for blending with fossil fuels. Data presented here will address this gap and I trust it will add scientific knowledge that could aid all present and future stakeholders involved in the biofuel genre.

Due to the involvement of the Agricultural Research Council: Grain Crops Institute (ARC: GCI) in the Sweetfuel Programme, sweet sorghum genotype evaluation trails were planted in South Africa since 2010. Dryland agricultural practises were applied at various locations and the genotypes were selected at random as to include as many genotypes as possible. An average of 20 genotypes were planted at the various locations across a number of years to determine the best lines for biomass yield, juice yield and Brix% values to be introduced into the sweet sorghum based EtOH production environment. Nitrogen trials were also conducted under dryland conditions and in a glasshouse. The same genotypes were planted and their reaction to the different N levels were recorded to determine whether N has an effect on biomass yield, juice yield and the Brix%. Rondomised block designs with three replications were used in the genotype trial layouts and two replications were applied in the N application trials.

The amounts of fermentable and non-fermentable sugars produced by the sweet sorghum were determined by high-pressure liquid chromatography by the North West University (Potchefstroom, South Africa) and these values were used to calculate the potential EtOH that can be produced from sweet sorghum and be blended into the existing fossil fuels. During 2010 / 2011, one trial was planted at the ARC: GCI at Potchefstroom (North West Province) and one at Taung (Northern Cape Province). Thereafter, the genotype trails were extended and trials were planted at the Agricultural Research Institute (ARC: SGI) at Bethlehem (Freestate Province), the Agricultural Research Institute (ARC: IIC) at Rustenburg (North West Provinve), Vaalharts

(Northern Cape Province), the ARC: GCI and Wilgeboom (10 kilometres outside Potchefstroom, North West Province), to cover different climatic and soil conditions. The best performing genotypes (between 18 to 20) were planted consecutively over three years, stretching across 2011/12 to 2013/14. This trial-based data was collected and analysed. In an attempt to allow comparisons regarding the data amongst the genotypes and the countries involved in the Sweetfuel project, the layouts of the trials were determined by the Sweetfuel Consortium in attempted to standardise the agronomical specifications across the six countries who were involved in the Sweetfuel project (www.sweetfuel-project.eu).

Fertilisers applied for the genotype trials applied was merely to standardise the soil nutrient content and to supply the necessary additional nutrients that were required for proper plant growth. The applications also took the clay content of the different soils into consideration. Planting started as soon as 50 mm of rainfall measured, usually from mid October to mid December. Different randomisation of the genotypes was applied at each location. The genotypes were planted in four rows of 5 m each. The inter-row spacing was 0.6 m and the intra-row spacing was 8 cm. A plant population of 207 500 plants per hectare was achieved. Chemical and mechanical weed control were executed and insecticides used to control stalkborer and aphids were applied when necessary. Harvesting was done when the seed reached the physiological maturity stage, which usually was from day 90 to day 120, depending on the genotype. Representative samples (54 stalks) from each genotype were processed and the data was recorded and anaysed. A three-roller hydraulic press was used to extract the juice from the stalks.

During the genotype evaluation trials, the biomass yield (mass), the juice yield (mass) and Brix% were determined, and the potential EtOH production was calculated from the synthesised sugars. The best biomassa yield produced by ss 003, ss 007, ss 017, ss 120, Hunnigreen (HG) and Supa. The highest calculated total EtOH potential produced from the bagasse was 71.1 kL ha^{-1} and obtained from HG during the 2014 season in Potchefstroom, as well as the highest calculated amount of EtOH (83.09 kL ha^{-1}) from bagasse, juice and residual sugars. Supa produced the best juice yield (57.38 t ha^{-1}) with a Brix% value of 20.84% at Rustenburg in 2014.

To study the effect of different N fertiliser application levels on the genotypes, overall eight N fertiliser application rates were applied with five levels per locality. Although ss 007 produced best at 200 kg ha^{-1}, it was clear from the recorded data that except for a few outliers, the effect of N fertiliser applications did not produce economical viable higher EtOH yields at very high N levels.

However, when looking at the conclusions drawn from this dissertation, sweet sorghum proved to be most viable on the subject of the production of EtOH in South Africa, when compared to other crops such as sugarcane and sugar beet compared to sweet sorghum (Table 18). When the decision by the stakeholders is in favour of the industry, it will be worthwhile to cultivate sweet sorghum.

Keywords

sweet sorghum, potential energy crop, bio-ethanol potential, nitrogen applications, residual sugars, first and second generation

Opsomming

Die rasionaal agter die studie was nie om soet sorghum genotipes en die effek van verskillende N toediengs op produksie te kwalifiseer en te kwantifiseer nie. Dit was bloot ’n studie om te bepaal of soet sorghum aangewend kan word vir bio-etanol produksie en of N toedienings die produksie sal beïnvloed.

‘n Tekort bestaan aan wetenskaplik gefundeerde inligting in Suid Afrika bestaan aangaande die verbouing van die beste soet sorghum genotipes en die optimale stikstof kunsmis toedienings op soet sorghum wat ’n invloed kan hê op die produksie van biomassa, stroop en Brix%. Dit is belangrik vir bio-ethanol (EtOH) produksie wat ten doel het om met fossiel brandstof vermeng te word. Data wat hier aangebied word, sal die tekort aanspreek en wetenskaplike gefundeerde inligting verstrek wat alle rolspelers in die dissipline kan aanwend, indien hulle betrokke wil raak in EtOH produksie.

Soet sorghum genotipe evalueringsproewe was vir die doel van die studie aangeplant in Suid Afrika vanaf 2010. Die genotipes wat by die proewe ingesluit was, was uitgesoek om soveel moontlike genotipes by die proewe in te sluit. Droëland proewe was geplant en 20 genotipes was aangeplant by verskillende plekke, wat gestrek het oor ’n aantal jare, om die genotipes ten opsigte van produksie (biomassa, Brix% en stroop) te bestudeer. Stikstof (N) proewe was ook aangeplant onder droëland toestande en een proef in Potchefstroom (2016/17) was in ’n glashuis geplant. Dieselfde genotipes as in die genotipe proef was gebruik en die reaksie op verskillende N toedieningsvlakke was gemonitor om te bepaal of N ’n invloed het op die produksie van biomassa, stroop en Brix% waardes. ’n Gerandomiseerde blok ontwerp is gebruik in die uitleg van die proewe en drie repetisies per proef is geplant. Die hoeveelheid fermenteerbare en nie-fermenterbare suikers wat produseer was, is bepaal en die waardes was gebruik om die hoeveelheid potensiële EtOH te bereken wat dan met fossiel brandstof vermeng kan word.

Gedurende 2011/2012 is twee proewe by Potchefstroom en Taung aangeplant, waarna die proewe uitgebrei is na Bethlehem:SGI, Rustenburg:IIG, Potchefstroom:IGG, Vaalharts en Wilgeboom (10 km buite Potchefstroom) om sodoende ’n verskeidenheid klimaatsomstandighede en verskillende grond tipes se effek ook te evalueer. Die beste genotipes was gedurende agtereenvolgende jare geplant wat gestrek het vanaf 2011/12 tot 2013/14 en die proef gebaseerde data was opgeteken en geanaliseer. Die uitleg van die proewe was bepaal deur die “Sweetfuel Consortium” om soedoende gestandardiseerde agronomiese spesifikasies neer te lê vir die ses lande wat ook by die internasionale projek betrokke was (www.sweetfuel-project.eu).

Stikstof toedienings was gedoen by die genotipe evalueringsproewe om die voedingstowwe in die grond te standardiseer en om die nodige voedingstowwe toe te dien wat nodig is vir optimale gewasgroei. Die kunsmistoedienings het ook die klei persentasie van die grond by die verskillende lokaliteite in aanmerking geneem. Aanplantings het begin nadat 50 mm reën gemeet is, en was gewoonlik vanaf middel Oktober tot middel Desember. Die genotipes is geplant in vier rye van 5 m elk. Die tussen-ry spasiëring was 0.6 m en die binne-ry spasiëring was 8 cm wat ’n plantestand van 207 500 plante per hektaar teweeggebring het. Chemiese en meganiese onkruid beheer is toegepas. Insekdoders was toegedien om stamboorders en luise te beheer. Die oes van die gewas het plaasgevind sodra die soet sorghum fisiologies ryp was en het gewoonlik na 90 tot 120 dae begin, na gelang van die genotipe. Die stingels is 20 cm bo die grond afgesny waarna die stroop uitgepers is met ’n drie-roller-hidroliese pers.

Die biomassa en stroop opbrengs is bepaal en die potensiële EtOH produksie is bereken van die gesintetiseerde suikers wat in die stroop en biomassa teenwoordig was. Die beste biomassa opbrengste is gelewer deur ss 003, ss 007, ss 017, ss 120, HG en Supa. Die beste stroop opbrengs (57.38 t ha^{-1}) met ’n Brix% van 20.84% is in 2014 deur Supa gelewer. Die genotipe HG het tydens die genotipe ondersoek die beste EyOH produksie vanaf biomassa (71.1 kL ha^{-1}) gelewer, asook die hoogste berekende hoeveelheid EtOH (83.09 kL ha^{-1}) gelewer vanaf bagasse plus stroop en residuele suikers.

Om die effek van N toedienings op die produksie van soet sorghum te evalueer is agt verskillende N vlakke toegedien, nl. 0 kg ha^{-1} (as kontrole), 30 kg ha^{-1}, 50 kg ha^{-1}, 60 kg ha^{-1}, 90 kg ha^{-1}, 120 kg ha^{-1}, 150 kg ha^{-1} en 200 kg ha^{-1}. Tydens die N kunsmis proef het die genotipe ss 007 die beste presteer met ’n berekende hoeveelheid EtOH van 9978.23 L ha^{-1} vanaf suikers in die stroop teen ’n N toediening van 200 kg ha^{-1}. Dit was duidelik uit die proef gefundeerde data in die studie, afgesien van ’n paar uitskieters, dat die toediening van hoë vlakke van N nie noodwendig hoër ekonomies lewensvatbare opbrengste gelewer het nie.

Volgens die gedateerde data en verwerking daarvan dui dit daarop dat die opbrengste van die biomassa, stroop, Brix% en EtOH hoër is as die van gewasse soos suikerriet en suiker beet. Soet sorghum is dus ’n baie goeie alternatiewe hernubare gewas is vir die produksie van EtOH.

Sleutelwoorde

soet sorghum, potensiële energie gewas, residuele suikers, bio-etanol potensiaal, stikstof toedienings, eerste en tweede generasie bio-etanol

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C_4 plant	C_4 plants producing a four carbon sugar
C_3 plant	C_3 plants produce two molecules of three-carbon compound
%, w/v, % **v/v**	weight/volume percent / volume per volume: used where both chemicals are liquids / weight (of solute) per volume (of solvent)
% ww	percentage wet weight
g/L	gram per litre
mg g^{-1}	milligram per gram
°Brix / Brix% / °Bx	sugar content
wt	weight
kg N/ha	kilogram nitrogen per hectare
N kg ha^{-1}	nitrogen kilogram per hectare
kg P/ha	kilogram phosphorous per hectare
L/ha	litre per hectare
L ha^{-1} (l ha^{-1})	litre per hectare
p.a.	per annum
g/m^2/day	gram per square meter per day
L/ha/harvest	litre per hectare per harvest
m^3 ha^{-1} p.a.	cubic meters per hectare per annum
g g^{-1}	gram per gram
g L ha^{-1}	gram per litre per hectare
kJ g^{-1}	kilojoules per gram
kg	kilogram
mm	millimeter
cm	centimeter
m^3 t^{-1}	cubic meter per tonne
m^3 ha^{-1}	cubic meter per hectare
g L^{-1}	gram per litre

R 19.79/L	South African currency per litre; nineteen rand and seventy-nine cents per litre
pH	the measure of the acidity of alcalinaty of a solution
mm p.a.	millimeter per annum
%	percentage
ºC	degrees Celsius
kg ha^{-1} (kg/ha)	kilograms per hectare
g	gram
t ha^{-1}	tonnes per hectare
m	metres
kg N ha^{-1}	kilograms nitrogen per hectare
g kg^{-1}	gram per kilogram
kg ha^{-1} N	kilogram per hectare nitrogen
m^3 ha^{-1}	metric meters per hectare
m^3 t^{-1}	metric meters per tonne
w/w	describe the concentration of a substance in a mixture or solution
ml	millilitres
g/block	gram per block
ton EtOH/ha	tonne ethanol per hectare
kg EtOH/ha	kilogram ethanol per hectare
L EtOH/ha (L ethanol/ha)	litre ethanol per hectare
Ethanol/kg	ethanol per kilogram
EtOH/ha	ethanol per hectare
yield/ha	yield per hectare
kg/ha	kilogram per hectare
t/ha	tonne per hectare
ton/ha (tonne ha^{-1}, tonnes/hectare)	tonne per hectare

ML ha^{-1}	megalitres per hectare
kL ha^{-1}	kilolitres per hectare
”E	degrees east
”S	degrees south
$mm.pa^{-1}$	millilitres per hectare
$(NH_4)_2SO_4$	ammonium sulphate
KH_2CPO_4	potasium dihydrogen phosphate
t ha^{-1} $^oC^{-1}$	tonne per hectare per degree Celsius
kg/ha/mm/ ^oC	kilogram per hectare per millimetre per degree Celsius
tce a^{-1}	ton fuel per ton of coal equivalent per hectare
ha	hectare
g ethanol/g sugar	gram ethanol per gram sugar

List of abbreviations

EU FP7	European Union FP 7 Research Programme
EN 228	European Standard specifies requirements and test methods for marketed and delivered unleaded petrol
EtOH	bio-ethanol
TSS	total soluble solids
CO	carbon monoxide
PIU	period of industrial utilisation
FAN	free amino nitrogen
DM	dry matter
RE	renewable energy
USA	United States of America
USDA	United States Department of Agriculture
SSF	simultaneous saccharification and fermentation
NDA	National Department of Agriculture
KAN	potassium ammonium nitrate
MAP	mono ammonium phosphate
HPLC	High-performance liquid chromatography
NDF	neutral determined fibre
ADF	acid determined fibre
ADL	acid determined lignin
ARC: GCI	Agricultural Research Council: Grain Crops Institute
ARC: SGI	Agricultural Research Council: Small Grains Institute
ARC: API	Agricultural Research Council: Animal Production Institute
ARC: IIC	Agricultural Research Council: Institute for Industrial Crops
HG	Hunnigreen genotype
SG	Sugargraze genotype
BMR	Brown midrip genotype

SK	Silage King genotype
E10	blend / addition of 10% biofuel to fossil fuel
LUC	land use change
iLUC	indirect land use change
dLUC	direct land use change
LCA	life cycle assessment
SFF	safe food and fertiliser
GHG	green house gases
CIRAD	French Agricultural Research Centre for International Development
ICRISAT	The International Crops Research Institute for the Semi-Arid Tropics
GxE	Genotype and environment interaction
RF	rainfall
HU	heat unit(s)
WUE	water use efficiency
NUE	nitrogen use efficiency
RUE	resource use efficiency
ANOVA	analysis of variance
ABB	algae based biofuel
VHG	very high gravity
Tx	average maximum temperature
Tn	average minimum temperature
HFCS	high fructose corn syrup
NWU	North West University
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuaria
BFAP	Bureu for Food and Agriculture Policies
NPK	Nitrogen, Phosphorous, Potassium
DAFF	Department of Agriculture, Forestry and Fisheries

Chapter 1

1.1 Background and motivation

First generation biofuel production from sugar rich feedstock, such as sugarcane, started in the 1960's and continued to the 1990's. A gradual increase in crude oil prices, a drop in market prices for starchy crops such as wheat, maize, and an increased awareness of the environmental impact of fossil fuel, has initiated investigation into first (1^{st}) generation EtOH production from starch after 1990. The food vs. fuel debate and efforts to increase economical sustainability of fuel ethanol plants initiated research into EtOH production form non-edible biomass such as lignocellulose.

According to Bryan (1990), the genus *Sorghum* is a complicated genus belonging to the sub-family (tribe) Andropogoneae of the grasses Poaceae with 24 species also subdivided into five sub-generic sections based upon morphology. Intensive research efforts are in progress in various countries viz., USA, China, India, Africa, Indonesia, Iran and Philippines to asses the agronomical and economical potential of sweet sorghum. Sweet sorghum (also called Sorgo) is an African plant belonging to the genus *S. bicolor* (L) Moench and is widely cultivated in the United States as an alternative crop for biofuels. The five basic races include *bicolor, guinea, caudatum, kafir and durra* and the ten intermediate races are those between any two of those types, classified primarily based on grain shape, glumes and panicles (Dogget, 1970). In the studies "Taxonomy of Sarga, Sorghum and Vacoparis (Poaceae: Andropogomeae)" by Spangler (2003) and in "Sweet sorghum: From theory to practice" by Srinivas (2013), both authors refered to the name *Sorghum bicolor* (L.) Moench, which was proposed by Clayton (1961) as the correct name currently in use for those cultivated sorghum types. Sweet sorghum is the general name for those varieties of sorghum, which has a juicy and sweet stem and is mainly cultivated for juice production. Other sorghum cultivars, such as *kafirs and milos*, are cultivated for grain and forage (Srinivas *et al*, 2012). Ripe sweet sorghum typically consists of about 75% cane, 10% leaves, 5% seeds and 10% roots by weight (Harlan and de Wet, 1972). In the search for suitable crops for EtOH production, different types of sorghums were investigated, i.e. grain sorghum, dual purpose (grain and fodder) sorghum, fodder sorghum, forage sorghum and sweet stem sorghum (Reddy *et al*, 2012). Sweet stem sorghum is a C_4 plant with high photosynthetic efficiency and high dry matter production, and is furthermore considered an important energy crop for production of EtOH. It can yield significant amounts of readily soluble fermentable sugars (Reddy *et al*, 2005). Crops with sugars

in the stalks, such as sugarcane and sweet sorghum, has the advantage over other EtOH crops that contain starch, because the sugar can easily be accessd for direct fermentation during the 1st generation EtOH production process and the bagasse (plus residual sugars in the bagasse) can be used as a source for second (2nd) generation biofuel or as animal feed (Srinivas *et al*, 2012; Braconnier *et al*, 2013). Figure 1 shows a sweet sorghum varieties, developed by The International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) (Srinivas *et al*, 2012).

Figure 1. Improved sweet sorghum varieties, ICSV 25274 & NTJ 2 (Source: ICRISAT)

Sorghum is also called "the camel of crops" because of its ability to grow in arid soils and its inate ability to withstand prolonged droughts. Globally it is the fifth largest cereal crop after wheat, rice, maize and barley (Srinivas *et al*, 2012). Specified biofuel, in the form of EtOH, can be produced through the fermentation of sugars from raw materials such as sweet sorghum, sugarcane, corn, wheat and sugar beet (Smith, 2007). A number of scientists (Reddy *et al*, 2005; Kumar & Reddy, 2009; Geng *et al*, 1989; Braconnier *et al*, 2013) also identified various feedstocks, viz sugarcane, maize, sweet sorghum, cassava and sugar beet as the most important renewable resources for worldwide EtOH production. Further, it is stated, that sweet sorghum is the most promising because it is a rugged crop, which can be cultivated under diverse agronomic conditions and require relatively less N fertiliser and water, when compared to sugarcane and maize. Sweet sorghum can also tolerate low precipitation levels, even as low as 450 mm per year. Sweet sorghum is also well adapted to all types of soil (prefering sandy and/or heavy soils with

high clay content - up to 30 %) and has a tolerance to a low pH and saline soils – optimum 5.5 to 8.5. The ideal temperature for germination is between 10 – 15 ºC and the optimum growing temperature is 27 ºC – 30 ºC. It therefore does well under dryland production systems. Research in Europe, Australia, Brazil and Zimbabwe has shown that sweet sorghum is an excellent crop for ethanol production because of its characteristics (Ferraris, 1981; Krishnaveni *et al*, 1990; Hills *et al*, 1990; Belletti *et al*, 1993; Woods, 2001; Fernandes, 2014 and Reddy, 2005, 2009, 2010). By using and fermenting the total soluble solids (TSS) directly, it eliminates the costly starch to sugar processes before fermentation of the sugars and ethanol production can start. What's more, sweet sorghum is a crop that is not a threat to food security issues. Bio-ethanol, from sweet sorghum, can be successfully introduced into the biofuel production programme of the sugarcane companies (Srinivas *et al*, 2009) and a blend of between 2% to 10% of biofuel with fossil fuel is possible (Brent *et al*, 2009). It was mentioned in research (Jihong *et al*, 2013) that sweet sorghum is considered to be a cost-effective feedstock for EtOH production due to its higher drought tolerant ability, lower production costs, and higher biomass yield compared to agricultural waste from other crops. However, the correct technology must be applied where the TSS in the juice and stalks are to be fermented to make EtOH production economical viable. Sweet sorghum juice accounts for a large part of the feedstock/substrate that contains abundant soluble sugars used directly as a substrate for EtOH production (1st generation ethanol), but the bagasse (2nd generation biofuel substrate) also provides efficient nutrient supplementation for microbe fermentation after which the residue can be used as animal feed.

Processing of sweet sorghum juice and the stalks, ensure that there are convertible lignocellulose materials available to produce EtOH (Dolciotti *et al*, 1998). Sweet sorghum juice contains 43-58% soluble sucrose, glucose, fructose and 22.6 to 47.8% in-soluble cellulose and hemicellulose. Some of the sugars in the sweet sorghum juice may include xylose, arabinose, sorbose, galactose and mannose. The sugar content in the juice differs between production years, soil condition and sweet sorghum variety (Billa *et al*, 1997). Yeap (2008), from the Faculty of Engineering of the University of Putra in Malaysia, explained the term ‘biofuel’ and ‘bio-ethanol’ as fuel and ethanol which is produced through fermentation of biological material such as starch, sugars and lignocellulosic biomass. Yeap mentioned that the production of EtOH could be categorised into three generations (first, second, third) which are differentiated by various raw materials. To validate sweet sorghum as an alternative crop for biofuel production, energy and economic input-output-relations have to be considered. To assess the energy efficiency of the sweet sorghum-biofuel process, the crop's adaptibility to climatic conditions and effective biofuel producing procedures are needed. This includes the entire value chain, from cultivation to processing and the

use of the whole plant with consideration of how the process effects changes in the soil. Exploitation of the advantages of sweet sorghum (*Sorghum bicolor* L. (Moench)) as energy crop is well researched through the development of 1st and 2nd generation EtOH production processes from sweet sorghum that is cultivated in temperate and semi-arid regions through genetic enhancement and the improvement of cultural and harvest practices for optimised yields (Yeap, 2008). There are many sweet sorghum cultivars being cultivated throughout the world, providing a diverse renewable resource for EtOH. It is highly productive and improvement through breeding approaches is an important future prospect (Srinivas *et al*, 2011). A biofuel substitute for petrol is EtOH and as little as 2% to 5% can be blended with fossil fuel, which is certified as EN228 by EU specifications. In terms of energy production, de Vries *et al* (2010), demonstrate that oil palm, sugarcane and sweet sorghum performed best against resource use efficiency (RUE) indicators due to their implicitly high energy yields compared to the very low nett energy production of other biofuel crops in regards to production methods.

A supportive environment is necessary to assist small-holder farmers in realising the potential of available land and this is often lacking in areas seemed 'suitable' in Sub-Saharan Africa (Kojima *et al*, 2007). This matter was also addressed in the paper by Florin *et al* (2013) where the question, "What drives sustainable biofuels?" was asked, and was answered by stating that although the largest bulk producer today is the USA, about 90% of the area planted under sorghum lies in developing countries. In a review by the Plant Production Systems at Wageningen University who has done research on indicator-based assessments of biofuel production systems involving small-holder farmers, the proposal was that research should aim more at sustainable processes rather than static detail. The diversity amongst small-holder farmers allows for accommodation of farmers across the biofuel production chain. Small-holder farmers were already producing sweet sorghum in Africa, Asia and Latin America. Sweet sorghum is a multi-purpose crop, yielding food in the form of grain, fuel in the form of EtOH from the juice in the stem, and fodder from its leaves and bagasse. These indicators are related to achieving productivity efficiency high enough for a sustainable agro-processing business (Florin *et al*, 2013).

According to Kering *et al* (2017) sweet sorghum is rated amongst the top crops for EtOH production, because it produces more fermentable sugars per kg of feedstock, requires less N fertiliser and less water than most energy crops. However, there exist various cultivation procedures, viz field management differences. Deheading of the panicles and removal of tillers can have an effect on juice yield and sugar concentrations. If the photosynthesised energy, used to produce grain, is diverted into the stem more ethanol is produced and the juice quality

improves. Plants cultivated with reduced tilling activities had on average thicker main stems, which contributed towards increased biomass and juice yields per plant (Kering *et al*, 2017). Studies aimed at determining hexoses at physiological plant maturity stage, established that sucrose is one of the major components in sweet sorghum juice, followed by glucose and fructose (Smith *et al*, 1987; Hunter and Anderson, 1997; Almodares *et al*, 2008). Hunter and Anderson (1997) reported that the total soluble solids (TSS) in sweet sorghum has the potential to yield up to 8000 L ethanol/ha of ethanol, which is double the amount compared to ethanol yields from maize grain and 30% more than the ethanol yield from Brazil's sugarcane industry. Guigou (2011) analysed the juice of three genotypes (Topper, M81 and Theis) and found that sucrose concentration in the juice, compared to glucose and fructose concentrations, was consistently higher. The results further showed that ethanol yields in the range of 0.35 - 0.48 g ethanol/g sugar was obtained, which compared well to the theoretical yield (68% - 94%). A correlation was thus evident between the TSS and the Brix%, which is a useful tool to estimate the potential ethanol yield from the raw material.

In the light of the arguments regarding the environmental impact and sustainability of biofuel production, it is worthwhile to shortly look at eutrophication. It has been argued (Quayle *et al*, 2010) that land use change (LUC) caused by agro-processing for biofuels can lead to eutrophication and will have a negative effect on the environment. Eutrophication is the process whereby normal limiting nutrients become more available to the environment and cause an imbalance in plant- and waterlife. Abnormal nutrient concentrations are the result of cultural and natural eutrophication of which natural eutrophication processes are affected by the impact of human activities. Studies carried out throughout South Africa indicated that N and phosphates (P) are the main contributors to eutrophication. Since sweet sorghum requires less N than most other energy crops, it could thus contribute to reducing eutrophication associated with energy crop production. Furthermore, the higher EtOH yields from sweet sorghum implies less arable land is required to produce the same amount of EtOH currently produced from crops such as maize and sugarcane. Sweet sorghum, as energy crop, can thus reduce the impact of LUC associated with alternative fuel production. In future, the applications of biomass for renewable energy, should it be for energy or biofuels, will rise and the effect of agro-processes will play a major role in indirect land use change (iLUC) in the form of impacts on habitats and soils. In an attempt to reduce risk, the production of bio-energy should be done sustainably (Fritsche, 2011). Another question "How sustainable are biofuels?" was asked by Stoeglehner (2009) in the report on the ecological impact of producing biofuels. The reason for the question lies in the fact that the production of renewable bio-energy needs bio-productive land to produce bio-energy and biofuel

crops, and the production of bio-energy will compete with food production. The effect of bio-energy production has a social implication, which one must take into consideration.

1.2 Problem statement

The ARC: GCI was one of eight consortium members of the European Union FP 7 Bio-ethanol Project (www.sweetfuel-project.eu) during 2010 to 2015 investigating sweet sorghum as an alternative energy crop. The project's aim was to establish the viability of sweet sorghum (*Sorghum bicolor* L (Moench)) as an alternative renewable resource in the production of 1st and 2nd generation EtOH. Due to research done it became evident that there is little progress made in the biofuel industry in South Africa and that a lack of science-based data exists regarding the effect of N fertiliser application levels to local soils to optimise TSS contents in sweet sorghum juice, which is needed for the production of 1st (and 2nd) generation EtOH. Therefore, in this study, the best sweet sorghum genotypes and the effect of N fertiliser application levels on biomass yield and sugar content of juice was investigated in order to provide guidelines regarding the optimum N fertiliser application levels to be applied by energy crop producers. Figure 2 shows members of the consortium visiting a sweet sorghum field at ICRISAT (India) where EtOH was produced.

Figure 2. Sweetfuel Consortium members visiting a sweet sorghum trial site at ICRISAT, India

1.3 Aims and objectives

1.3.1 Aim

The aim of this study is to produce evidence-based data to quantify the production of sweet sorghum genotypes and to investigate the effect of N fertiliser applications on fermentable sugars and biomass yield for EtOH production from sweet sorghum.

1.3.2 Objectives

- Evaluate the suitability and production of different sweet sorghum genotypes over a five-year period (2010-2015) for bio-ethanol production at different locations in South Africa.
- Determine the effect of different nitrogen fertiliser application levels during cultivation on biomass, juice and sugar yield (Brix%) for optimum bio-ethanol production.
- Determine if a statistical relationship exits between the application of nitrogen fertiliser levels during cultivation and the biomass yield, juice yield, Brix% and sugar content of the juice.

1.4 Scope of study

A lack in scientific information exists in South Africa regarding the propagation of the best sweet sorghum genotypes and the application of optimum levels of N fertilisers during cultivation of sweet sorghum which will have an effect on producing the optimum biomass yields, juice yields and sugars (Brix%) to be utilised in EtOH production. In this study various sweet sorghum genotypes were evaluated over a five-year period to determine the biomass yields, juice yields and Brix% for EtOH production. Furthermore, different sweet sorghum genotypes and eight N application levels were evaluated to determine the effect of different N fertiliser applications on the juice yield, biomass yield and Brix% that are the determinants in the quality and quantity of EtOH to be produced. The genotype evaluation trails and N fertiliser application trials were done at the ARC: SGI (Bethlehem), the ARC: IIC (Rustenburg), Vaalharts, the ARC: GCI (Potchefstroom) and Wilgeboom, to cover different climatic zones as to legitimise the results and to generate sound data for analyses.

1.5 Contribution of this study to the South African bio-ethanol industry

From information supplied in Chapters 1 and 2 it is evident that research on sweet sorgum as an alternative renewable resource for EtOH production, has mainly been globally conducted.

However, through involvement in the Sweetfuel Project and investigations into the South African scenario, it became clear that inadequate information is available to determine the best sweet sorghum genotypes to be cultivated, and the optimum N fertiliser application levels to be applied for optimum bagasse and juice (sugar) yields for the production of EtOH. The applicable N fertiliser levels for optimum juice production is emphasised by Hartemink (2006) and in addition to that it was stated that total availability of N, phosphorous (P) and optimum pH levels are very important chemical parameters in producing EtOH from sweet sorghum. The results from this study reveal that a number of genotypes are suitable for EtOH production based on the high juice yields, sugar yields and Brix%. The economic application levels of N fertiliser for optimum crop yields and EtOH production, suggested a guaranteed economic viable biofuel enterprise. This study will supply evidence-based data to address the lack of information regarding the EtOH-fossil fuel-blending market in South Africa, and to act as a tool for stakeholders considering entry into the EtOH industry.

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Chapter 2

Literature Study

2.1 Introduction

The production of biofuels from energy crops, such as sweet sorghum, is one of the most immediate and feasible solutions to meet the food, fuel, feed, and fibre demands of the increasing population. However, to date the scientific information available on its cultivation and sustainability seems disperse, insufficient, and sometimes inconsistent. Sweet sorghum is a hardy crop that grows very successfully on marginal land. Based on existing literature, discussions are continuing regarding the potentials, limitations and bottlenecks in order to solve and optimize sweet sorghum productivity (Monti *et al*, 2003). Moreover, amongst the sweet types, sugar and syrup sorghum subtypes have been developed by breeders to become one of the leading crops in EtOH production. Sugar and syrup production varieties produce a mixture of glucose and fructose, but newer developed cultivars are now also utilsed as a 2nd generation fuel crop due to the huge amounts of bagasse, which is produced (Monk *et al*, 1984). No other species show the flexibility of sorghum in producing similar amounts of starch, sugars or cellulose in the grains and stems.

The sweet sorghum genotypes grown for biofuel production will depend on the environmental conditions and the type of conversion processes used. Research to develop sweet sorghum cultivars started in the late 1960's and peaked during the 1970's and mid 1980's, and once the best genotypes have been identified for the production of 1st or 2nd generation biofuels numerous sorghum characteristics can be manipulated by traditional or improved agronomic approaches. It could be incorporated as needed in order to optimize its yields (Rooney *et al*, 2007). According to Thompson (1979), various other crops should be beared in mind for EtOH production such as maize, sugarcane, cassava and sugar beet. Sugar beet is less preferable as a source of EtOH because of its susceptibility to some pests and diseases like leaf spot. The gains will have to exceed losses through the development of better varieties and management due to a build-up of unfavourable effects caused by monoculture crops. In South Africa, sugarcane and sweet sorghum are more viable when compared to the poorer yields of cassava, different farm production costs and different crop nutrient requirements. More research on cassava will improve the status thereof as an energy crop, and certainly, it should be considered, as a long-term competitor. Cassava is an annual crop, and the topography of the Natal coastal belt makes production difficult. It would probably have to be irrigated to compete economically with sugarcane and sweet sorghum in

South Africa. More experience with cassava and improved production and processing technologies might make this crop more viable in parts of South Africa. The production of ethanol in Australia, using sweet sorghum, is an entirely new venture and research showed that production cost appears to be significantly less than that of ethanol from sugarcane. When existing mills and distilleries are used to produce EtOH from sweet sorghum, the cost of EtOH production is likely to be lower than the cost in a new project. An added advantage of sugarcane and sweet sorghum is the fact that a fibrous residue is available after removal of the fermentable solids from the crops. The fibrous residue can be used as furnace fuel or for 2nd generation EtOH production. Current EtOH production from sugarcane in South Africa is more than the average current production per hectare from cassava in Brazil, and is more than the predicted production from cassava in Australia. The production of EtOH from maize, sweet sorghum, cassava and sugar beet is more seasonal than that from sugarcane. Continuous annual production of EtOH from sugarcane is a problem due to the demand for sugar. Yields of sucrose, estimated recoverable sugars and Brix% are important variables for EtOH production. If Brix% is an acceptable measure of total fermentable solids, sugarcane and sweet sorghum proved to be the more acceptable feedstocks for EtOH production (Thompson, 1979). Research done on EtOH production from sweet sorghum bagasse using microwave irradiation (Marx *et al*, 2014) illustrates the demand to increase the research on the conversion of alternative (non-conventional) biomass sources for renewable energy production.

2.2 Environmental impact of bio-ethanol production from sweet sorghum

In the light of the global trends, and regarding sustainability as one of the the general aims of biofuel production, it is noteworthy to look at the effect of LUC caused by agricultural pratices. Even though the buzzword today is “sustainability” and numerous attempts are in place to reduce the negative impact of human activity on the environment, whether the activities lead to direct land use change (dLUC) and/or to iLUC, the damage can be slowed down. Callisto *et al* (2014) stated that the concept of producing biofuels from renewable energy sources to reduce LUC, green house gasses (GHG) emissions, global warming, etc., is questioned when the effect of the agricultural practices involves in biofuel production also increase eutrophication. Investigations showed that cultural eutrophication is related to human, social and economic activities and this form of eutrophication can be controled, but it speeds up natural eutrophication which is a natural process caused by runoffs of nutrients from natural sources into catchment areas. Natural eutrophication is a slow process and is part of environmental processes, but it can be made worse by human activities. Callisto *et al* (2014) further determined that the minimalisation of

eutrophication is possible because better management of natural resources can be implemented. Cultural (anthropogenic) eutrophication can be controlled to some extent because the environmental impact of humans can be minimised. It was reported that eutrophication could include the dangers of infectious diseases caused by water-related diseases from the overlaoding of P, N and hazardous bacteria. Life cycle analysis (LCA) should be executed for every bio-energy alternative, because it produces a magnitude of end-products. LUC can increase the effect of eutrophication based on increased GHG emissions, contamination of healthy water sources and nett energy balances disturbances. Eutrophication is mainly caused when the fertilisers, containing N and P, are washed off through rainwater and/or irrigation water and when the runoffs and stream flow (iLUC) contaminating downstream water sources such as rivers, lakes, wetlands and estuaries (Schindler *et al,* 2008). Golterman and De Oude (1991) reported that the clearing of natural vegetation and deforestation are contributing to the emmisions of GHG's. Lands that are more open are created and are exposed to erosion and accelarated run-offs, resulting in increased levels of P and nitrates caused by LUC. They also mentioned that chemicals applied by farmers in the form of fertilisers, insecticides and herbicides are washed into fresh water sourses, wetlands and estuaries and add to the increase of eutrophication. Accesive algal growth also occurs and this leads to the depletion of oxygen in lakes, rivers, and coastal waters. To combat or reduce eutrophication, systems should be applied to restore the positive conditions through the reduction of N and P into water resources (Golterman and De Oude, 1991). Biofuel production also has a dLUC effect due to direct impacts on the environment, eg. water -, air – and soil pollution as was reported by Cornelissen *et al* (2009) in ECOFYS. It was further reported by Cornelissen *et al* (2009) that a common law explanation is that the iLUC comes into effect when residues of existing resources are used to produce biofuels, and dLUC's is the effect of the production of crops to produce biofuel and therefore more natural resources are used and affected by these agricultural activities. LUC as result of crop production and biofuel production activities, displace impacts on the environment to other areas causing dLUC which is more controllable than iLUC's, because the indirect effects are sometimes hidden by the point of entry when the environment is affected and when the changes come into competition with food production. The production of biofuels therefore has an indirect effect on LUC's because and it becomes an important issue when global food supply is under discussion where the conversion of natural environments into croplands has a direct effect on the sustainabilty of our environments. Biofuel production is still less harmfull to the environment compared to fossil fuel production.

Apart from a series of international studies concluding that agricultural activities have an effect on LUC, Ansara-Ross *et al* (2012) did a South African study where the effect of pesticides

contaminating South African fresh waters was investigated. Point and non-point sources of pesticides pollution from agricultural activities lead to contamination of canals, dams, ponds, pans, streams and rivers. Miller (2010) mentioned that N fertiliser applications and land use impacts are notable causes of eutrophication, whether the agricultural activities are related to crop and/or lifestock production or not. The contamination of aquatic ecosystems leads to public concerns. A study by Jansen and Rutz (2012) also addressed the sustainability, restoration of degraded land, reduced land abandonment and the mitigation of GHG's. It showed that the expansion of bio-energy in Sub-Sahara Africa could have a negative socio-economic and environmental impact. Regulatory frameworks were put in place to ensure environmentally, economically and socially sustainable production processes of biofuels, of which the most advanced frameworks exist in South Africa and Mozambique. The paper by Jansen and Rutz (2012) mentioned that crops proposed for biofuel production includes sugarcane, sugar beet, sunflower, canola and soybeans, whereas maize was excluded, due to food security reasons and jatropa was excluded, due to invasive and poisonous reasons. In a study on the river water quality in South Africa done by Van der Laan *et al* (2012) it was concluded that agricultural activities, whether for food or bio-energy, could have a negative affect on water quality. Sugarcane and other fertilised and irrigated crops in regards to cultivated areas, play a role in eutrophication due to increasing salt, N and P deposits in run-off waters over time. Areas, which were investigated, include areas around the Tugela River, Malelane and Komatipoort (Crocodile and Komati rivers) and Pongola (Pongola and Bivane rivers). Results showed an increase of salt concentrations that indicated high anthropogenic inputs. These results can be applied to all areas throughout South Africa whether the cultivation of crops are used for food or bio-energy/biofuel crops.

The production of sweet sorghum and the specific effect thereof on the environment, was studied by Olukoya *et al* (2014). According to the study, GHG's can be reduced when ethanol is produced from sweet sorghum feedstock, but under certain conditions. It also showed that the effect of sweet sorghum-bio-ethanol is only detected on a small, decentrilised basis.

2.3 Bio-ethanol from other natural resources

A number of crops have been studied in regards to biofuel and/or bio-ethanol production. In India bio-ethanol is mainly produced from molasses ethanol, but other options for 1st generation ethanol include starchy biomass like grains or tubers. All plant and plant derived materials have great potential to provide renewable energy for the future. Huge amounts of agro and forest residues are feedstocks generated annually, but the availability of these for bio-ethanol production has to be increased (Sukumaran and Pandey, 2010). Blanchard *et al* (2015) mentioned in a study that oil-

seed bearing trees, a number of woody materials, agricultural and municipal waste and sweet sorghum are other feedstocks used in India in the biofuel industry. The Indian Government did set a target of 10% blending with fossil fuels in 2008. Roughly 60% of world ethanol production is from sugar crops, both sugarcane and sugarbeet. Unfortunately, distillation does consume a great deal of energy, especially when ethanol is produced from starch feedstocks where 75% of the energy is used in producing the fuel, leaving a 25% energy positive process. Due to availability of arable land sugarcane is mainly used in Brazil's bio-ethanol fuel programmes and in 2016 an amount of 98.3 billion litres was produced. A mixture of 78% gasoline and 22% anhydrous ethanol is currently used as vehicle fuel throughout Brazil. Ethanol production in the United States (USA) has grown from a small amount in 1978 to 6.4 billion litres in 1998 of which more or less 3.9 billion litres were consumed in the domestic fuel mix. In France the most important single agricultural feed stock for the production of ethanol is sugarbeet, from which roughly 50% of the total is manufactured (Tyagi, 2002). Below reference is made of a few prefered crops more often mentioned in literature.

2.3.1. Sugar beet

Sugar beet is a C_3 crop and is regarded as a very good alternative natural resource for producing biofuel. One of the drawbacks in using sugar beet is due to its vulnerability to diseases. The production thereof must be moved to new fields every season. Thompson (1979) published an article in a journal "The proceedings of the South African Sugar Technologists' Association", where McCann and Prince (1978) was cited, stating that the average yields in Europe are about 45 tonnes sugar beet or 6.3 tonnes of sucrose per hectare, and in the USA 7 tonnes sucrose per hectare. Therefore, vast areas must be available to produce sugar beet every season to supply a biofuel refinery of raw material on a sustainable basis. The USA is currently the leader in sugar beet production, followed by China and Europe. However, this crop is restricted to high rainfall areas to maintain high yields. Sugar beet is also susceptable to diseases, like leaf spot, and the chemical treatments decrease the economical viability of sugar beet as an energy and ethanol crop. The adaptibility to more tropical climates and storage of the raw material is also a problem. Another limitation in the use of sugar beet as an EtOH source, is the fact that it has very little fibrous residue which is suitable to provide the heat energy for processing the ethanol can be used as a 2^{nd} generation ethanol source (Funkenstadt, 2013; Panella, 2012; Lipinsky, 1977; Inman-Bamber, 1978).

In an article by Marx (2012), the Biofuel Strategy of South Africa (2007) was cited, describing the usefulness of sugarcane and sugar beet and the huge contribution it could make in penetrating

the biofuels markets. The study stated that sugar beet is adapted to a wider climatic range than sugarcane, which makes it more viable than sugarcane and still has a sugar (sucrose) content similar to that of sugarcane.

2.3.2. Sugarcane

In the study by Ravindranath *et al* (2011) is was mentioned that although sugarcane as feedstock dominates ethanol production across the world, other crops as maize, sweet sorghum, sugar beet, cassava, rice and wheat are also used as feedstock for ethanol production in developing countries. In Brazil sugarcane is the main feedstock for 1st generation biofuel and produces 5476 litres of EtOH per hectare per year with a global average of 5005 litres per hectare per year. The maize yield is 3651 litres per hectare per year in USA, whereas the global average is around 2372 litres per hectare per year. Indian distilleries use molasses, derived from sugarcane, as the feedstock for ethanol production and the annual supply of molasses is sufficient only for producing approximately 2.7 billion litres of ethanol, of which only a minor share is available for fuel use. Surplus ethanol from molasses is therefor limited and India's cane production can barely supplement the current demand of ethanol even at 5% blending (Sukumaran and Pandey, 2010).

In South Africa, sugarcane is less viable as an energy crop due to the limited areas where it can be cultivated to produce high yields. Furthermore, most areas in South Africa where sugarcane can be cultivated is currently dedicated towards the production of sugar. In The Bureau for Food and Agriculture Policy Report (BFAP, 2005) Thompson (1979) was cited stating that to consider EtOH production from sugarcane, it is important to keep in mind that the major sugar producing areas in South Africa are located in Kwazulu-Natal, Mpumalanga and a small area in the Eastern Cape. Small-scale farmers produce around 13% sugarcane, milling companies produce 2% and large-scale commercial growers produce 75% of the total crop. Brazil's sugar production is shared by the household and biofuel markets, which to the same extent is not possible in South Africa. In the report, the importance of the unit "Brix%" was also refered to because it is an indication of the sugar content of the sugar and the soluble sucrose (TSS) in the sugar which are needed during fermentation. The levels of N fertiliser applications are important because it has an effect on the sugar content of sugarcane and sweet sorghum juice, which in turn determines the fermentation processes and the amount of EtOH, which will be produced. Two fermentation processes are applied namely, aerobic and anaerobic fermentation, and are divided regarding the yeast bacteria or fungus that is used during fermentation and which will determine the end-product. Thompson (1979) mentioned that the programmes and management techniques in South Africa should aim at producing the maximum amounts of sucrose. If the national sugar harvest

was to be shared by the household market and the EtOH market, it will be important to produce the maximum sucrose from the juice and biomass. It becomes a rather complicated process when EtOH is produced from sugar and bagasse, which starts when the sugarcane is being transported to the sugar mill where the cane gets crushed and the sugar juice is then divided into two paths i) high quality for sugar production and ii) juice with low quality sugars for the production of EtOH. From the juice and bagasse, approximately 5500 litres of ethanol can be produced from one hectare of sugarcane. Goldemberg *et al* (2007) investigated the sustainability of ethanol production from sugarcane and reported that huge markets have opened internationally. In Brazil EtOH prices are no longer controlled by the government and therefore the expansion of ethanol production and exports are envisaged which raised concerns regarding sustainability. In the USA, the E10 blends from sugarcane indicated reductions in CO_2 emmissions during winter. However, in South Africa, sugarcane production is restricted to tropical climatic regions and therefore not enough sugar can be produced to support the EtOH markets as well.

2.3.3. Maize

According to the BFAP Report (2005) an amount of 25.4012 kg of maize can produce 9.55 litres of EtOH which indicates that to run one ethanol plant an amount of 370 00 tonnes of maize will be needed to produce 150 million litres of ethanol. Food security became an issue and therefore the South African Government put a ban on the use of maize for EtOH production.

Either wet or dry milling processes are used for ethanol production from cornstarch. A dry-grind process is simpler and will require less capital than wet milling. A dry-grind process entails grinding the corn into a fine powder, which is then cooked, hydrolyzed, and fermented. In a wet-milling process, the numbers of co-products are more due to the separation of the corn kernel into germ, starch, and other components. Starch makes up less than half of the weight of maize and about 40% to 50% of the theoretical yield of EtOH of a maize plant is obtained from starch. The majority of the wet milling end-products are utilized in the EtOH industry (Shukla *et al,* 2000).

According to Bothast *et al* (2005), EtOH has been used as a renewable fuel source across the world, especially in the USA since the turn of the century. The involment of farmers in rural areas also renewed the interest in the production of EtOH by either the dry and/or wet milling processes. It was indicated that additional research is needed to improve the long-term viability of the use of maize to improve the characteristics of the kernel and other higher-valued by-products to keep maize competitive against other crops like sweet sorghum, sugar beet, miscanthus, etc.

2.3.4. Grain sorghum

Grain sorghum (*Sorghum bicolor*) is an important cereal crop in the world and was explored for biofuel production on a worldwide scale. Grain sorghum is utilised in more than 30 countries and makes it very viable to be included into the EtOH program. The research station, ICRISAT, also developed disease resistance in various cultivars, which largely contributes to improved hybrids to be included into a EtOH programme. However, sweet sorghum seems to be best suited for EtOH production because of its higher fermentable sugar content in the stalk, when compared to sugarcane (Reddy *et al*, 2010). As an annual and high biomass-producing crop, grain sorghum fits well into the mix of dedicated energy crops. A synergy is provided by applying what is known from sorghum starch properties to the biofuel sector. Grain sorghum will be a 2nd generation biofuel source because it supplies a lignocellulosic-based raw material, which must be fermented into EtOH to be transformed into a commercially successful venture. A goal was set by the USDA to replace fossil fuels with 30% liquid fuels produced from lignocellulosic-based raw material by the year 2030. Sorghum is important to farmers because its adaptations to marginal rainfall areas make it viable regarding the expansion of grain-based EtOH distilleries. A lot of research already went into the utilisation of the whole-plant concept where the leaves, grain and stems can be used in the production process of EtOH, but there is still work to be done to fully utilise grain sorghum as bio-energy crop (Sarath *et al*, 2008). Sorghum improvement programmes in South Africa started at least 30 years ago and were aimed at both the commercial and small-holder farmer sectors. A variety of sorghum accessions were tested and consisted out of 23 landraces from South Africa, 13 from ICRISAT and 5 newly bred varieties from the National University of Lesotho in Maseru. The study showed that cellulose is the major fibre component in grain sorghum, followed by hemicelluloses and lignin (Uptmoor *et al*, 2006).

According to Dicko (2006), the selection of sorghum varieties is very important to meet specific local food and industrial requirements, especially in developing countries, and plays an important role in food security in African countries. In South Africa and Nigeria, the starch component of grain sorghum is also used for the production of beer and EtOH. Dolciotti *et al* (1998) reported that grain sorghum produces up to 15 t ha^{-1} structural polysaccharides and can be considered as an interesting crop for biofuel production. To improve the performance of sorghum it was recommended by Kaye *et al* (2007) that sorghum should be intercropped with soybeans. The nitrification characteristics of soybeans will supply N to the sorghum plants and it was recorded that this system, together with the correct water regime, increased the amount of panicles per square meter.

There are currently still aspects like protein digestability, levels of extractable proteins, protein and starch interaction, mash viscosity, amount of phenolic compounds, ratio of amylase to amylopectin and the formation of amylase-lipid complexes in the mash that are affecting the EtOH fermentation efficiency of grain sorghum. Grain sorghum should be enhanced to have a higher starch content because a differential of 64% to 74% in starch can result in a 15% calculated difference in EtOH volume per unit grain sorghum used. Researchers and EtOH producers indicated that sorghum is a feasible feedstock for biofuel production and therefore the bio-processing of sorghum grain could benefit both grain producers and the biofuel industry (Wang *et al*, 2008).

2.3.5. Algae

Algae can be used in a third (3^{rd}) generation biofuel production system and is investigated in China because of their shortage of arable land. Studies carried out to estimate the economical viability and the potential of energy production from microalgae, compared well to traditional biomass resources. Areas in the Southwest of China are important regions where developments of biofuel activities are currently taking place, because other areas can only be utilised in winter and will jeoperdise the supply of raw material to the refinaries. The potential energy production from algae estimated to be able to reach 4.19 billion tce a^{-1}, is hindered by transport costs due to the sloapy geology of China. It is estimated that the number of vehicles will increase from 130 million to 150 million by the year 2020 in the People's Republic of China, which will increase the demand on fuel availability. Micro-algae with a 35% lipid content will be able to produce 18.16 t ha^{-1} to 31.62 t ha^{-1} biofuel, which is the equivalent of up to 38.76 t ha^{-1} produced by standard coal. The biodiesel - algae industry will be in a position to supply 34% of the demand for fossil fuels by 2030 (Zhang *et al*, 2012). In a study by the Global Bioenergy Partnership Orginisation, "Algae-based biofuels: A review of challenges and opportunities for developing countries" it was mentioned by Van Iersel *et* al (2009) that algae-based biofuel (ABB) is very viable because of the smaller effect the climatic conditions, land types, water types and space will have on ABB. The process is also more environmentally friendly because the LUC effect is reduced, GHG emissions will be less, fresh water usage can be avoided and it can be produced in synergy with fish cultivation. Both micro-algae and macro-algae (seaweed) can be used as raw material for ABB and algaculture should be economical viable to make the conversion into energy feasible. A number of by-products, such as food-additives, colorants and omega-3-fatty acids, will become available throughout the processing, which contributes to the value-chain of ABB. Limited resources, such as capital and technology, will make the adaptation of ABB less likely.

Klassen *et al* (2016) pointed out another option in which biogas can be produced from micro-algal substrates. It is reported that through anaerobic digestion of biomass, the production of biogas is possible and when the combustion of the biogas takes place, the energy produced is efficient for electricity and fuel. Research done by Singh *et al* (2014) resulted in the importance of the sustainable approaches in the utilisation of plant and micro-algae raw material in the processing of biodiesel. The cost to produce biodiesel from algae-based raw material is higher compare to biodiesel from plant oil, therefore it is recommended that these two raw materials should be utilised together in the production of biodiesel.

2.3.6. Grasses

Porensky *et al* (2014) from the Department of Natural Resources and Environmental Science, University of Nevada, Reno (USA) stated that research done on cool-season grasses (particularly *E. elongate* and *L. cinereus*) indicated that further attention might be worthwhile to add these grasses to the crop list as potential raw materials for biofuel supplied by cold desert agriculture. It was mentioned that it is still unclear that annual and perennial grasses, adapted to regions that are more arid, will be able to produce enough raw material to be regarded as a renewable biomass crop. The feasibility of the transitioning of grasses from traditional crops to low-input biofuel crops should get more attention to gain a better understanding of which grasses are best suited for arid-land biofuel crop development. Due to water use efficiency (WUE) characteristics, it could be expected that cool-season species will produce more biomass than warm-season species. In trials executed to investigate grasses for biofuel purposes, all plots were fertilised annually in late April with ammonium sulphate (21-0-0) at 533.7 kg ha^{-1} which added ± 112 kg ha^{-1} of N. Cool-season and warm-season grasses were compared and differences occured due to the effect WUE had on the root architecture of the plants. However, despite the variances in production levels amongst the grasses, which were evaluated, it was stated that when more emphasis is put on phenology and physiology traits, grasses can be used as potential biofuel crops. Mentioned results are also supported by Leimu & Fischer (2008) as determined through their study on local adaptations in plants, especially now that the current climate change situation influences the performances of plants. Regarding the constant supply of raw materials, it is an important principle to apply in chosing the right crops because local crops produce more biomass than foreign species. Wilsey *et al* (2011) conducted trials to test the hypothesis that there exists a greater richness amongst native specie diversity compared to exotic grassland communities. The research further indicated that exotic specie diversity decreases across grasslands. Another aspect of significance is that above-ground biomass was higher in native grasslands. Regardless of the

slight variances, it is impotant factors to consider when grass species are taken into account for 2nd generation biofuel production. In a paper by Yeap (2008), it was mentioned that EtOH can be produced through three different processes, depending on the raw material. First generation biofuels produced from sucrose-containing raw materials, 2nd generation biofuels from lignocellulose and hemi-cellulose, and 3rd generation biofuels from lignocellulosic algae-based biomass. Yeap (2008) did research as to determine the viability of Napier grass as EtOH raw material source. It is cultivated in tropical countries to serve as feedstock for 2nd generation biofuel production and it can produce three times more EtOH compared to 1st and 3rd generation processes. A weakness in producing EtOH, as with other 2nd and 3rd generation alternative crops, is the complexity and costs involved and explain why it has not played a leading role in comparison to cheaper fossil fuels, even though Napier grasses are a very viable renewable energy source.

2.4 Cultivation of sweet stem sorghum

Sweet sorghum is cultivated through different methods, but row agronomic management can be adapted and will give the best yield. Sweet sorghum needs low input requirements, such as low production costs, is drought tolerant, is versatile, and the high yields give sweet sorghum the edge regarding a better energy balance compared to other competing energy crops, especially if bagasse is included into 2nd generation energy production (Monti *et al*, 2003). In temperate climates of Europe where productivity/adaptation improvements through genetically modified crops are not allowed, sustainable agricultural practices are the options to improve yields. Research efforts seem particularly in want on the subject of harvesting techniques, handling and storing. Therefore, Zegada-Lizarazu and Monti (2012), Universtity of Bologna (Italy), asked the question, "Are we ready to cultivate sweet sorghum as a bio-energy feedstock?" Row width seems to have a significant effect on productivity. In fact, Martin and Kelleher (1984) indicated that regardless the plant density, narrow rows result in higher yields. Higher planting densities associated with narrower than conventional planting rows should result in higher stalk and sugar yields and the improved control of weeds (Lueschen *et al*, 1991; Broadhead *et al*, 1980). According to recorded results from trials by Turgut *et al* (2005), Da Silva *et al* (2018) and Mahmoud *et al* (2013), there are too many variables influencing the production of biomass and juice merely to evaluate yields according to plant densities, viz climatic conditions, agronomic practices, leaf area indexes, stem diameter, stem height and the forming of tillers. Countries across the world are experiencing increasing pressure regarding their commitments in supplying efficient and improved energy. China is such a place, experiencing rapid economic growth the

past thirty odd years, and is trying to keep population growth below 8%. The increase in China's energy supply resulted in increased oil imports and environmental polution. To combat this, investigations into non-edible renewable resources started and sweet sorghum showed potential and an estimated production of 30 million tonnes of ethanol on 8 million hectares of land is envisaged (Li *et al*, 2009). In a study by Buxton *et al* (1999) regarding sweet sorghum yield, the effects of different agricultural practices on the performance of sweet sorghum were investigated and confirmed that double cropping of sweet sorghum with winter rye might improve soil and water conservation, but not the sweet sorghum yield as such.

Sweet sorghum is a sugar-rich crop and due to its efficient C_4 photosynthesis process, a short production cycle, effective nitrogen efficiency use (NUE) and WUE, high tolerance to environmental stresses and adaptability to marginal lands, proved to be an excellent alternative source of raw material for 1st generation ethanol-producing systems. Although WUE was also noted by Rolz *et al* (2014) as important, it was indicated by Schaffert and Gourley (1981) that all the agronomic management practices, such as the use of cultivars with different maturities and sowing the same variety at different times, may help to extent the period of industrial utilisation (PIU), which is the period of time in which the maximum sugar extraction is economicaly viable. Results showed that for several sweet sorghum cultivars the PIU varied from 20 to 40 days. This limited time constitutes a management problem that restricts the raw material supply, and needs future research. Observations were made by Rolz *et al* (2014) in a study where four sweet sorghum genotypes were used in a trial, and results showed that at harvest time there were differences amongst varieties, sites and years regarding sugars and TSS. An inverse correlation was found between stalk sugar content and the ratio between hexoses and sucrose at a physiological maturity stage. Ethanol production was between 200 and 250 grams EtOH/kg of dry stem for Sugar Drip, Top 76-6, and Umbrella genotypes. Ethanol productivity was higher for Umbrella and Top 76-6 and equal to approximately 2,500 L/ha/harvest.

2.5 Studies on biomass / bagasse yields and the effect of nitrogen fertiliser on biomass yields

According to Mastrorilli *et al* (1999) the final EtOH yield per hectare of sweet sorghum (juice plus bagasse) planted will determine the EtOH yield per hectare obtained in any particular agricultural area of the world. Sweet sorghum grown on marginal land, can produce a biomass yield as high as 35 t ha^{-1}, while when grown on irrigated land it can produce up to 130 t ha^{-1}. Ethanol can thus be produced from as little as 0.252 m^3 t^{-1} biomass cultivated on marginal land. The investigation furthermore showed that the productivity and WUE of sweet sorghum, when

affected by soil water deficit, occurring at different vegetative growth stages, could be crucial. A solution might be the use of raw materials that can produce both food (in the form of grain) and fuel (from bagasse) in a single crop (Edenhofer *et al*, 2011).

Results from a study by Sowinski *et al* (2018) on Brunic Arenosols soil in the southwestern region of Poland determined that improved N management is necessary to optimise NUE for sorghum production on sandy soils. Although the biomass did not show a significant response to various N fertiliser application levels (0, 90 and 180 kg ha^{-1}), there were yearly differences. Higher nitrate concentrations in the biomass occured, which is an important by-product in the EtOH based industry. A possible explanation can be that sweet sorghum extracted N from the soil which was present in the soil before the trial was planted. According to Regassa and Wortmann *et al* (2014) in a trial in Louisiana, it showed that sweet sorghum had a lower response to N compared to maize due to lower N uptake. The NUE was higher when the produced energy and biofuel yields were compared. Lower N requirements to produce the same amount of EtOH, compared to maize, makes sweet sorghum more efficient regarding EtOH production (Wortmann *et al*, 2010). Proportionally more N uptake occurred early in the season with a more gradual rate of uptake of other nutrients during the growing season. For energy purposes, however, it seems that the timing of fertilisation is more important than the N application level. Almodares and Darany (2006) indicated that with sweet sorghum the plant height, stem diameter, and dry matter yield increased when N fertilisation occurred at vegetative stage rather than reproductive stage. Almodares and Taheri (2007) reported that N applications have a significant effect on sweet sorghum production. Even though N availability generally exerts the greatest effect on yield, research results are somewhat contradictory. A possible explanation might be the different fertilisers as source of N, which is applied across the world. Moreover, the results supplied by Wiedenfeld (1984) support the majority of publications regarding the reaction of sweet sorghum on NUE where it demonstrated that excessive N fertilisation levels could reduce the juice quality and, consequently, the EtOH yield. Although the threshold of increased biomass yields per N uptake rate differs amongst genotypes, in general, the juice quality expressed as the total dissolved solids, decreases with the highest fertilisation level. In addition, it was stated that N uptake efficiency was found to decrease with the N dosage, whilst the computed EtOH yields would increase with fertilisation to a certain threshold. It is therefore important to reduce biomass growth with lower N dosages, which can lead to higher sugar content in the stalk juice. Zegada-Lizarazu and Monti (2012) indicated that the production and accummulation of the sugar content in sweet sorghum stems are complex processes. It is important to have a good understanding of approriate and sustainable agricultural practises to optimise productivity. It is even more

imparative in countries where genetically modified crops are not allowed. What's more, mentioned in the report is that excess N fertilisation applications can be detrimental to sugar production, as was seen from the results where the threshold of increased biomass yields per N uptake varied amongst genotypes. Different biomass yields in reaction to different N fertiliser applications and on different soil types can be used as a starting point for sweet sorghum fertilisation programmes. The results also showed that moderate to low fertilisation rates are more effective in producing comparatively high EtOH yields. The different uptakes and results regarding biomass yields indicated that there is a need for further studies to determine the potential of sweet sorghum cultivation on various soil types. It is possible to reduce the N supply by rotating sorghum with legume crops, which will provide a percentage of N through nitrification by legume plants. Results by Varvel *et al* (2008), Blevins *et al* (1990) and Wortmann *et al* (2007) showed that legumes could contribute up to about 140 kg N ha^{-1} to the soil when intercropped and rotated with soybeans, either nodulated or not, and it will increase grain yields of grain sorghum. The rotation enhanced sorghum yield due to the fixation of N, or by a preceding soybean crop, up to 35 to 41%. It should be mentioned that the increased yields of sorghum is likely to not be the only reason for increased sorghum yields (Kaye *et al*, 2007). Bagayoko (2000) reported that the infections by *Arbuscular mycorrhizae* of sorghum roots grown in rotation with legumes significantly contribute to increased yields, compared to sorghum monoculture systems. The detrimental effects on stem sugar production by excess N have to improve, because the N contribution by legumes is not that significant. The biomass yield reacts positively to N fertiliser applications, but only up to some point. On the other hand, rotations with winter cover crops such as rye may have positive effects on soil properties, thus reducing soil erosion problems (Ferraris *et al*, 1981; Wortmann *et al*, 2010). Another nutrient usually applied together with N is P, but research indicated that the response of sweet sorghum to P is limited. It is applied when necessary to support early vigour of the seedlings and eventually the EtOH yield. Potassium (K) is a more sensitive nutrient because its availability is necessary for sugar accumulation, as was researched in other sugar crops such as sugarcane and sugar beet (Guiying *et al*, 2000; Saballos, 2003; Wiedenfeld, 1984). Additionally, although sweet sorghum is most suitable as renewable EtOH crop, the advantage lies in the fact that all the parts of the sweet sorghum plant can be utilised in the form of food, animal feed and fibre (eg. paper and board manufacturing) when the by-products are processed (Almodares and Hadi, 2009).

Another reason, extremely important for non-oil-producing developing countries, is that sweet sorghum also produces grain (up to 2.6 t ha^{-1}), which is a valuable product currently used as animal feed. The contribution of sweet sorghum to the combined food-ethanol-fodder value chain

is therefore substantial (Blümmel, 2009). What's more, sorghum is used as human food and is a good source of vitamins and minerals. Sorghum is very suitable for specific food processing practises and is the staple food in Africa. This makes sweet sorghum a multipurpose crop that allows not only for energy production, but also for rural food security (Dicko, 2006).

2.6 Studies on juice yield and Brix% and the effect of nitrogen fertiliser on juice yields and Brix% levels

In a study by Ratnavathi *et al* (2010), it was indicated that the primary advantages of sweet sorghum are (i) its high EtOH productivity 3.1–5.6 m^3 ha^{-1} p.a., (ii) its adaptability to diverse climatic and soil conditions and (iii) its reduced need for N fertiliser and water when compared to corn and wheat. Ratnavathi *et al* (2010) evaluated five sweet stem sorghum genotypes for EtOH production from stalk juice. Data was collected from Keller, SSV 84, Wray, NSSH 104 and BJ 248 for biomass yield, sugar yield from stalks and EtOH production. The TSS was fermented by a distiller's strain of *S. cerevisiae* and EtOH production of 9.0% w/v was obtained from Keller. Similar experiments were conducted with unsterile sweet sorghum juice (15% sugar concentration) and 6.47% w/v EtOH was produced. The total juice obtained is between 20.7 m^3 ha^{-1} and 34.3 m^3 ha^{-1}. In an article by Smith and Buxton (1993), published in the Bioresource Technology Magazine, the data showed that 0.33 g g^{-1} sugar yielded through fermentation and 4.31 g L^{-1} ha^{-1} EtOH was produced. The average EtOH yield across two years was above 3100 L ha^{-1} and did not differ significantly between irrigated and natural rainfed trials.

It was mentioned that sugars can be converted to EtOH directly and starches are utilised in the 1st generation production processes, but the starches must first be hydrolyzed to fermentable sugars by the action of enzymes from malt or molds. The yeast *S. cerevisiae* is the predominant micro-organism employed in industrial molasses fermentations, but the bacterium, *Zymomonas mobilis*, also has potential in this regard (Senthilkumar and Gunasekaran, 2008). Weitzel *et al* (1989) reported juice yields between 46% and 54% if non-stripped stalks were pressed by roller mills, and yield increased to 58% if stalks were stripped before pressing. In India results on sweet sorghum studies showed that 60%, 33%, and 7% of sucrose, glucose and fructose can occur in the juice. The TSS content varied during the growing season with a Brix% of 12.5% early in the season and reaches a value higher than 17% when matured. Sugar content and the sugar profile in different varieties of sweet sorghum juice can be very different (Prasad *et al*, 2007). It is evident that in the past important information has been generated, but was inconsistent and sometimes with limited applicability, therefore important information gaps need to be filled and/or updated regarding the best agricultural practices. Several studies showed that plant density and N fertiliser

application rates have insignificant effects on yield and sugar concentration. (Ferraris *et al*, 1986; Wortmann *et al*, 2010). Unfortunately, sweet sorghum has little breeding history and thus the potential production of EtOH from sweet sorghum through genetic enhancement is very high. The Brazilian sugar and EtOH sector are combinding sugarcane and sweet sorghum. This method extends the operation period of distilleries for EtOH production and reduces overhead costs (Braconnier, 2013; personal communication).

Almodares and Hadi (2009) also pointed out the suitability of sweet sorghum because of its growing pattern characteristics. The storage of large quantities of non-structural carbohydreates (sucrose, glucose, and fructose) in the stem, which can be converted into biofuel, as well as the higher tolerance to heat, salt and drought, make it a better crop compared to sugarcane. It was further pointed out that the input factors in each individual year interacted inconsistently to sucrose and sugar yield. Sweet sorghum bagasse contains cellulose and hemicellulose, which can be converted into EtOH using a variety of technologies and the processing costs of this 2nd generation EtOH makes it less viable than 1st generation EtOH production. The research showed that the sugar yield increased significantly with an increase in sulphuric acid concentration from 50 to 70 g kg $^{-1}$ during fermentation. A potential EtOH yield fermented from of 480 g kg^{-1} total sugar is obtainable after 24 hours, using a mixed culture of organisms. By using a 50 g kg^{-1} sulphuric acid solution in water, with a power input of 43.2 kJ g^{-1} of dry biomass, the sugar yield can be increased up to 820 g kg^{-1} (conversion efficiency of 94%.). These results show that 2nd generation biofuel of 0.252 m^3 t^{-1} or 33 m^3 ha^{-1} EtOH is obtainable using the lignocellulose part of the stalks which is high enough to enjoy more commercial support. Although Limtong *et al* (2006) researched EtOH production from sugarcane, the results can apply to sweet sorghum due to the similarities of the juice. It is reported that EtOH production decreases at sugar concentrations higher than 22% and a possible reason is that various other factors, such as temperature and osmotic pressure, can be responsible for the decrease. It was reported that N deficiency reduces biomass concentration and lead to stuck fermentation. As early as 1992, McCaig *et al* reported the importance of N and that an addition of free amino nitrogen (FAN) leads to higher final EtOH concentrations in the fermented medium. The objective of the study by Breisha (2010) was to produce EtOH through fermentation by using a high sucrose concentration. Breisha (2010) further reported that the fermentation slow down when the sucrose concentration is 25% or less. Different from N fertiliser added to the soil, N in the form of ammonium sulphate can be added during fermentation at a rate of 5 mg g^{-1} of consumed sucrose; this addition is constant at various sugar concentrations and will produce an estimated 11.55% of EtOH. Supplementations during fermentation was also investigated by Laopaiboon *et al* (2009) and

showed the effect of carbon and N supplementations on sweet sorghum juice using very high gravity (VHG) technology. Supplementations to the yeast can be toxic. The correct yeast strains should therefore be used that can tolerate the high EtOH levels to ensure high EtOH production (Phukoetphim *et al*, 2017). Laopaiboon *et al* (2009) and Deesuth *et al* (2015) indicated a decrease in capital and energy cost to produce EtOH. Mei *et al* (2008) and Asli (2010) stated that the supplementation of $(NH_4)_2SO_4$ as N source and KH_2PO_4 (potassium dihydrogen phosphate) as P source increased the EtOH yield to a level of 93.83% when *S. cereviciae* is used during the fermentation of the juice.

Results from a trial where four cultivars (USA 1, USA 2, Hunnigreen and Sugar Graze) were considerd, showed that the sugars (glucose, sucrose and fructose), hemicellulose and cellulose of sweet sorghum are suitable for EtOH production. Reference was made to variations in the concentrations of the sugars amongst the cultivars. Results showed that the overall sugar content decreased toward 6 month's maturity of the plant. A possible explanation for the decreases might be that some genotypes, at the six-month stage, is past the physiological maturity stage and have dried off considerably resulting in much less diluted sugars (Mutepe, 2012). Mentioned observations were supported in a statement indicated that changes in free reducing sugar, total reducing sugars and ethanol are positively correlated in sweet sorghum juice (Ratnavathi, 2010).

Whereas in another study where the genotype Keller was tested and the yeast strain CFTR 01 of *S cerevisiae* was the fermentation agent, it was shown that when the stems along with leaves were used, the EtOH production increased from 0.42 to 0.45 g g^{-1}. It was also reported by Sipos *et al* (2009) that the sweet sorghum juice has sufficient amounts of nutrients for cell growth and increasead EtOH fermentation. Previous research showed that common EtOH fermentation yeasts, such as strains of *S. cerevisiae*, utilize sugars in mixtures of fermentable sugars in a certain order. With over 25% sugars, normal brewery yeasts will always leave significant amount of residual sugars in finished beers. It was found that the major portion of the residual sugars from concentrated juices was fructose. Fructose (1.0–5.1%, w/v) was still in the finished beers made from juices (25% and 30% sugars) and stayed unchanged for some time after the completion of the normal fermentation process. This indicated that, amongst the three kinds of sugars in the concentrated sweet sorghum juices, sucrose and glucose were consumed best by the yeast (Meneses *et al*, 2002). When sweet sorghum juice, together with mixed sugars, is used as raw material during EtOH production, the yeast *S. cerevisiae* is usually used for fermentation because of its preference in utilizing sucrose and glucose to fructose (Bvochora *et al*, 2000; Laopaiboon *et al*, 2009; Berthels *et al*, 2004).

According to Wu *et al* (2009) sweet sorghum is an ideal feedstock for EtOH production in the Southeast and Midwest USA. It contains approximately 16 – 18% fermentable sugars, which makes this crop an ideal feedstock for EtOH production. Increasing the juice yield or making proper use of remaining sugars in the bagasse is crucial for realizing the high EtOH yield of sweet sorghum and is of important economical value (Wu *et al*, 2009). Other views shared by researchers indicated that normal yeast used for EtOH production (brewing and distillers yeast) can ferment all the sugars (glucose and maltose) of similar concentrations in a normal SSF process of maize mash, but might not convert all the sugars in concentrated sweet sorghum juices into EtOH (Devantier *et al*, 2005).

Anglani (1998) stated that sweet sorghum is separated according to the sugar composition into saccharin and a juice type. The saccharin type with high sucrose content is mainly used for refined sugar production and the latter with higher glucose concentration is used for syrup production. However, it is important to apply the correct source of N fertiliser to the soil since even the remaining sugars in the bagasse are influenced by the applied N. Attention paid to these factors will also reduce capital cost, as well as the energy cost, to produce EtOH (Deesuth *et al*, 2015).

Additional proof from trials carried out in the USA during 2008 and 2009 supports the results published in other research papers regarding the effect of N fertiliser on juice production. Juice yields increased from 7481 to 12626 L ha^{-1} and 8587 L ha^{-1} to 13368 L ha^{-1} in 2008 and 2009. Variations amongst seasons and genotypes occurred, but overall there were positive responses to N in 2008. The increase in N increased the juice yields in both cultivars M81E and Topper. In 2009, the juice yield of Topper was not significantly affected by different N rates. Persisting weed competition from pigweed and crabgrass resulted in M81E producing lower juice yields in 2008 (Mosali *et al*, 2010). Holou (2011) conducted trials in Missouri to determine the effect of N fertiliser applications on juice yield. The results indicated that the juice yield (average 68.8±6.1% by weight and P=21) did not depend that much on N applications, but the production year had a significant influence. The density of the juice as determined by the TSS content was not affected by the N fertiliser rates. The amount of juice varied between 15.2 and 71.1 m^3 ha^{-1} depending mainly on the year, but soil type and N fertiliser rate had an effect ($P< 0.0001$). It was further reported that N fertiliser applications also improved the sugar content (Brix%) of the juice, especially in clay soils.

The Brix% is very important as it is a direct measurement of how the plant is performing, as all plants use six molecules of water and six molecules of carbon dioxide, together with the radiation

from the sun, to make one molecule of basic sugar and six molecules of oxygen. When the sugar levels in plants are measured, it directly corresponds to how much sugar production has taken place in the plant. Various definitions are used by researchers to describe the Brix measurement, of which a few is explained here. The unit of measurement for sugars is degrees Brix (Brix% or °Bx is used in scientific literature) which is a measurement of the mass ratio of dissolved sugars to water in a liquid, eg. 25 Brix% solution has 25 grams of sucrose per 100 grams of solution 25% w/w, or in simpler terms it means that there are 25 grams of sugar and 75 grams of water in the 100 grams of solution. The Brix% is determined by the refractive index of light against a sucrose source. Antoine Brix introduced the Brix measurement (Shearer, 2010).

In a trail done by Soileu and Bradford (1985) in Mississippi the results of N fertiliser on Brix% showed that no trend could be established because many variables, such as lime or nonlimed soils, silt loam soils, climatic and management practises, etc., affected a precise determination of the N effect. In general, it appeared that N fertiliser had an effect on the sugar content of the sweet sorghum juice. Four amounts of NPK fertiliser were applied and the juice yield varied between 1 886 kg ha^{-1} to 2 732 kg ha^{-1}, with the highest yield at the third highest N application. The pattern/trend regarding the effect of N application rates on Brix% is recorded in a number of papers, stated that although the N rates do affect biomass and juice yields, there is no significant effect on Brix% (Maw *et al,* 2016; Russo and Fish, 2011; Garafalo *et al*, 2016; Dubey and Kewalanand, 2018; Kurai *et al*, 2015)

2.7 Concluding remarks

Even though a lot of research had been done on planting the best genotypes and the effect of N fertilisers on sweet sorghum production, no data is available to be recommended to the South African agricultural sector. It is clear from the literature study that sweet sorghum is more suitable for the production of biomass, juice and EtOH than other crops. Tables 18 compare the worldly production of EtOH from some of the main crops, indicating sweet sorghum to be superior. Proof is supplied in Chapter 4 that sweet sorghum performed better where potential EtOH production values as high as of 9,978 kL/ha from sugars in the juice and 83,09 L/ha from sugars in the bagasse had been implied.

Evidently sweet sorghum competes well with other feedstocks to be used as renewable alternative crop for the production of EtOH.

2.8 References

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Chapter 3

Materials and Methods

For the purpose of this study sweet sorghum was investigated as an alternative renewable resource for EtOH production and not as such to identify the best genotypes or to recommend specific fertiliser programmes. Two kinds of trials were executed. Firstly, all-in-all 20 genotypes were studied to determine their adaptibilily to various climatic conditions and their suitability to produce enough raw material to be used as feedstock for bio-ethanol production and secondly, some of the same genotypes were used in trials to investigate whether different N fertiliser application levels might have an effect on the production of the genotypes.

3.1 Genotype evaluations regarding biomass yields, Brix % and juice yields

Sweet sorghum genotype evaluation trails were planted in South Africa under dryland conditions since 2010. Randomised block design with three replications were used to screen genotypes. The genotypes screened at the various locations were selected randomly so as to include as many genotypes as possible.

Table 1. List of genotypes used in research

Genotype origin			
ARC	**PANNAR**	**AGRICOL**	**K_2-Agri**
ss 001	Hunnigreen (HG)	E3	Sugar Graze (SG)
ss 003	p 175	SUPA	
ss 007	P 40197	BMR	
ss 008	p 225		
ss 016	p 249		
ss 017	p 868		
ss 019	p 888		
ss 081	p 893		
ss 120	p 895		
ss 27	Silage King (SK)		
ss 56	px 174		
ss 63			
L001			
sswd			
ss 506			

During 2010 – 2011, two trials were planted at Potchefstroom ARC: GCI (26°43'50.19"S and

27°04'51.85" E, altitude 1349 m) and Taung (27°34'43.55"S and 24°44'21.91"E, altitude 1349 m). Thereafter, the genotype trails were extended and trials were planted at Bethlehem ARC: SGI (28°09'54.62"S and 28°17'46.74"E, altitude 1721 m), Rustenburg ARC: IIC (25°43'36.63"S and 27°17'21.53"E, altitude 1130 m), Vaalharts (27°56'46.52"S and 24°50'41.37"E, altitude 1180 m), Potchefstroom ARC: GCI and Potchefstroom Wilgeboom (26°45'33.18 S and 27°06'42.46 E, altitude 1329 m) to cover different climatic and soil conditions. The best performing genotypes were planted consecutively over three years stretching across 2011-12 to 2013-14 and the trial data is presented in Chapter 4. New genotypes were introduced over the three years to investigate alternative genotypes as was exchanged within the Sweetfuel Consortium and against the previous years' best performers. Trials were conducted in different climatic zones as to legitimise the results and to generate sound data for analyses.

Tables 2 to 5 represent a summary of prevailing weather conditions at the locations where trials were conducted. The data presented in these Tables were used in Figures 21 to 23 and 25 to 31. The daily distributions of the climatic conditions are available in Appendix K and data was supplied by Me I Joubert from ARC: Institute for Ground, Climate and Water in Pretoria.

Table 2. Climatic conditions at Vaalharts where the trials were planted

Year	**Tx** (average maximum temperature, °C)	**Tn** (average minimum temperature, °C)	**RF** (rainfall, mm pa^{-1})	**HU** (heat units, °C)
2012	29.39	9.05	317.25	8.73
2013	30.31	9.63	259.59	9.61
2014	32.83	13.69	121.16	12.27
2015	30.6	9.87	257.05	9.74
2016	30.33	10.2	410.21	9.59
2017	29.32	9.14	353.82	8.56

Table 3. Climatic conditions at ARC: GCI and Wilgeboom where the trials were planted

Year	**Tx** (average maximum temperature, °C)	**Tn** (average minimum temperature, °C)	**RF** (rainfall, mm pa^{-1})	**HU** (heat units, °C)
2012	26.18	9.51	648.21	7.39
2013	26.27	9.66	758.95	7.52
2014	25.90	9.63	626.87	7.28
2015	27.29	10.30	543.24	8.48
2016	26.61	10.30	665.99	8.00
2017	25.75	9.82	542.04	7.15

Table 4. Climatic conditions at ARC: IIC where the trials were planted

Year	**Tx** (average maximum temperature, °C)	**Tn** (average minimum temperature, °C)	**RF** (rainfall, mm pa^{-1})	**HU** (heat units, °C)
2012	28.20	12.32	518.16	9.87
2013	28.48	12.61	450.09	10.13
2014	27.78	12.36	774.95	9.54
2015	32.51	12.7	254.51	12.10
2016	28.69	13.78	98.81	10.94
2017	27.83	12.69	710.44	9.87

Table 5. Climatic conditions at ARC: SGI where the trials were planted

Year	**Tx** (average maximum temperature, °C)	**Tn** (average minimum temperature, °C)	**RF** (rainfall, mm pa^{-1})	**HU** (heat units, °C)
2012	22.91	6.82	477.00	4.15
2013	22.54	6.61	699.27	3.96
2014	22.59	6.74	713.22	4.00
2015	24.11	7.54	522.73	5.22
2016	23.60	8.52	615.70	5.18
2017	24.08	6.63	729.23	4.45

An example of variations in plant growth amongst the different genotypes can be seen in Figure 3.

Figure 3. Typical variations in plant growth of different genotypes (A-ss 27, B-ss 120) in Vaalharts 2013-14

The layout of the trials was determined by the Sweetfuel Consortium in an attempt to standardise the agronomical specifications across the six countries who were involved in the Sweetfuel project (www.sweetfuel-project.eu). Examples of the trial layouts are illustrated in Tables 6 and 7.

Table 6. Layout of genotype evaluation trials at the different locations

Rep 1 **Block 1** **Genotype # 12 (4 rows per genotype)** 	--1.5m--	**Block 2** **Genotype # 7** 	----------5m----------	**Block 3** **Genotype # 19** 		**Block 4** **Genotype # 3** 		…etc …etc		….to 22 ….to 22
----2 m----	-1.5m-									
Rep 2 **Block 1** **Genotype # 22**		**Block 2** **Genotype # 14**		**Block 3** **Genotype # 11**		**Block 4** **Genotype # 4**				
Rep 3 **Block 1** **Genotype # 1**		**Block 2** **Genotype # 10**		**Block 3** **Genotype # 16**		**Block 4** **Genotype # 21**				

The same layout was used at all the locations where the genotypes were tested. Different randomisations of the genotypes were used at each location. The genotypes were planted in four rows of 5 m each. The inter-row spacing was 0.6 m and the intra-row spacing was 8 cm. A plant population of 207 500 plants per hectare was achieved.

The average sand, silk and, clay content (soil textures) at the various locations where the trials were conducted is given in Table 7.

Table 7. The soil type indicating the average sand, silk and clay percentages at the various locations

Soil type	Wilgeboom	Vaalharts	Rustenburg	Bethlehem	Potchefstroom
Sand %	73	92	47	77	53
Silk %	7	2	10	3	10
Clay %	19	7	43	20	37

Fertilisers were applied according to the recommendations of Mr W. Deale (Researcher, ARC: GCI). The applications done at the genotypes trials were merely to standardise the soil nutrient content and to supply the necessary additional nutrients which were required for proper plant growth. According to the analyses of the soil samples and recommended fertilisers to be applied, the required fertilisers were applied to make it possible to evaluate the genotypes and their reaction to different N fertiliser levels (see Appendices E 1 to E 10). The applications also took the clay content of the different soils in consideration, e.g. the average clay content of the soil at Potchefstroom is 37%, Bethlehem 20% clay and Rustenburg has an average silk content of 10% and a clay content of 43%. At Potchefstroom, the fertilisers that were applied for the genotype trials were 150 kg ha^{-1} super phosphate applied with planting, together with topdressing of 100 kg ha^{-1} ammonium sulphate. During the N fertiliser trial in Potchefstroom during 2016/17 NPK 3:2:1 (25) was applied to the soil in the glasshouse. At Bethlehem 320 kg ha^{-1} KAN (28) was applied. At Rustenburg 200 kg ha^{-1} MAP (33) and 220 kg ha^{-1} KAN were applied. Vaalharts fertiliser applications were 150 kg ha^{-1} super phosphate and 470 kg ha^{-1} ammonium sulphate. Wilgeboom received 140 kg ha^{-1} MAP (33) and 230 kg ha^{-1} KAN. The size of each block/plot was 9 m^2, as was indicated in Tables 6 and 7. The fertiliser recommendations were calculated on an application-per-hectare basis and were recalculated to the size of the blocks/plots. Data was statistically analysed with Anova's and AMMI-byplots by using Microsoft: Genstat for Windows (2015 & 2018), 18th Edition. Planting time started as soon as 50 mm of rainfall was measured; usually from mid October to mid December. Chemical weed control was executed by using Sorgomil (active ingredient: terbuthylazine + S-metolachlor) applied at 35 L ha^{-1} and Basagran (active ingredient: sodium salt of bentazon) applied at 2-3 L ha^{-1}. In addition weeding was done manually. Insecticides used to control stalkborer and aphids were Bulldock (active ingredient: beta-cyfluthrin) applied at a rate of 0.6 ml per 100 m row and Metacystox (active ingredient: oxydemeton-methyl) at an application rate of 1.75 – 2.25 L ha^{-1}, respectively. Harvesting was done when the seed reached the physiological matured stage, which usually was from day 90 to day 120, depending on the genotype. Stalks were cut with a thumper cutter at a height of about 20 cm above the ground. Representative samples (54 stalks per genotype per replication) from the

inner two rows were harvested and processed. The panicules were removed and not considered as part of the measurables, and only the stalks with the leaves were processed. The stalks with leaves were weighed and then the juice was pressed from the stalks with a three-roller hydraulic press. The biomass yield (mass) and juice yield (mass) was determined with an electronic scale (I'Can Precision Scale OCS-20B, accurate 2 decimals) and the Brix% was measured with a refractometer (Atago Pocket Refractometer PAL-1). The roller press used in South Africa (ARC: GCI) is shown below in Figure 4.

Figure 4. The three roller hydraulic press used at ARC:GCI to extract the juice

The bagasse (stalks) material that was left after the juice has been extracted can be seen in Figure 5. The bagasse still contained some residual sugars and juice, therefore TSS from the bagasse and the extracted juice are fermented separately when EtOH is produced. The amounts of bagasse EtOH and juice/sugar EtOH is added to obtian the total calculated EtOH from the sweet sorghum genotypes under investigation.

Figure 5. Image of sweet stem sorghum bagasse (uniform for all locations)

3.2 Trials to investigate the potential ethanol production (calculated) from sweet sorghum when various nitrogen levels are applied at various locations

The fertiliser application trials stretched over a couple of years viz. 2011/12 to 2013/14 and 2016/17, which were planted in Wilgeboom, Potchefstroom ARC: GCI and Vaalharts, respectively. Various genotypes were planted which are listed in Table 8.

Table 8. List of genotypes planted during 2011-2014 and 2016/2017 seasons

2011/2012	2012/2013	2013/2014	2016/2017
HG	HG	ss 027	ss 007
p 229	ss 03	ss 120	HG
ss 506	ss 56	HG	SG
sswd	ss 120	SK	
BMR	ss 081	p 893	
ss 017	ss 008	ss 017	
ss 016	ss 016	E3	
ss 120	ss 007	ss 003	
ss 019	SUPA	p 868	
p 175	BMR	ss 007	
ss 007	p 868	ss 008	
p 40197	p 204	ss 016	
L001	SK	ss 001	
p 304	ss 017	p 249	
		ss 081	
		SUPA	
		p 225	
		p 895	

The layout of the trials are shown below in Table 9.

Table 9. Layout of the nitrogen fertiliser trial at the Potchefstroom (2016/17)

N application NPK 3:2:1 (25) (kg/ha)	Area (m^2)	N application (g/block)
0	9	0
50	9	45
100	9	90
150	9	135
200	9	180

Hg /150 135g 14 11	SG /200 180g 10 12	007 /200 180g 5 13	Hg /0 0g 11 14	007 /50 45g 2 15						1 007/0 2 007/50 3 007/100 4 007/150 5 007/200 6 SG/0 7 SG/50

SG /50 45g 7 10	007 /150 135g 4 9	Hg /50 45g 12 8	SG /150 135g 9 7	Hg /100 90g 13 6	007 /100 90g 3 5	SG /100 90g 8 4	Hg /200 180g 15 3	SG /0 0g 6 2	007 /0 0g 1 **REP3** Block1
007 /200 180g 5 6	Hg /100 90g 13 7	SG /100 90g 8 8	007 /100 90g 3 9	Hg /50 45g 12 10	007 /150 135g 4 11	SG /200 180g 10 12	007 / 0 0g 1 13	Hg / 0 0g 11 14	SG /50 45g 7 15
SG /150 135g 9 5	Hg /150 135g 14 4	SG /0 0g 6 3	007 /50 45g 2 2	Hg /200 180g 15 **REP2** Block1	Hg /200 180g 15 15	007 /0 0g 1 14	Hg /150 135g 14 13	SG /200 180g 10 12	007 /100 90g 3 11
SG /0 0g 6 **REP1** block1	007 /200 180g 5 block2	SG /50 45g 7 block3	Hg /100 90g 13 block4	007 /150 135g 4 block5	SG /150 135g 9 block6	Hg /50 45g 12 block7	SG /100 90g 8 block8	007 /50 45g 2 block9	Hg /0 0g 11 block10

8 SG/100
9 SG/150
10 SG/200
11 HG/0
12 HG/50
13 HG/100
14 HG/150
15 HG/200

The trials were cultivated under dryland conditions and a randomised block design and two repetitions were applied. The genotypes were planted in four rows of 5 m each, the inter-row spacing was 0.6 m, and the intra-row spacing was 8 cm. Soil analysis was done and fertiliser recommendations were made by Mr W. Deale to apply the correct N levels. Fertilisers were applied according to the soil analysis. The applications were calculated on a basis to neutrilise the N residue from previous years (as control at 0 kg ha^{-1} and counted as one of the applications) and to apply the additional fertilisers at the different levels to accommodate the N fertiliser levels to study the effect of N levels on biomass yield, sugar content and juice yield. To study the effect of different N fertiliser application levels on the genotypes, eight N fertiliser application rates were applied across the time span of this study, namely 0 kg ha^{-1}(as control and was counted as a application level), 30 kg ha^{-1}, 50 kg ha^{-1}, 60 kg ha^{-1}, 90 kg ha^{-1}, 100 kg ha^{-1}, 120 kg ha^{-1}, 150 kg ha^{-1} and 200 kg ha^{-1}. At Vaalharts 150 kg ha^{-1} super phosphate was applied, together with ammonium sulphate at a 0 kg ha^{-1}, 30 kg ha^{-1}, 60 kg ha^{-1}, 90 kg ha^{-1}, 120 kg ha^{-1} rate. At Wilgeboom a 200 kg ha^{-1} level was added and 285 kg ha^{-1} super phosphate was applied, together with KAN (28) at a 0 kg ha^{-1} (as control), 30 kg ha^{-1}, 60 kg ha^{-1}, 90 kg ha^{-1} and 120 kg ha^{-1} rate, and 200 kg ha^{-1} in 2014. At the Potchefstroom (2016/17) trial, a 150 kg ha^{-1} level was applied with planting and 50 kg ha^{-1} as top dressing, and NPK 3:2:1 (25) was applied at the different levels.

Germination after 10 days of planting and top dressing application of the fertiliser and are shown in Figures 6 and 7 respectively.

Figure 6. Image of germination ten days after planting in glasshouse (Potchefstroom, 2016-17)

Figure 7. Image of fertiliser application (top dressing of NPK 3:2:1 (25)) in glasshouse (Potchefsatroom, 2016-17)

Figure 8 shows the variation in the growth performances of the sweet sorghum genotypes at the same growth stage in reaction to different N fertiliser application levels (2016-2017 season). Figure 9 illustrates the height the plants can reach at physiological maturity stage (2016-2017 season). The genotype SG shows lodging in Figure 9, which is the result of a thinner stem that cannot support the height this genotype reached in the glasshouse.

Figure 8. Image of genotype variations and reaction to fertiliser levels (0 kg ha^{-1} to 200 kg ha^{-1}) in Potchefstroom, 2016-17

Figure 9. Image of plant height at physiological mature (harvesting) stage in Potchefstroom, 2016-17

Planting time started as soon as 50 mm of rainfall was measured. Chemical weed control was executed by using the same herbicides as were used in the genotype trial. Weeding was also done manually. Insecticides used to control stalkborer and aphids were the same as were used in the

genotype trial. Harvesting was done when the seed reached physiological matured stage, which usually is from day 90 to 120, depending on the genotype. Stalks were cut with a thumper cutter at a height of about 20 cm above the ground and representative samples from the inner two rows were taken and processed. Juice was pressed from stalks with the three roller hydraulic press (Figure 8). Representative samples (54 stalks) from each genotype were processed and the data was recorded and anaysed. The panicules were removed and not considered as part of the measurables, and only the stalks with the leaves were processed. The stalks with leaves were weighed and then the juice was pressed from the stalks with a three-roller hydraulic press. The mass of the biomass and juice was determined and Brix% was measured with a refractometer. Data was statistically analysed by using Genstat (data analysis programme for Windows 18th Edition).

3.3 Determination of sugar content of juice and bagasse

Compositional analysis of the extracted juice from the genotypes, which were planted during 2016/2017, was done at the North-West University (NWU) using high-pressure liquid chromatography (HPLC) (see Appendix G).

Table 10. Compositional analysis (g/L) of the juice of some cultivars

Genotype: ss 007										
N (kg ha^{-1})	Sucrose	Citric acid	Glucose	Xylose	Arabinose	Succinic acid	Glycerol	Acetic acid	methanol	Ethanol
0	11.62	1.91	51.03	62.19	0.56	10.53	0.5	2.02	0.95	1.52
50	30.27	0	101.38	94.79	0.49	11.7	0.41	0.97	1.48	0.67
100	12.39	3.08	117.15	95.63	0.81	10.18	0.38	0.41	1.02	0.74
150	3.51	0	72.6	74.55	0.62	8.72	0.62	5.62	0.86	2.93
200	21.2	0	34.8	108.06	0.37	9.2	0.48	1.38	0.86	2.93
Genotype: Hunnigreen (HG)										
N (kg ha^{-1})	Sucrose	Citric acid	Glucose	Xylose	Arabinose	Succinic acid	Glycerol	Acetic acid	methanol	Ethanol
0	2.64	1.1	50.37	49.59	0.64	10.32	0.31	2.09	0.59	0.44
50	5.2	2.12	71.11	65.79	0.55	9.27	0.3	0.65	0	0
100	2.59	0.98	57.55	53.02	0.47	11.29	0.22	1.83	0.7	0
150	3.33	0.63	37.22	41.87	0.67	9.38	0.4	1.22	0	2.82

200	5.76	1.83	57.08	57.57	0.36	11.79	0.46	1.04	0	0.61
Genotype: Sugar graze (SG)										
N (kg ha^{-1})	Sucrose	Citric acid	Glucose	Xylose	Arabinose	Succinic acid	Glycerol	Acetic acid	methanol	Ethanol
0	3.74	1.07	50.48	54.77	0.54	12.18	0.35	1.46	0	1.28
50	3.08	1.99	31.98	51.34	0.43	6.56	0.48	2.71	1.16	8.23
100	3.37	1.06	5.72	28.53	0.47	8.24	1.52	3.83	0.58	13.57
150	3.55	1.03	36.13	41.65	0.53	4.79	0.24	1.2	0	1.82
200	3.79	2.29	49.34	61.34	0.76	9.3	1.44	1.38	0.83	13.49

The compositional analysis of the bagasse which was done by the ARC: API in Pretoria (see Appendix F). The cellulose and hemicellulose content is an indication of 2nd generation sugar/ethanol potential. The sugars and juice that remains in the pressed stalks after the majority of the juice has been extracted, contribute to the total sugar yields, resulting in higher EtOH production levels.

Table 11. Comparison of compositional analysis of the bagasse of three genotypes at N applications of 0 and 200 kg ha^{-1} (wt. % on a wet basis)

Component	**Method**	**0 kg N/ha**	**200 kg N/ha**	**0 kg N/ha**	**200 kg N/ha**	**0 kg N/ha**	**200 kg N/ha**
Dry matter	ASM013	86.87	88.70	87.87	89.06	87.96	86.69
Moisture	ASM013	13.13	11.30	12.13	10.94	12.04	13.31
Ash	ASM048	7.58	6.46	10.70	8.91	7.01	4.20
Protein[a]	ASM078	5.26	7.53	7.96	3.81	5.07	4.42
Fat[b]	ASM044	0.66	0.87	0.95	1.22	0.96	1.04
Carbohydrates	ASM075	73.37	73.84	68.26	75.12	74.92	77.03
NDF	ASM060	57.25	64.62	58.14	61.39	61.86	50.63
ADF	-	36.35	42.51	35.59	34.74	34.80	28.60
ADL	-	8.08	11.95	6.92	6.19	7.27	10.14
Cellulose[c]	Calculated	28.27	30.56	28.67	28.55	27.53	18.46
Hemicellulose[d]	Calculated	20.90	22.11	22.55	26.65	27.06	22.03
Lignin[e]	Calculated	8.08	11.95	6.92	6.19	7.27	10.14
Residual sugars[f]	Calculated	16.12	9.22	10.12	13.73	13.06	26.40

a. Protein = N x 6; b. Ether extract; c. ADF-ADL; d. DNF-ADF; e. Acid soluble lignin;
f. Residual sugars = Carbohydrates – Cellulose – Hemicellulose - Lignin

The genotypes HG, SG and ss 007 were chosen due to the fact, that these genotypes performed well throughout the genotype and N fertiliser application trials. The performence of SG varied amongst the three variables (biomass, Brix%, juice), yet high yields were still delivered.

3.4 Statistical Analysis

Data were analysed using the statistical program GenStat (2015, 2018). All trials were designed as randomised block designs. The genotype trials had three repetitions and the N trials had 2 repetitions. The Anova' and AMMI-byplots were run using this programme. Differences between entries were tested for in an analysis of variance. Because analysis of variance was done, the standard error of the mean (SEM) was accommodated in the Figures in Chapter 4 and not the standard deviation. The least significant difference (LSD) values were added below the Figures as footnotes. The data was was acceptably normal with homogeneous treatment variances. Treatment means were separated using Fishers' protected t-test least significant difference (LSD) at the 5 % level of significance (Snedecor & Cochran, 1980), if the F-probability from the ANOVA was significant at 5 %.

3.5 References:

VSN International (2015, 2018). Genstat for Windows 18th Edition. VSN International, Hemel Hempstead, UK. Web page: Genstat.co.uk

SNEDECOR, GW & COCHRAN, WG. 1980. Statistical methods (7th Ed.). Iowa State University Press

Chapter 4

Results and Discussion

4.1. Genotype evaluations regarding biomass yield, juice yield and Brix% at three locations during 2011-2012 to 2013-2014

4.1.1. Biomass yield during 2011-2012 to 2013-2014

A total of 20 sweet sorghum genotypes were planted and tested at Potchefstroom, Rustenburg and Bethlehem in the genotype evaluation trials during the 2011-2012 planting season. The biomass yield, Brix index and juice yield obtained for the best performing genotypes planted at Bethlehem, Potchefstroom and Rustenburg are given in Figure 10, 11 and 12 respectively. Performance yields for the genotypes not shown here can be found in Appendix A1. The statistical analysis for the genotype evaluations can be found in Appendix J.

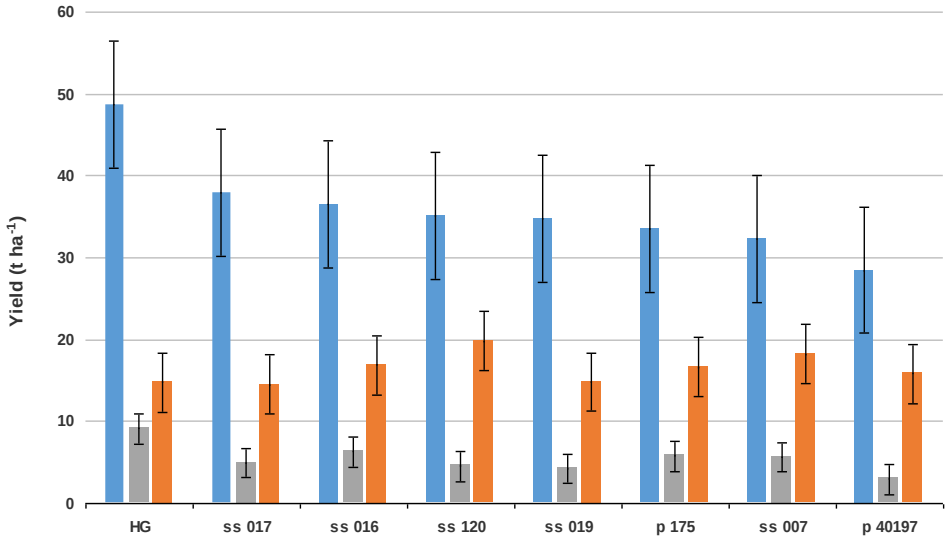

Figure 10. Biomass yield (t ha^{-1}) (■, a), Brix index (%) (■, b) and juice yield (t ha^{-1}) (■, c) from the different genotypes planted at Bethlehem during 2011-2012

a) biomass LSD (p=0.05): 12.769
b) Brix% LSD (p=0.05): 5.946
c) juice LSD (P=0.05): 2.99

The values in Figure 10 are based on the data capturing of the raw materials and was recording as such that indicates that HG produced the highest biomass (48.6 t ha^{-1}) and juice (9.1 t ha^{-1}) yields. The best Brix% (19.8%) was measured from ss 120. When the F pr – value for Bethlehem is considered for the three measurables (mass : 0.325; Brix% : 0.156; juice : 0.416), it appears that there are no significant differences amongst the genotypes. In all the trials the biomass yields

were determined by weighing the fresh stalks.

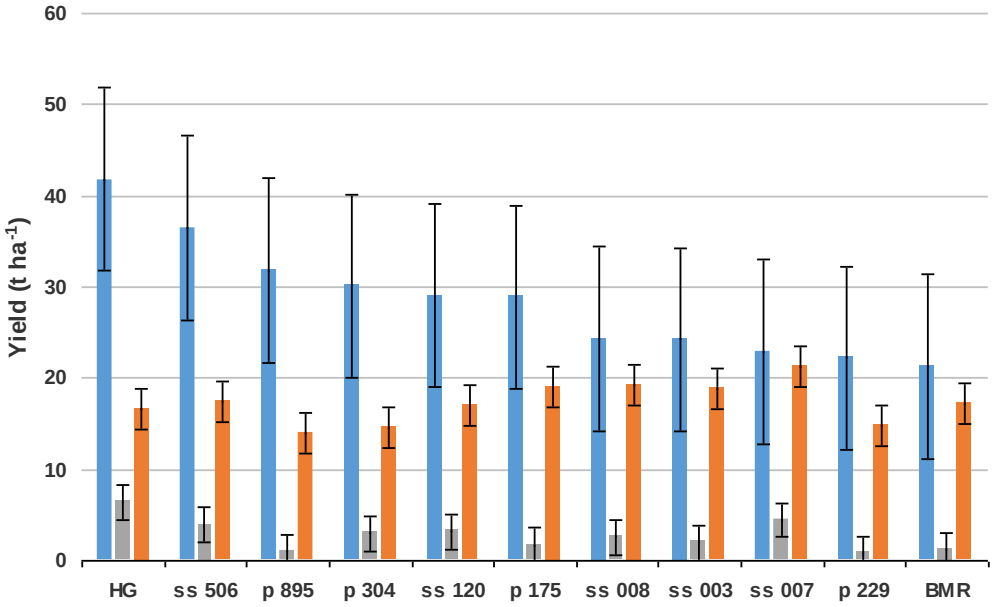

Figure 11. Biomass yield (t ha^{-1}) (■, a), Brix index (%) (■, b) and juice yield (t ha^{-1}) (■, c) from different genotypes planted at Rustenburg during 2011-2012

a) biomass LSD (p=0.05): 16.63
b) Brix% LSD (p=0.05): 3.652
c) juice LSD (P=0.05): 3.103

The values in Figure 11 are based on the data capturing of the raw materials and was recording as such that indicates that HG also produced the highest biomass (41.8 t ha^{-1}) and juice (6.4 t ha^{-1}) yields. The best Brix% (21.3%) was measured from ss 007. When the F pr - value for Rustenburg is considered for the three measurables (mass : 0.049; Brix% : <0.001; juice : 0.05), then there are significant differences amongst the genotypes.

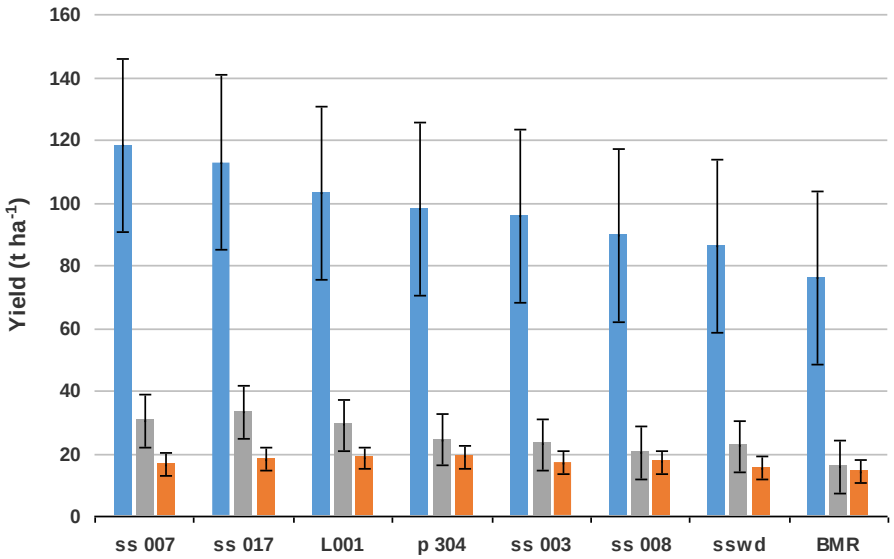

Figure 12. Biomass yield (t ha^{-1}) (■, a), Brix index (%) (■, b) and juice yield (t ha^{-1}) (■. c) from different genotypes planted at Potchefstroom during 2011-2012

a) biomass LSD (p=0.05): 45.62
b) Brix% LSD (p=0.05): 6.009
c) juice LSD (P=0.05): 13.58

The values in Figure 12 are based on the data capturing of the raw materials and was recording as such that indicates that ss 007 produced the highest biomass (118.4 t ha^{-1}) and highest juice (33.3 t ha^{-1}) was yielded by ss 017 at Potchefstroom. The best Brix% (18.8%) was measured from p 304. When F pr-value for biomass yield in Potchefstroom is considered for the three measurables (mass : 0.289; Brix% : 0.171; juice : 0.151), then there are no significant differences amongst the genotypes.

Genotypes HG, ss 017, ss 120, p 175, p 304, ss 007, ss 008 and ss 003 performed well at two of the three locations. Although the highest biomass yield was produced by ss 007 at Potchefstroom the Brix% (16.5%) only just made the benchmark for viable EtOH production during 2011/12. It can therefore be said that the biomass might not be the determining factor when it comes to EtOH production from sweet sorghum. The highest Brix% (21.32%) was recorded from the juice of genotype ss 007 at Bethlehem during 2011-2012. This makes this genotype very viable for EtOH production, because almost twice as much EtOH can be produced from the same volume of extracted juice. Figure 11 indicates that at Rustenburg genotype HG out performed the other genotypes regarding biomass (41.82 t ha^{-1}), but the average juice and Brix% levels were low. The average rainfall (RF) across the seasons was higher at Bethlehem, but the biomass production at Bethlehem was lower than Potchefstroom. The soil type at Bethlehem is sandy. The heat units (HU) at Potchefstroom (average 7.63) was higher compared to Bethlehem (average 4.49) and

could be a possible explanation for the higher yields at Potchefstroom. The Brix% of the majority of the genotypes are higher than 16%, which is the minimum benchmark for viable EtOH production from sweet stem sorghum (Schaffert, 2011: personal communication). Only ss 007 had a constant production across the three locations and three production years. It is evident from Figures 10 to 18 that although the biomass yield is decreasing, the Brix% and juice yields almost stayed constant. The best average juice yield across all seasons was recorded at Potchefstroom. The variances amongst the genotypes indicate that the soil, photoperiod effect and water (rainfall/irrigation) might have played a role in the performances of the genotypes. This phenomenon can be applied to all the variances amongst genotypes and climatic conditions, yet it still appears that the internal genetic physiology of the plant determines the production. It is clear from the recorded data that huge variances amongst the genotypes exist, even though management practises were the same at all the locations.

The biomass yield, Brix index and juice yield obtained from the best performing genotypes planted at Bethlehem, Potchefstroom and Rustenburg during the 2012-2013 planting season are given in Figure 13, 14 and 15 respectively. Data for gentotypes not shown here can be found in Appendix A2.

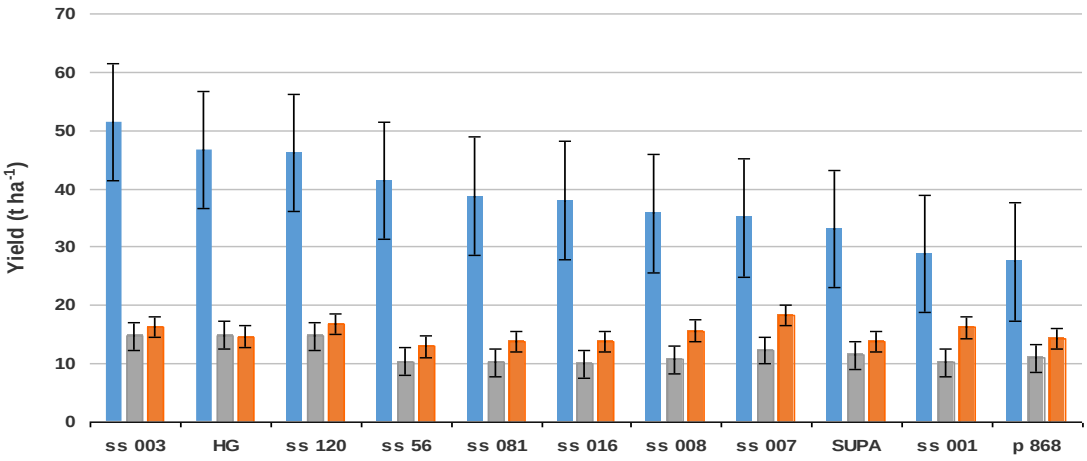

Figure 13. Biomass yield (t ha^{-1}) (■, a), Brix index (%) (■, b) and juice yield (t ha^{-1}) (■, c) from the different genotypes planted at Bethlehem during 2012-2013

a) biomass LSD (p=0.05): 16.61
b) Brix% LSD (p=0.05): 2.985
c) juice LSD (P=0.05): 3.911

A huge difference (24.01 t ha^{-1}) between the best (51.49 t ha^{-1}) and worst (27.48 t ha^{-1}) biomass yield was recorded at Betlehem. The juice yield only differs with 4.89 t ha^{-1} and the Brix% with 5.43 t ha^{-1}, which indicates that although more biomass will supply more juice the biomass is not specifically determining the produced amount of juice and Brix%. When the F pr - value for Bethlehem is considered for the three measurables (mass : 0.007; Brix% : <0.001; juice : <0.001),

then there are significant differences amongst the juice yields and Brix%.

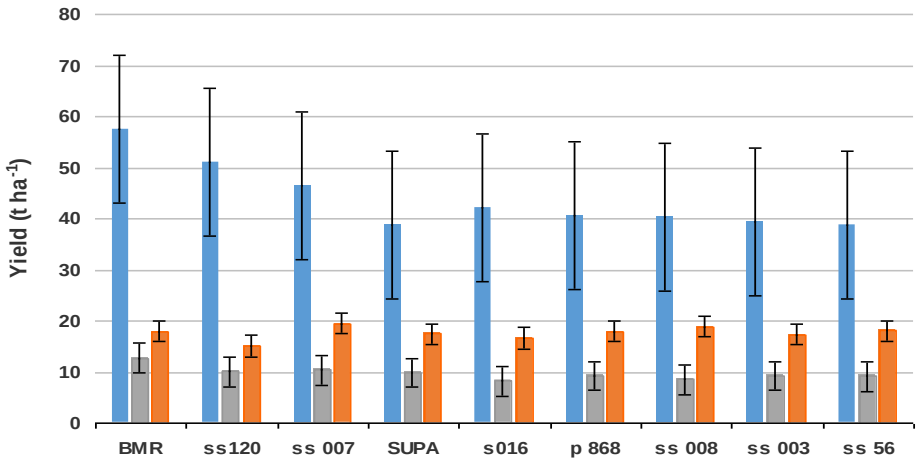

Figure 14. Biomass yield (t ha^{-1}) (■, a), Brix index (%) (■, b) and juice yield (t ha^{-1}) (■, c) from different genotypes planted at Potchefstroom during 2012/2013

a) biomass LSD (p=0.05): 23.88
b) Brix% LSD (p=0.05): 3.34
c) juice LSD (P=0.05): 4.638

The same phenomenon is also visible in Figure 14 where the juice and Brix% variances were not affected by the biomass production. Genotypes ss 008 and ss 003 produced some of the best juice yield and Brix% with lower biomass yields. When the F pr - value for Potchefstroom (2102-13) is considered for the three measurables (mass : 0.303; Brix% : 0.008; juice : 0.408), then there are no significant differences amongst the genotypes regarding biomass and juice yields.

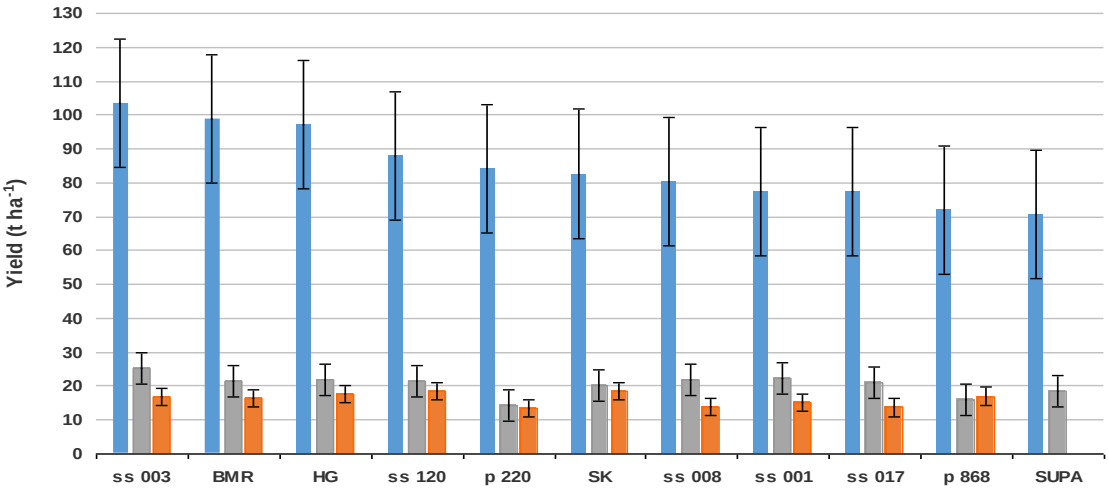

Figure 15. Biomass yield (t ha^{-1}) (■, a), Brix index (%) (■, b) and juice yield (t ha^{-1}) (■, c) from different genotypes planted at Rustenburg during 2012/2013

a) biomass LSD (p=0.05): 31.28
b) Brix% LSD (p=0.05): 4.303
c) juice LSD (P=0.05): 7.635

More genotypes performed well across the three locations during 2012-2013, compared to the previous season. Good biomass production levels were maintained by five genotypes (ss 003, ss 120, ss 008, p 868 and Supa) across the three locations. The treatments stayed the same as in 2011-2012, and the trend of the biomass yield, Brix% and juice yield was very similar. The best performing genotype regarding biomass yield during 2012-2013 was ss 003 with 103.44 t ha^{-1} at Rustenburg. The best juice yield (25.05 t ha^{-1}) was achieved at Rustenburg by ss 003 and the Brix% (16.87%) just made the benchmark (Figure 15). The biomass and juice yield were exceptional, taking into account that this production was achieved under dryland conditions and a soil type with high clay content. The highest Brix% (19.44%) was produced by ss 007 at Potchefstroom, although the juice yield (10.35 t ha^{-1}) was low compared to the other genotypes. Eleven out of all measured Brix% values were below the benchmark during this production year.
The biomass yield, Brix index and juice yield obtained for the best performing genotypes planted at Bethlehem, Potchefstroom and Rustenburg during the 2013-2014 planting season are given in Figure 16, 17 and 18, respectively. The best performing genotypes' data are shown in the figures and the accommodating data for the genotypes not shown here can be found in Appendix A3.

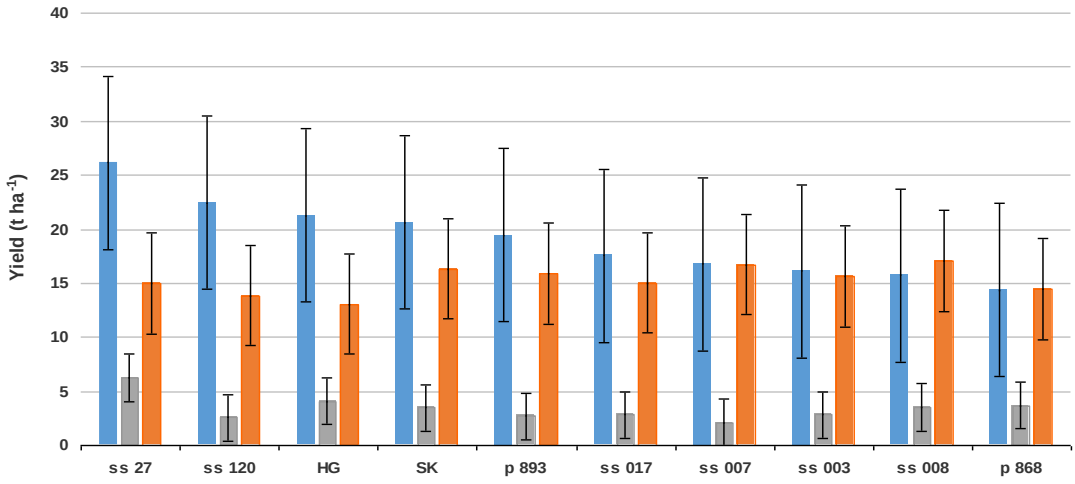

Figure 16. Biomass yield (t ha^{-1}) (■, a), Brix index (%) (■, b) and juice yield (t ha^{-1}) (■, c) from the different genotypes planted at Bethlehem during 2013/2014

a) biomass LSD (p=0.05): 13.35
b) Brix% LSD (p=0.05): 7.774
c) juice LSD (P=0.05): 3.644

An interesting picture is presented by the data in Figure 16. The measured Brix% values were extremely high compared to the juice yields and biomass yields, even though only five genotypes reached the benchmark for acceptable Brix% values.

Due to the complexity of the genotypes' performances accross locations and seasons, no explanation can be given.

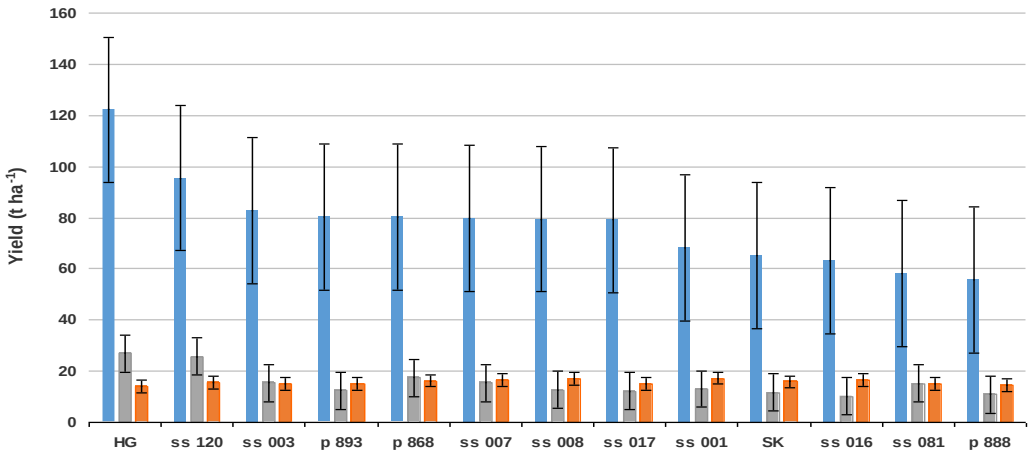

Figure 17. Biomass yield (t ha^{-1}) (■, a), Brix index (%) (■, b) and juice yield (t ha^{-1}) (■, c) from different genotypes planted at Potchefstroom during 2013/2014

a) biomass LSD (p=0.05): 47.4
b) Brix% LSD (p=0.05): 4.031
c) juice LSD (P=0.05): 12.1

Although the amounts of the data represented in Figure 17 differ from those in Figures 10 to 15, a similar picture is visible indicating the high biomass yields and almost stable juice yields and Brix% values. This is, however, not a disqualifying characteristic of sweet sorghum, because the measured amounts are still high and it will be the sugars from the biomass (bagasse) and the sugars from the juice that will ultimately be fermented, and that will determine the total amount of EtOH that will ultimately be produced.

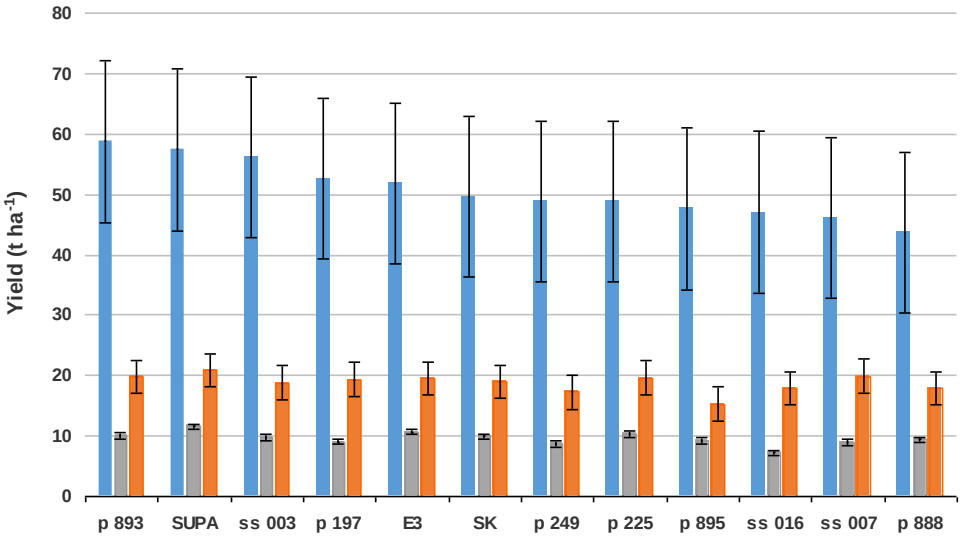

Figure 18. Biomass yield (t ha^{-1}) (■, a), Brix index (%) (■, b) and juice yield (t ha^{-1}) (■, c) from different genotypes planted at Rustenburg during 2013/2014

a) biomass LSD (p=0.05): 22.23
b) Brix% LSD (p=0.05): 4.631
c) juice LSD (P=0.05): 61.64 (transformation square root: 0.8142)

Out of 20 genotypes which were tested during 2013-2014 four genotypes (SK, p 893, ss 007, ss 003) produced the best during the 2013- 2014 season across the three locations. During the 2013-2014 season the best biomass yield (122.16 t ha $^{-1}$) and a juice yield of 26.86 t ha^{-1} by HG were produced in Potchefstroom. The biomass yield was an exceptional high yield, although the Brix% (14.14%) was below the benchmark of 16%. Of all the genotypes, which were tested during 2013/14, only ss 003 also performed well during 2012-2013. Worthwhile to mention that HG did not perform well during the two previous seasons and produced the lowest Brix% (13.07%), which is still in close proximity to the benchmark.

A compilation of the performances of the genotypes across seasons and locations is shown in Figure 19.

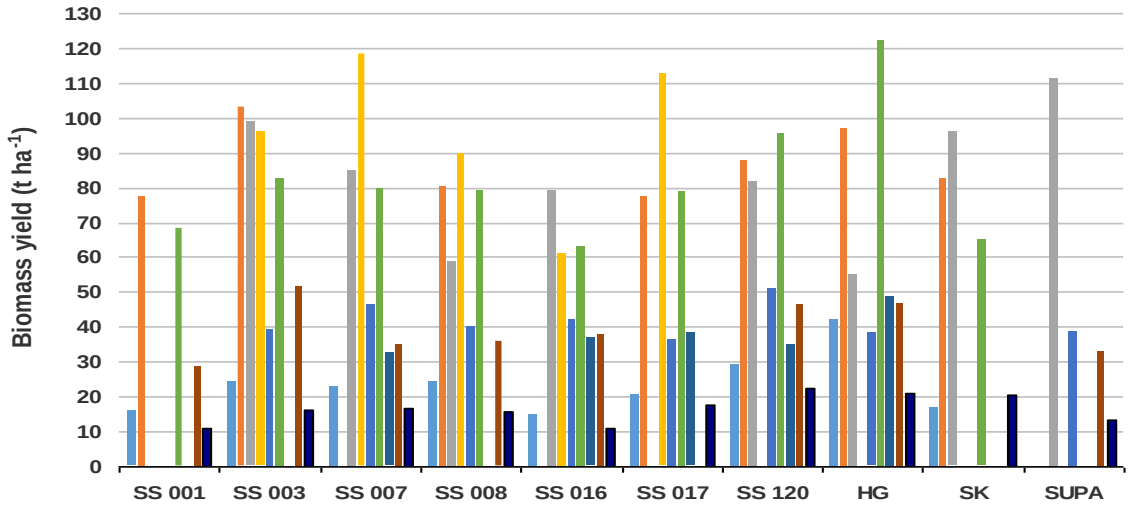

Figure 19. Biomass yield of different genotypes planted at different locations from 2011 to 2014. Locations: , Rustenburg 2012; , Rustenburg 2013; , Rustenburg 2014; , Potchefstroom 2012; , Potchefstroom 2013; , Potchefstroom 2014; , Bethlehem, 2012; , Bethlehem, 2013; , Bethlehem, 2014

The genotypes HG (122.16 t ha^{-1}), ss 003 (103.44 t ha^{-1}), SK (95.94 t ha^{-1}), Supa (111.56 t ha^{-1}), ss 007 (118.43 t ha^{-1}) and ss 017 (112.9 t ha^{-1}) performed well across seasons and localities. The genotype HG (122.16 t ha^{-1}) planted at Potchefstroom during 2014 performed the best in terms of biomass yield, although the Brix% measurement was of the lowest across the seasons. Genotypes (ss 003, BMR, HG and ss 120) at Rustenburg produced on average the second highest biomass yield during 2012-2013, and also the second highest biomass during 2013-2014.

The environmental factors (RF and HU) were taken into consideration to investigate the effect it might have on the performance of sweet stem sorghum. The effects thereof on the performances of the different genotypes, planted at different locations during the period of 2011 to 2014 were combined and compared and results are represented in Figures 20 to 22. The biomass yield per

unit RF (mm pa^{-1}) and per HU (°C) were calculated by dividing the biomass yield per hectare by the average RF and average HU at each location during the relevant planting season. From the RF and HU data given in Chapter 3 (Tables 2 to 5) it can be seen that climatic conditions could have been the reason for the significant different biomass yields obtained from the same genotypes in different seasons and locations.

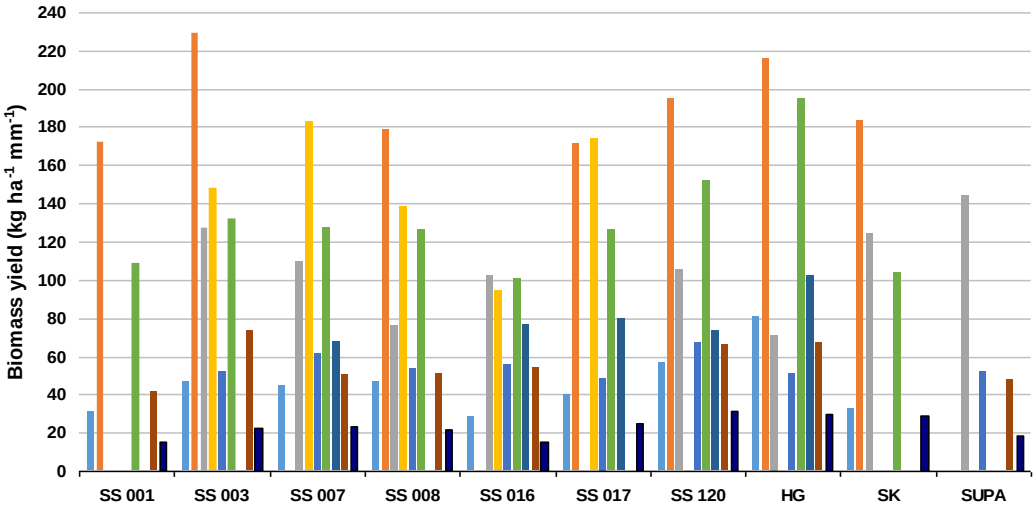

Figure 20. Biomass yield with only rainfall taken into account across different locations and different planting seasons. Locations: , Rustenburg 2012; , Rustenburg 2013; , Rustenburg 2014; , Potchefstroom 2012; , Potchefstroom 2013; , Potchefstroom 2014; , Bethlehem, 2012; , Bethlehem, 2013; , Bethlehem, 2014

When only rainfall is taken into account, most genotypes performed well for biomass yield at Rustenburg and in Potchefstroom. The genotypes HG (215.65 kg ha^{-1} mm^{-1}), ss 003 (229,82 kg ha^{-1} mm^{-1}), SK (183.67 kg ha^{-1} mm^{-1}), Supa (143.96 kg ha^{-1} mm^{-1}), ss 120 (195.6 kg ha^{-1} mm^{-1}), and ss 008 (182.7 kg ha^{-1} mm^{-1}) performed well, except ss 016 which might be an indication that this genotype is susceptive to RF. Although Supa featured often amongst the best performers, it did not perform well across all locations and seasons regarding its calculated EtOH potential due to the precarious nature of its sugar production. When the production patterns of the genotypes in Figure 19 are compared to those in Figure 20, changes are visible which indicate that rainfall affects the biomass yield. For example, ss 003 in Potchefstroom (2012) and ss 007 in Rustenburg (2014) produced less biomass.

When only HU's are taken into account most genotypes performed well in Potchefstroom in 2014 and Bethlehem in 2013. Interesting to note that the genotypes, which were some of the best

overall performers, did not do well when the effect of the HU is altered.

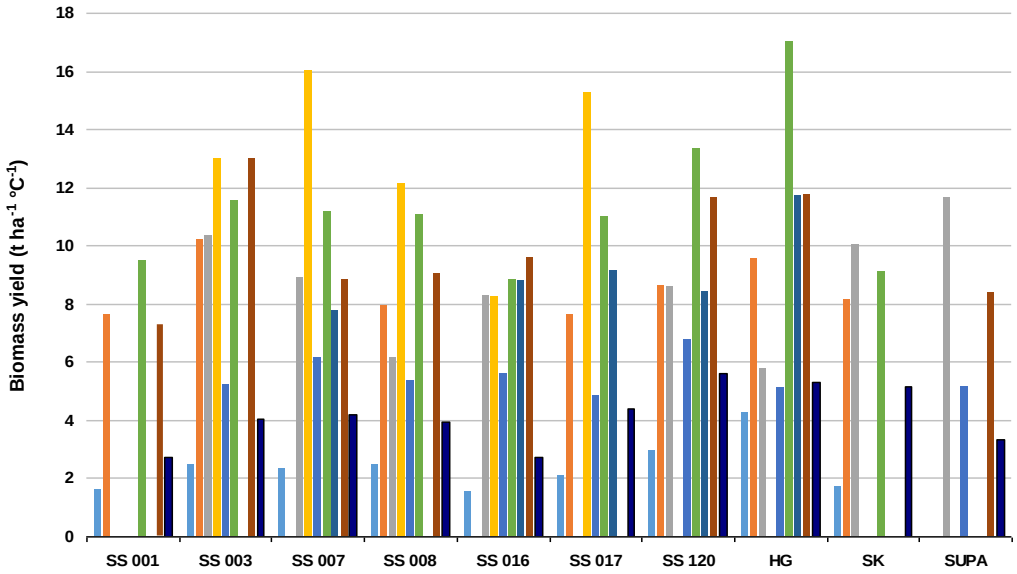

Figure 21. Biomass yield with only heat units taken into account across different locations and different planting seasons. Locations: , Rustenburg 2012; , Rustenburg 2013; , Rustenburg 2014; , Potchefstroom 2012; , Potchefstroom 2013; , Potchefstroom 2014; , Bethlehem, 2012; , Bethlehem, 2013; , Bethlehem, 2014

The genotypes HG (17.05 t ha^{-1} $°C^{-1}$) at Potchefstroom during 2014, ss 003 (13 t ha^{-1} $°C^{-1}$) at Bethlehem during 2013, ss 008 (12.14 t ha^{-1} $°C^{-1}$) at Potchefstroom during 2012, ss 017 (15.28 t ha^{-1} $°C^{-1}$) at Potchefstroom during 2012, and ss 007 (16.03 t ha^{-1} $°C^{-1}$) at Potchefstroom during 2012 performed well. These results show the importance of taking into account the average rainfall and environmental temperatures when cultivating energy crops in dryland conditions. When the production patterns of the genotypes in Figure 19 are compared to those in Figure 21, changes are visible which indicate that rainfall affects the biomass yield. For example, ss 016 in Bethlehem (2013) and ss 120 in Potchefstroom (2013) produced less biomass.

Furthermore, the sensitivity of the genotypes to the prevailaing average temperatures in the regions where it was planted, as seen in this study and as represented in Figure 22, corresponds to the heat sensitivity of sweet stem sorghum as a photoperiod crop reported by Dolciotti (1998).

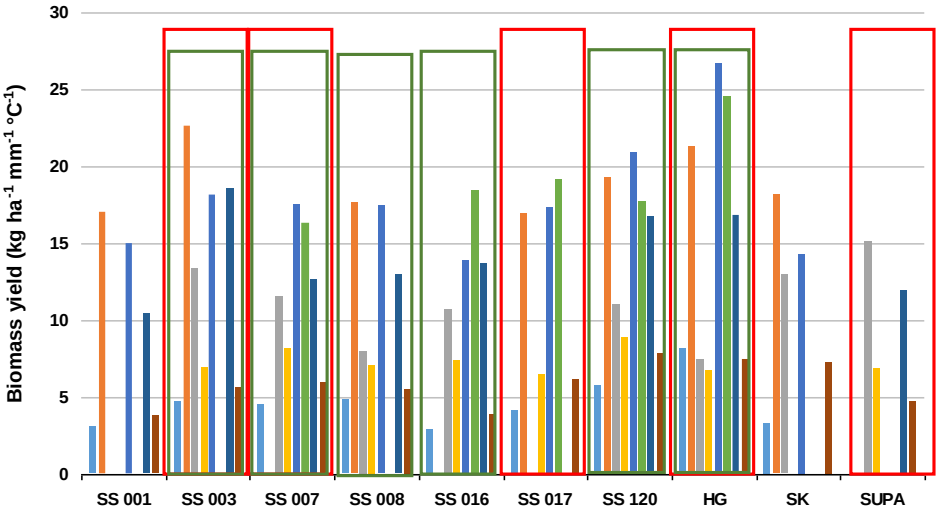

Figure 22. Biomass yield with rainfall and heat units taken into account across different locations and different planting seasons. Locations: , Rustenburg 2012; , Rustenburg 2013; , Rustenburg 2014; , Potchefstroom 2012; , Potchefstroom 2013; , Potchefstroom 2014; , Bethlehem, 2012; , Bethlehem, 2013; , Bethlehem, 2014

Figure 22 (Appendix B 6) is a summary of the patterns showed in Figures 20 and 21, and shows the performances of the genotypes when the biomass yield per unit RF (mm) and per HU (°C) were taken into consideration. The red blocks represent the highest biomass yields across the three years and were produced by ss 003, ss 007, ss 017, HG and Supa. The green blocks represent the biomass yields covering the majority of the nine production seasons, even though it was not the highest yields. The genotype ss 003, ss 007, ss 008, ss 120, HG and ss 016 did well across eight out of the nine seasons. Genotype ss 017 did well across seven out of the nine seasons and genotype Supa did well across four seasons. The data in Figure 22 indicates that ss 003, ss 120 and HG are the least susceptive to RF and HU changes and are adaptive to most climatic conditions/localities, and can therefore be recommended to farmers whose aim is biomass production and whoever wants to get involve in EtOH production.

It can be seen that biomass yield and juice yield differ between seasons and locations for the same genotypes, even though the measured Brix index remains approximately the same. The differences in biomass yield when rainfall is taken into account is indicative that most genotypes of sweet sorghum perform better in terms of biomass yield when the rainfall is higher, even though the crop itself is drought tolerant. These results make it difficult to recommend a specific genotype for a specific location.

4.1.2. Juice yield, Brix% and sugar yield during 2011-2012 to 2013-2014

Juice yield obtained from different genotypes at different locations and planting seasons without taking into account the effect of rainfall or ambient temperature are presented in Figure 23.

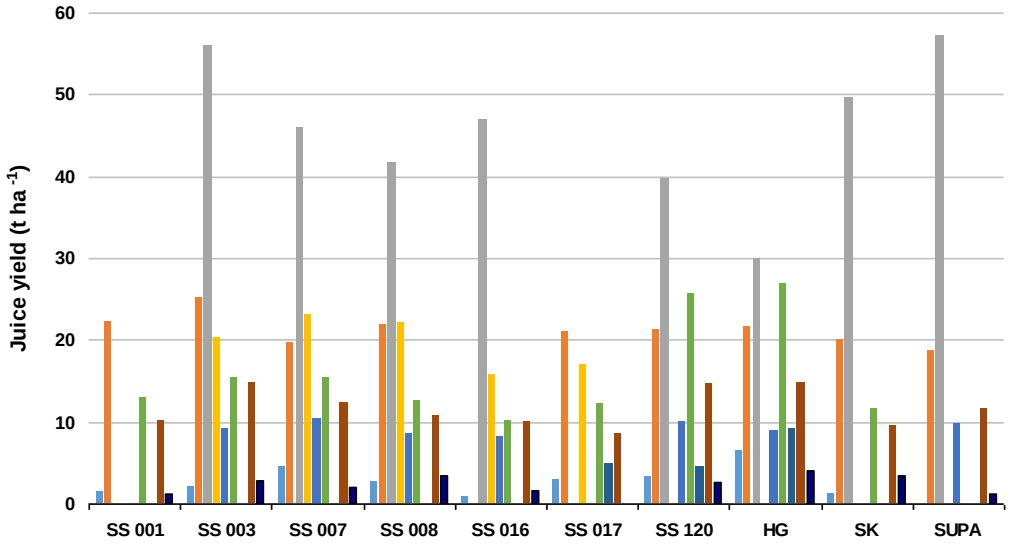

Figure 23. Juice yield across different locations and different planting seasons. Locations: ■, Rustenburg 2012; ■, Rustenburg 2013; ■, Rustenburg 2014; ■, Potchefstroom 2012; ■, Potchefstroom 2013; ■, Potchefstroom 2014; ■, Bethlehem, 2012; ■, Bethlehem, 2013; ■, Bethlehem, 2014

Genotypes ss 003, ss 007, ss 008, ss 016, ss 120, HG, SK and Supa produced the highest juice yields at Rustenburg during 2014. The best juice yield was 57.38 t ha^{-1}, produced by genotypes Supa in 2014 at Rustenburg with a high Brix% index of 20.84%. Supa was not constant in the production of the biomass, juice and Brix%. The lowest yield was produced during 2012 of 1.15 t ha^{-1} from SK, although the Brix% index was quite high (18.38%). Refering again to Tables 2 to 5 where the weather conditions are summarised, it can be deducted that the variances in average RF did have an effect on juice production.

The juice yield for different genotypes, with RF and ambient temperature taken into account, is compared in Figure 24 (Appendix B 7).

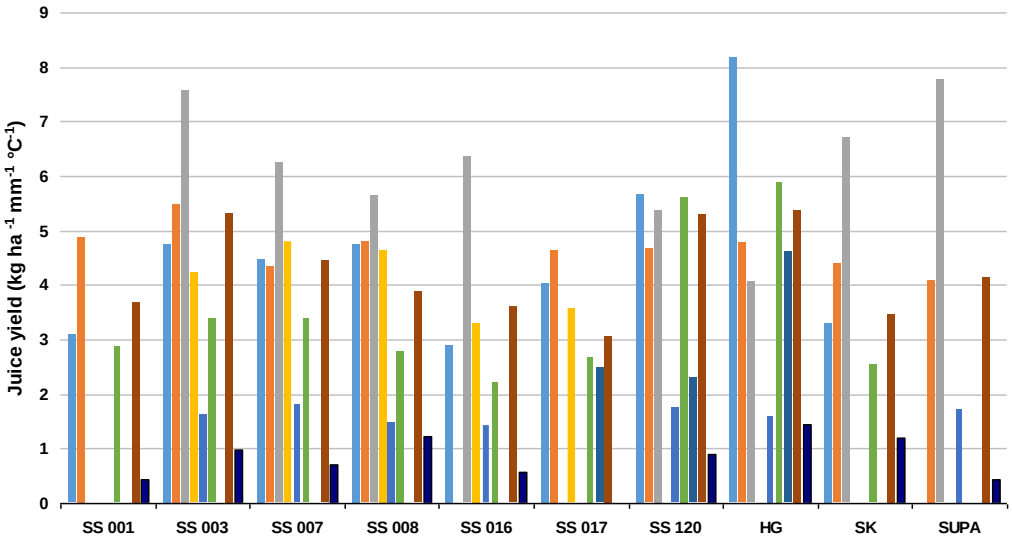

Figure 24. Juice yield with rainfall and ambient temperature taken into account across different locations and different planting seasons. Locations: , Rustenburg 2012; , Rustenburg 2013; , Rustenburg 2014; , Potchefstroom 2012; , Potchefstroom 2013; , Potchefstroom 2014; , Bethlehem, 2012; , Bethlehem, 2013; , Bethlehem, 2014

The genotypes HG, Supa, SK and ss 003 performed the best under conditions where RF and HU are included in the calculations to determine the genotypes' yields per unit rainfall and per unit temperature. The effect of only RF or only HU on juice yield is compared in Figures 25 and 26 respectively. When HU's are taken out of the equation (Figure 25) the same genotypes performed the best, but differences amongst six genotypes became evident.

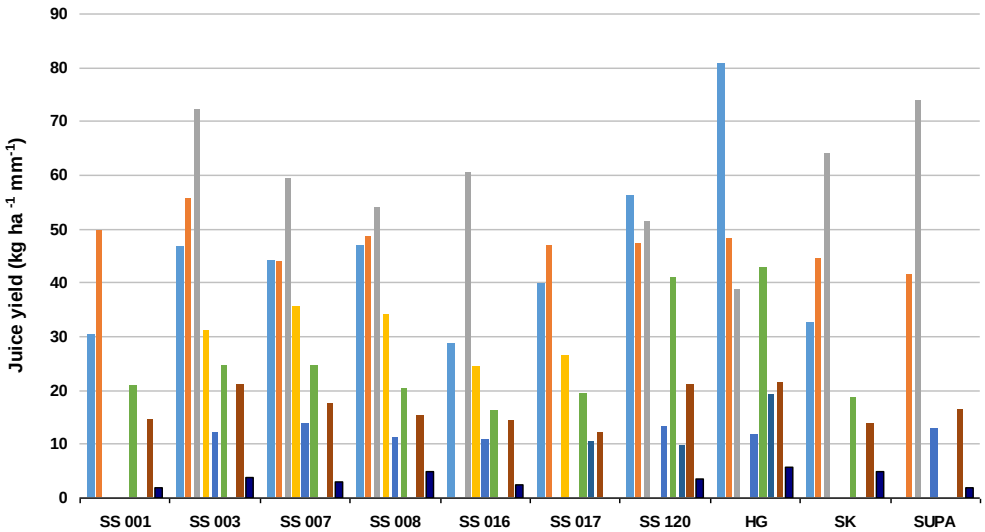

Figure 25. Juice yield with only rainfall taken into account across different locations and different planting seasons. Locations: , Rustenburg 2012; , Rustenburg 2013; , Rustenburg 2014; , Potchefstroom 2012; , Potchefstroom 2013; , Potchefstroom 2014; , Bethlehem, 2012; , Bethlehem, 2013; , Bethlehem, 2014

The six genotypes affected, when the HU component is ommited from the equation, are ss 001, ss 016, ss 017, ss 120, SK and ss 008.

The juice production from these genotypes was better in Bethlehem during 2013 compared to the yields in Potchefstroom 2014.

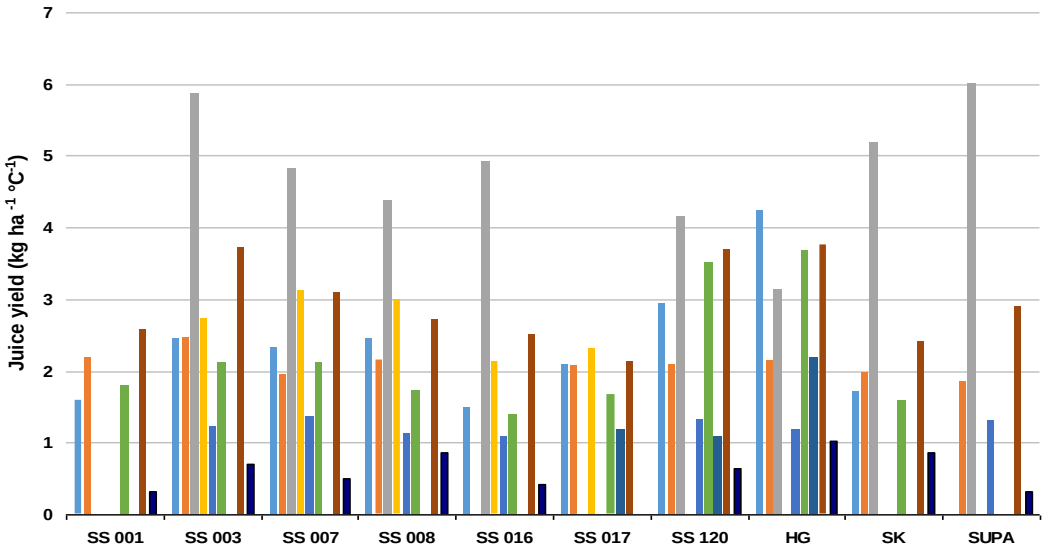

Figure 26. Juice yield with only ambient temperature taken into account across different locations and different planting seasons. Locations: , Rustenburg 2012; , Rustenburg 2013; , Rustenburg 2014; , Potchefstroom 2012; , Potchefstroom 2013; , Potchefstroom 2014; , Bethlehem, 2012; , Bethlehem, 2013; , Bethlehem, 2014

Figure 26 represents the juice yields when the RF factor is omitted. From these calculated expected yields, it was only ss 120, which were affected. Although ss 120 ranked amongst the best genotypes, it is shown that it is sensitive for climatic changes. If the juice yields are normalised for rainfall and ambient temperature, it can be seen that both RF and HU had an effect on juice yields and that genotypes ss 003, ss 120 and HG again performed the best across most of the locations and planting seasons. The best normalised juice yields were obtained at Rustenburg for all of the planting seasons.

Ethanol yield from an energy crop is not just dependent on the juice yield, but also the fermentable sugar content of the juice produced. The relationship between fermentable sugar yield (calculated from juice yield and Brix index) for three genotypes (ss 003, ss 120 and HG) is shown in Figure 27.

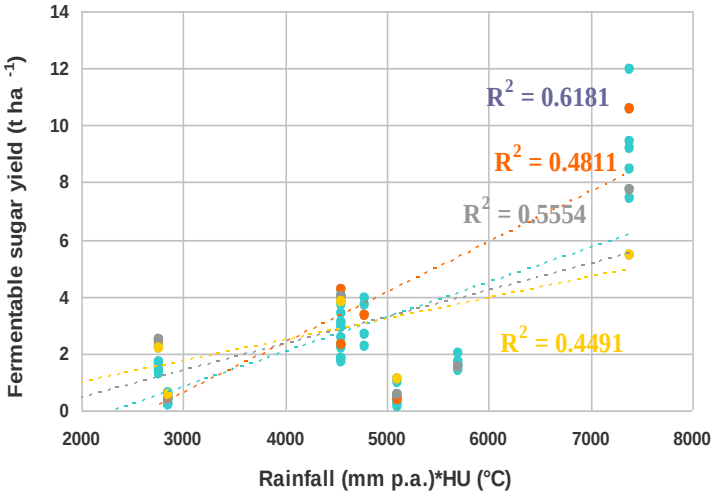

Figure 27. Relationship between fermentable sugar yield and product of annual rainfall and heat unit at the different localtions during different planting seasons.
Genotypes: ● , All genotypes; ● , ss 003; ● , ss 120; ● , HG

According to the data shown in Figures 24 to 27 it reveals that ss 003 (10.56 t ha^{-1}) and Supa (11.97 t ha^{-1}) proved to be recommendable genotypes for 1st generation EtOH. This yields were obtained at Rustenburg which is proof that sweet sorghum can perform well in areas where soils with a high clay content occur. Regarding 2nd generation EtOH production the genotypes ss 003 and HG showed the most promise across seasons and localities. From Figure 27 it can be seen that there is a relatively strong relationship between fermentable sugars and environmental conditions.

The fermentable sugar yield component from sugar yields of the juice and the bagasse for different genotypes across all locations and planting seasons was calculated and results are given in Figure 28 and Figure 29, respectively. Sugar yields form the bagasse was calcaulted based on the composition analysis (cellulose and hemicellulose) content as determined by the ARC: API analysis (Appendix F1).

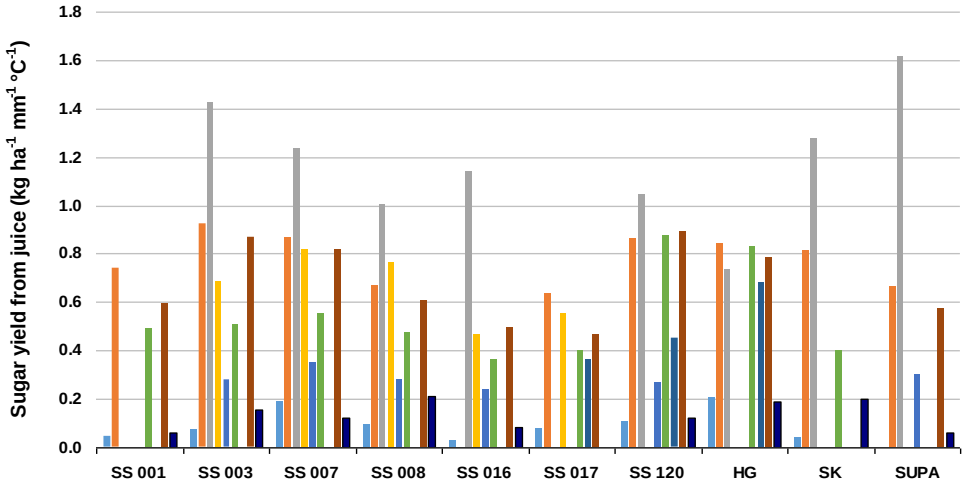

Figure 28. Fermentable sugar yield from juice (1st generation) with rainfall and ambient temperature taken into account across different locations and different planting seasons. Locations: , Rustenburg 2012; , Rustenburg 2013; , Rustenburg 2014; , Potchefstroom 2012; , Potchefstroom 2013; , Potchefstroom 2014; , Bethlehem, 2012; , Bethlehem, 2013; , Bethlehem, 2014

SK produced the lowest amount (0.04 kg ha^{-1} mm^{-1} $^oC^{-1}$) of fermentable sugars to be fermented during the 1st generation EtOH production process. In Figure 29 the SK yield from bagasse is 11.74 kg ha^{-1} mm^{-1} $^oC^{-1}$. It confirms the importance of combining the sugars in the juice and bagasse for optimum EtOH production. Supa, for example, produced the most sugars (1.62 kg ha^{-1} mm^{-1} $^oC^{-1}$) from the juice (Figure 28), but a low sugar yield (9.77 kg ha^{-1} mm^{-1} $^oC^{-1}$) was obtained from the bagasse (Figure 29). However, when the two values are added, it supplies a high amount of sugars to be fermented.

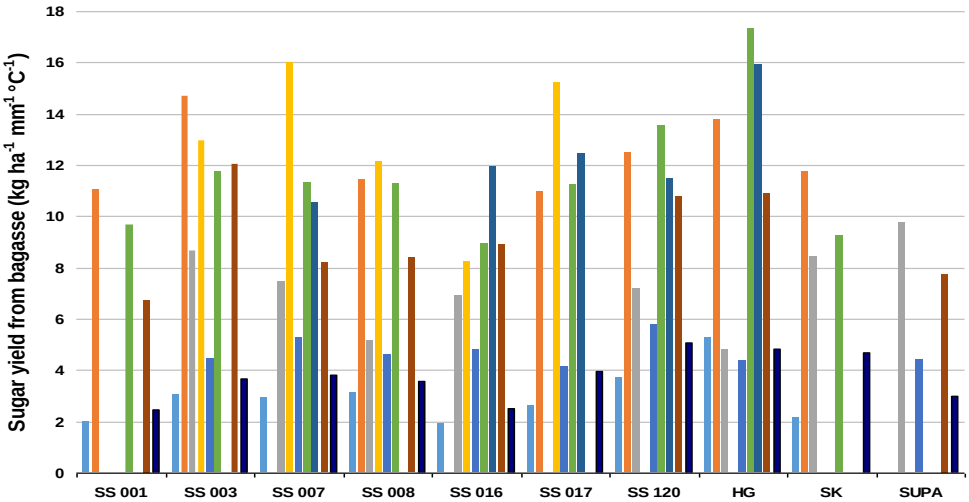

Figure 29. Fermentable sugar yield from bagasse (2nd generation) with rainfall and ambient temperature taken into account across different locations and different planting seasons. Locations: : , Rustenburg 2012; , Rustenburg 2013; , Rustenburg 2014; , Potchefstroom 2012; , Potchefstroom 2013; , Potchefstroom 2014; , Bethlehem, 2012; , Bethlehem, 2013; , Bethlehem, 2014

Figure 29 indicates that ss 003, ss 120 en HG are the best genotypes when sweet stem sorghum is to be cultivated for 2nd generation EtOH production. Genotype ss 003 performed better in soil with clay and sand. Genotypes ss 120 and HG adapt well to all soil types, but seems to prefer sandy soils. Genotype HG genotype can also tolerate soils with a higher clay content compared to ss 120. Apart from the RF and HU effect which are indicated in the graphical presentations, it appears that the soil, as another environmental factor, also plays a role in genotype performances and only then better results can be obtained from HG.

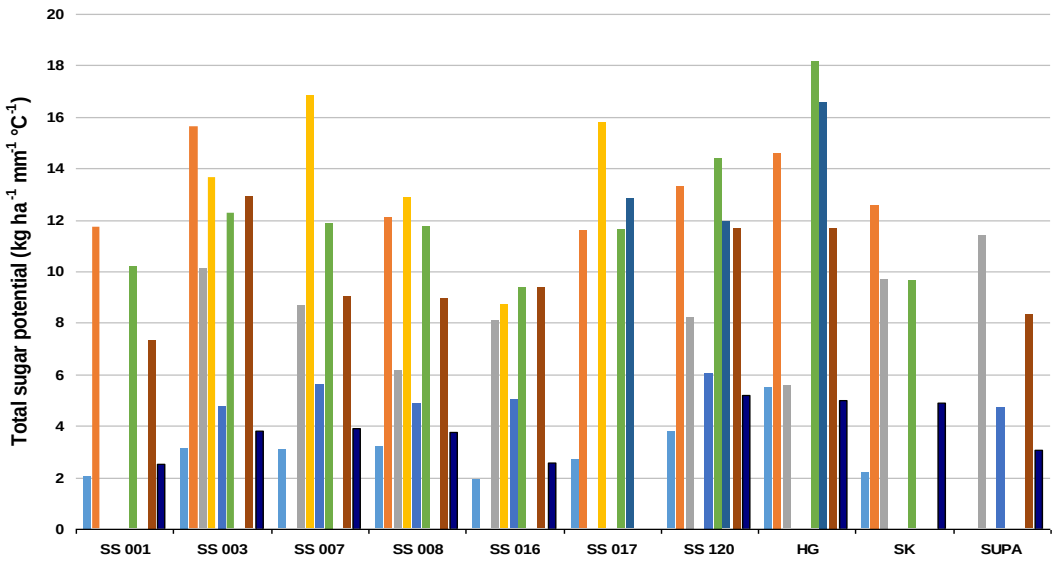

Figure 30. Total sugar potential (1st and 2nd generation) with rainfall and ambient temperature taken into account across different locations and different planting seasons. Locations: ■, Rustenburg 2012; ■, Rustenburg 2013; ■, Rustenburg 2014; ■, Potchefstroom 2013; ■, Potchefstroom 2014; ■, Bethlehem 2012; ■, Bethlehem, 2013; ■, Bethlehem, 2014

Figure 30 indicates that HG performed the best in regards to sugar production at Bethlehem during 2012 and 2013, and ss 007 performed the best in Potchefstroon during 2013.

Figures 31 to 33 are images of the three locations to give a visual representation of the differences amongst the locations and the genotypes. It can also be seen that the soil type at Rustenburg contains a high clay (43%) content. The soil in Potchefstroom has a higher percentage of sand and is more of a clay-loam type. The soil in Bethlehem is sandy (see Appendices E 1 to E 6).

Figure 31. Image of genotype differences at Rustenburg

Figure 32. Image of genotype differences at Potchefstroom

Figure 33. Image of genotype differences at Bethlehem

Figure 34 gives an indication of the height reached by some of the plants. Figure 35 is a picture of the panicle. Although the seed is not harvested nor used during the 1st generation EtOH production cycle, it supplies cellulose and hemicellulose to be used in the 2nd generation EtOH production process.

Figure 34. Illustration of plant height at Potchefstroom

Figure 35. Illustration of a panicle from a specific sweet stem sorghum genotype (Rustenburg)

4.2. Effect of nitrogen applications on biomass yield, Brix% and juice yield.

4.2.1. Season 2011 - 2012

During the 2011- 2012 season the effect of five different N applications on biomass yield, juice yield and Brix% were investigated using three genotypes (PX 174, ss 120, ss 27) at Vaalharts and three genotypes (BMR, ss 120, ss 27) at Wilgeboom. The image in Figure 36 gives and indication of the plant growth of different genotypes at Vaalharts, where the five different N fertiliser levels were applied.

Figure 36. Image of the effect of different N fertiliser levels on plant height at Vaalharts during the 2011-2012 planting season

The recorded data regarding the effect of the five different N applications applied at trials at Wilgeboom and Vaalharts during the 2011-2012 planting season on the different genotypes investigated, is shown in Figures 37 and 38 respectively (see Appendix D 1a and D 1b).

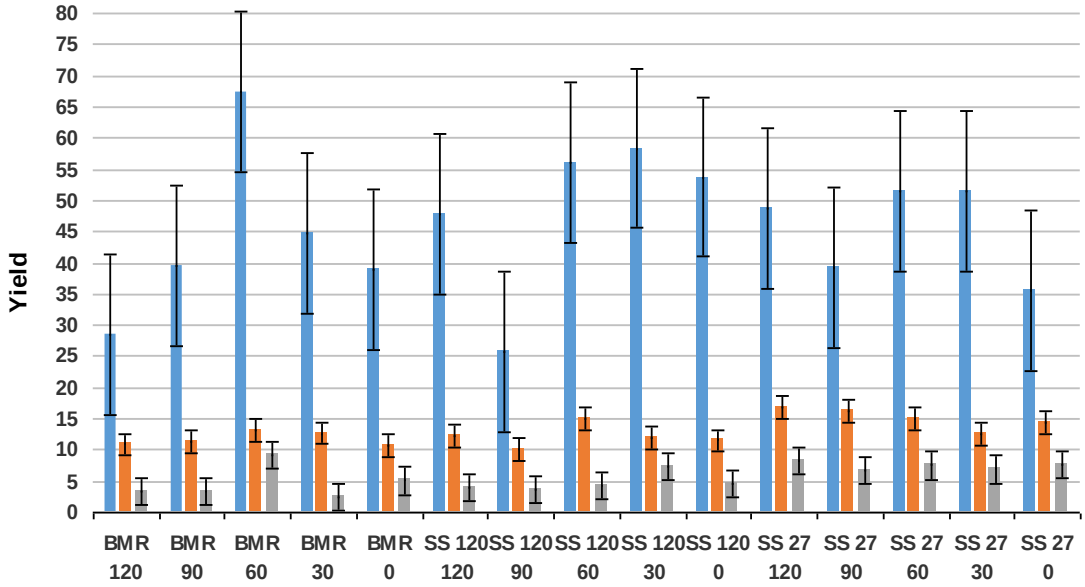

Figure 37: Effect of N application levels (0 to 120 kg ha^{-1}) on biomass yield (t ha^{-1}) (■, a), Brix index (%) (■, b) and juice yield (ton ha^{-1}) (■, c) obtained from different genotypes planted at Wilgeboom in the 2011-2012 planting season

a) biomass LSD (p=0.05): 27.58
b) Brix% LSD (p=0.05): 3.828
c) juice LSD (P=0.05): 4.662

The best biomass yields at Wilgeboom were obtained from all three genotypes at a N application rate of between 30 kg ha^{-1} and 60 kg ha^{-1}. Genotypes ss 120 and BMR produced low biomass yields at 120 kg ha^{-1}, indicating that very little will be gained at very high N application levels. At an application rate of 120 kg ha^{-1} only ss 27 yielded a substantial amount of biomass (48.64 t ha^{-1}).

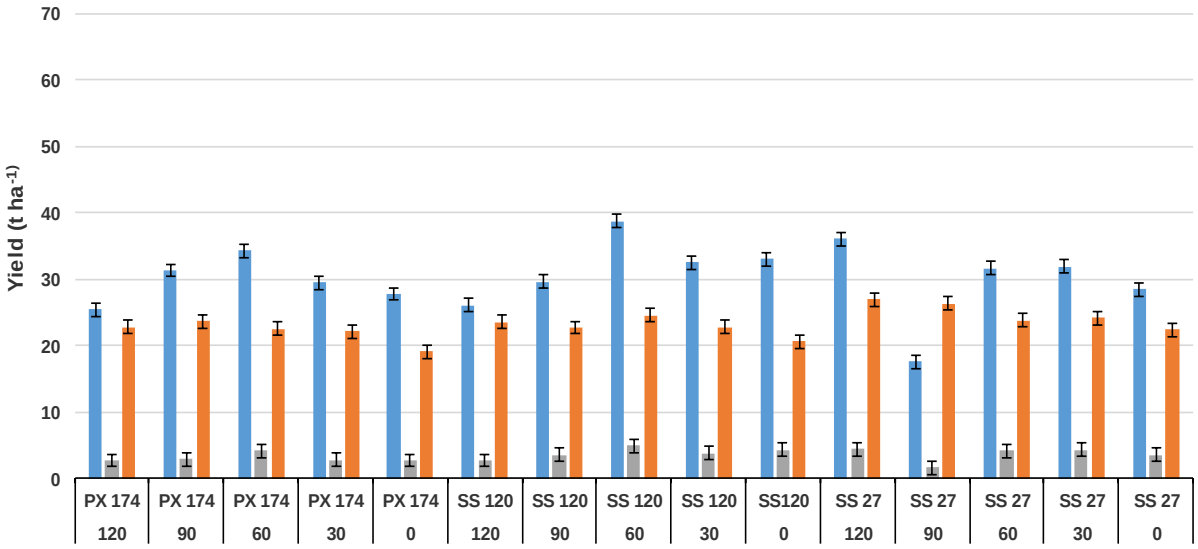

Figure 38: Effect of N application levels (0 to 120 kg ha^{-1}) on biomass yield (t ha^{-1}) (■ a), Brix index (%) (■, b) and juice yield (t ha^{-1}) (■, c) obtained from different genotypes planted at Vaalharts in the 2011-2012 planting season

a) biomass LSD (p=0.05): 7.335
b) Brix% LSD (p=0.05): 3.614
c) juice LSD (P=0.05): 1.278

The same pattern was observed at Vaalharts. On average, the best yields were obtained from the intermediate N application levels. At this location, the genotypes ss 120 and ss 27 reacted well to a higher N application level. Genotype ss 120 at 60 kg ha^{-1} N had the best biomass yield of 38.74 t ha^{-1} with the 0 kg ha^{-1} N that did even better than 120 kg ha^{-1} N. At a 120 kg ha^{-1} ss 27 yielded 36.1 t ha^{-1} as should be expected with the highest N application, although the second best yield of 31.63 t ha^{-1} at 60 kg ha^{-1} only produced 4.47 t ha^{-1} less biomass

The correlation between N applications and biomass yield, juice yield and Brix% is given in Table 12.

Table 12. Correlation matrix for biomass yield, Brix%, juice yield and N application levels for trials at Wilgeboom and Vaalharts in the 2011-2012 planting season

Vaalharts	**N application**	**Biomass yield**	**Brix%**	**Juice yield**
N application	1	0.149(p= 0,323)	0.555(p= 0,001)	0.067(p= 0,718)
Biomass yield	0.149(p= 0,323)	1	0.171(p= 0,189)	0.939(p< 0,0001)
Brix %	0.555(p= 0,001)	0.171(p= 0,189)	1	0.157(p= 0,392)
Juice yield	0.067(p= 0,718)	0.939(p<0,0001)	0.157(p= 0,392)	1
Wilgeboom	**N application**	**Biomass yield**	**Brix%**	**Juice yield**
N application	1	-0.185(p= 0,327)	0.142(p= 0,445)	-0.119(p= 0,53)
Biomass yield	-0.185(p= 0,327)	1	0.364(p= 0,048)	0.403(p= 0,027)
Brix %	0.142(p= 0,455)	0.364(p= 0,048)	1	0.449(p= 0,013)
Juice yield	-0.119(p= 0,53)	0.403(p= 0,027)	0.449(p= 0,013)	1

From the Table 12, a significant correlation is visible between Brix% and N applications, as well as between biomass yield and juice yield. A a mild correlation between biomass yield and the N application at Vaalharts. At Vaalharts, I see a relatively low correlation between biomass yield and the N application and a mild correlation between Brix% and juice yield. A viable correlation is visible between biomass yield and juice yield at Wilgeboom and Vaalharts. Both Vaalharts and Wilgeboom have a high content of sandy soil, with Wilgeboom having a little higher clay content. The results here would thus correlate with those findings in the genotype trials, especially for the genotype, ss 120. The genotype trials showed that this genotype is best for EtOH production in areas with sandy soils. The fact that genotypes did better in these N application trials in the soil with a lower sand content might point to the fact that N applications could be used to get higher yields in marginal areas that would have produced low yields otherwise. Although the data is scattered a percievable effect of higher N rates were visible. The highets biomass yield (67.4 t ha^{-1}) at Wilgeboom was produced by BMR at a N application of 60 kg ha^{-1}. An increase in the N rate from 30 kg ha^{-1} to 60 kg ha^{-1} resulted in an increase in a biomass of 22.75 t ha^{-1}. Genotype ss 27 yielded the best amount of Brix% (16,87%) at a N rate of 120 kg ha^{-1} and indicated an increase (1,95%) in the Brix% when the N rate was increased from 60 kg ha^{-1} to 90 kg ha^{-1} to 120 kg ha^{-1}. The juice yield indicates that there was an increase, eg. ss 27 increased from 6,62 t ha^{-1} at 90 kg ha^{-1} to 8,22 t ha^{-1} at 120 kg ha^{-1}. At Vaalharts the best biomass yield (38.74 t ha^{-1}) was produced by ss 120 at a N application of 60 kg ha^{-1}. An increase in the N rate from 30 kg ha^{-1} to 60 kg ha^{-1} resulted in an increase in biomass of 6,24 t ha^{-1}. Genotype ss 27 yielded the best amount of Brix% (26,93%) at a N rate of 120 kg ha^{-1} and also indicated an increase (3,18%) in the Brix% when the N rate was increased from 60 kg ha^{-1} to 90 kg ha^{-1} to 120 kg ha^{-1}. The juice yield indicates that there was an increase, eg. with ss 120 an increase was measured from 3.74 t ha^{-1} at a N rate of 30 kg ha^{-1} to 4,9 t ha^{-1} at a N rate of 60 kg ha^{-1}. These values reveal that when the increases in biomass, juice and sugars in the juice are taken into consideration, the slight increases in N applications (30 kg ha^{-1} to 90 kg ha^{-1}) will have a positive effect. Too much N will not increase the genotypes' performances and there will be no financial and/or no increases in production benefits when higher N rates are applied.

4.2.2. Season 2012-13 and 2013-14

During the 2012-13 and 2013-14 seasons N fertiliser application trials were again done at Vaalharts and Wilgeboom. The 2012-13 data of Wilgeboom could not be used due to a very bad

season and another trial had to be replanted during the 2013-14 season and data is represented in Figure 40 (Appendix D 2b). Figure 39 (Appendix D 2a) represents the data of the performances of the genotypes at the different N fertiliser levels at Vaalharts (2013).

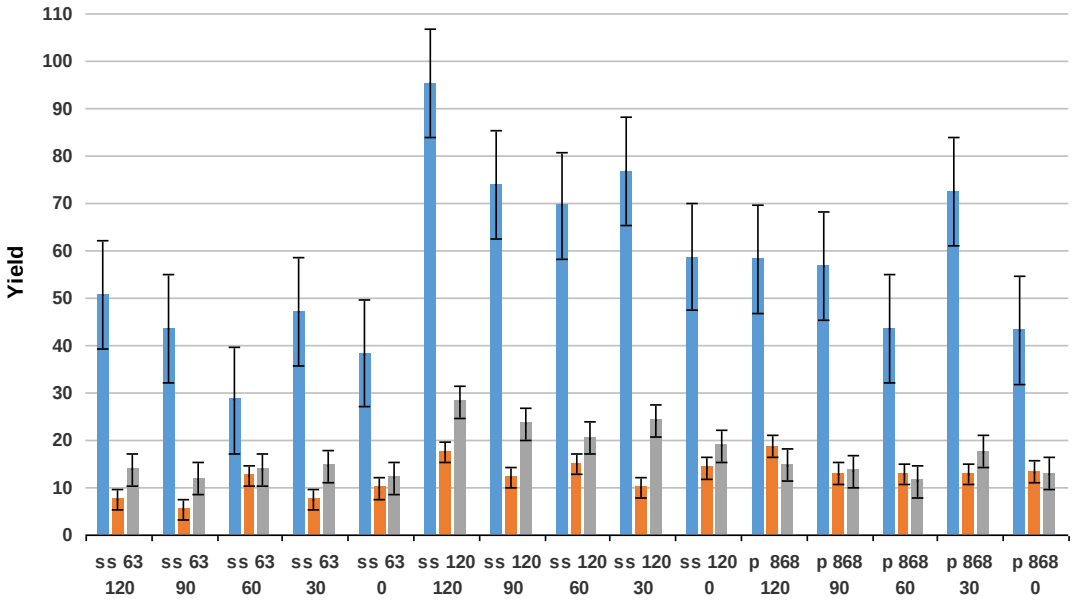

Figure 39. Effect of N application levels (0 to 120 kg ha^{-1}) on biomass yield (t ha^{-1}) (■, a), Brix index (%) (■, b) and juice yield (t ha^{-1}) (■, c) obtained from different genotypes planted at Vaalharts in the 2012-2013 planting season

a) biomass LSD (p=0.05): 24.39
b) Brix% LSD (p=0.05): 4.719
c) juice LSD (P=0.05): 7.243

At Vaalharts the best biomass (95.3 t ha^{-1})was produced by ss 120 at a 120 kg ha^{-1} N application rate. An increase of 21.48 t ha^{-1} occurred from an N increase from 90 kg ha^{-1} to 120 kg ha^{-1}. Apart from a few exceptions, it was shown that an increase in N application levels resulted in a slight increase of biomass, juice and Brix%.

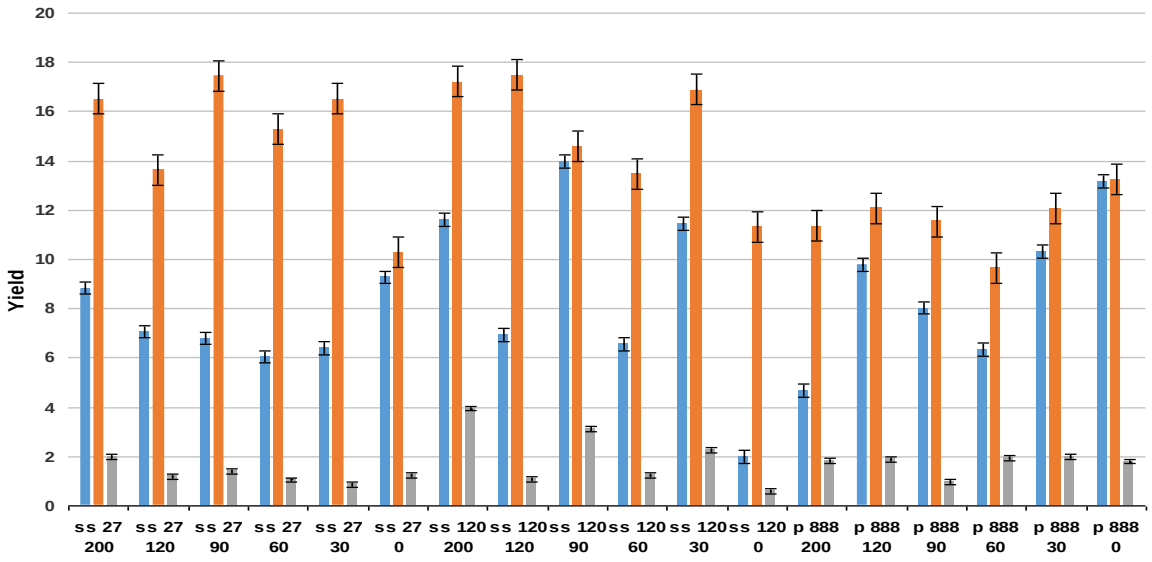

Figure 40: Effect of N application levels (0 to 120 kg ha^{-1}) on biomass yield (ton/ha) (■, a), Brix index (%) (■, b) and juice yield (t ha^{-1}) (■, c) obtained from different genotypes planted at Wilgeboom in the 2013-2014 planting season

a) biomass LSD (p=0.05): 0.543
b) Brix% LSD (p=0.05): 1.311
c) juice LSD (P=0.05): 0.2194

Figure 40 indicates that at Wilgeboom an increase of 7.4 t ha^{-1} biomass by ss 120 with a N application rate increase from 60 kg ha^{-1} to 90 kg ha^{-1} occured. The best Brix% (17.5%) was measured and an increase of 2.92% was obtained from an increase of 90 kg ha^{-1} to 120 kg ha^{-1}. The best juice yield (3.95 t ha^{-1}) was measured and an increase of 2.89 t ha^{-1} was obtained from an increase of 120 kg ha^{-1} to 200 kg ha^{-1}.

Table 13. Correlation matrix for biomass yield, Brix%, juice yield and N application levels for trials at Wilgeboom and Vaalharts in the 2012/2014 planting season

Vaalhart 2013	**N application**	**Biomass yield**	**Brix%**	**Juice yield**
N Application	1	0.237(p=0.208)	0.147(p=0.438)	0.151(p=0.426)
Biomass yield	0.237(p=0.208)	1	0.387(p=0.035)	0.879(p<0.0001)
Brix%	0.147(p=0.438)	0.387(p=0.035)	1	0.362(p=0.049)
Juice yield	0.151(p=0.426)	0.879(p<0.0001)	0.362(p=0.049)	1
Wilgeboom 2014	**N application**	**Biomass yield**	**Brix%**	**Juice yield**
N Application	1	0.005(p=0.978)	0.334(p=0.046)	0.426(p=0.010)
Biomass yield	0.005(p=0.978)	1	0.212(p=0.215)	0.719(p<0.0001)
Brix%	0.334(p=0.046)	0.212(p=0.215)	1	0.265(p=0.118)
Juice yield	0.426(p=0.010)	0.719(p<0001)	0.265(p=0.118)	1

Again, the data was too scattered to get good correlations, but there is still a mild correlation

observed between biomass yield and N application, and biomass and Brix%, at Vaalharts, as well as a mild correlation observed between Brix% and juice yield. The best correlation exists between biomass yield and juice yield, which you can also see if you follow the trends in the figures. Looking at both locations over 2 to 3 seasons, even though the data is typically scattered for planting data, it is shown that there are some correlations between biomass yield and Brix% when adding N, but only up to a certain dosage. At Wilgeboom a mild correlation exists between N application and Brix%, as well as between the N applications and juice yield. It might be that the soil at Wilgeboom was still recovering from the previous bad year and that is why the yields were so low and the sugar index so high compared to the previous trial.

The sugar potential shown in Figure 41 matches up well to the data in Figure 23 (juice yields), which is turned around by the data shown in Figure 24 and 25 where the calculated juice yield per mm rainfall and per heat unit was illustrated. A possible explanation might be that the Brix% determined by the refractometer also measured other impurities affecting the density of the juice and TSS contents, and that the values in Figure 24 are mere calculations. Through the chemical analysis the values are more concise as is represented in Figure 41.

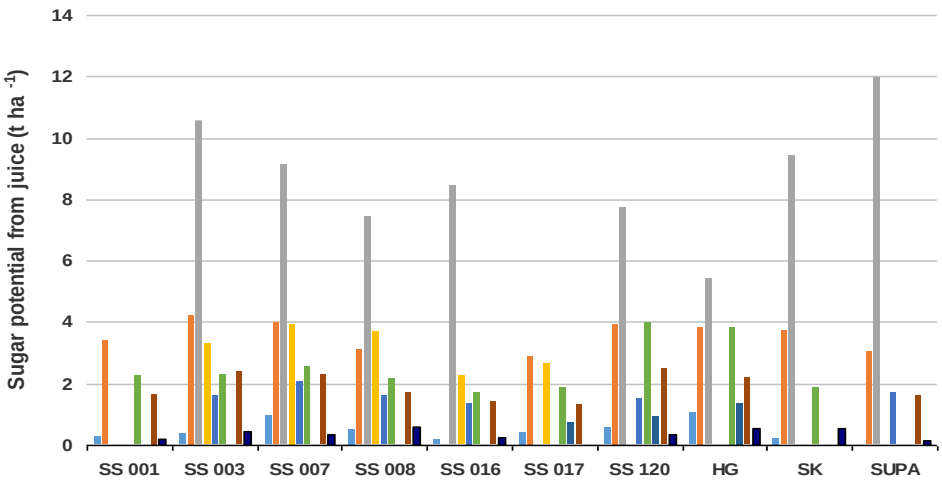

Figure 41. Graphical representation of the sugar potential from juice across locations and production year. Year and location: Bethlehem 2012(■), Bethlehem 2013(■), Bethlehem 2014(■); Rustenburg 2102(■); Rustenburg 2013(■); Rustenburg 2014(■); Potchefstroom 2012(■); Potchefstroom 2013(■); Potchefstroom 2014(■)

The calculated sugar potential (sugar=Brix%/100*measured amount) of the produced juice gives an idea of the amount of EtOH which can be produced. From data in Figure 41 (Appendix B 8.1) it is clear that very high amounts of sugars were produced, eg. sugar production in Rustenburg during 2014 was from Supa (11,96 t ha^{-1}), ss 003 (10,56 t ha^{-1}) and ss 007 (9,16 t ha^{-1}). The values

contained in Figure 41 (potential amount of sugar in the juice) and in Table 14 (potential amount of sugar in the bagasse), are used to calculate an estimated amount of total EtOH.

Table 14. Indication of total sugar potential (bagasse) 64.76 % cellulose, hemicellulose and residual sugar t ha^{-1} across locations and production years

	Rustenburg			Potchefstroom			Bethlehem		
Genotype	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
SS 001	10.19	50.23	n/a*	n/a	n/a	44.19	n/a	18.66	7.05
SS 003	15.68	66.99	63.97	62.09	25.48	53.61	n/a	33.34	10.45
SS 007	14.86	n/a	54.97	76.70	30.01	51.74	20.93	22.69	10.87
SS 008	15.73	52.12	38.07	58.09	26.14	51.43	n/a	23.16	10.20
SS 016	9.57	n/a	51.30	39.50	27.23	40.96	23.69	24.60	7.14
SS 017	13.37	50.02	n/a	73.11	23.61	51.20	24.62	n/a	11.37
SS 120	18.82	56.89	52.96	n/a	33.00	61.85	22.72	29.95	14.52
HG	27.08	62.86	35.58	n/a	24.87	79.11	31.50	30.19	13.77
SK	10.92	53.54	62.13	n/a	n/a	42.13	n/a	n/a	13.35
SUPA	n/a	n/a	72.25	n/a	25.15	n/a	n/a	21.48	8.63

*not available or not recorded due to very bad performance

The calculated EtOH potential from the produced sugars in the juice and sugars in the bagasse gives an idea of the amount of EtOH which can be produced and is shown in Table 15.

Table 15. Total ethanol potential (kL ha^{-1}) from juice, bagasse and residual sugars

	Rustenburg			Potchefstroom			Bethlehem		
Genotype	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
SS 001	10.65	53.89	n/a	n/a	n/a	46.95	n/a	20.28	7.38
SS 003	16.39	71.70	72.69	n/a	27.26	56.69	n/a	35.88	11.05
SS 007	15.91	n/a	62.52	82.20	32.20	54.91	n/a	24.82	11.41
SS 008	16.51	55.64	44.00	62.06	27.95	54.34	n/a	24.93	10.89
SS 016	9.95	n/a	58.28	n/a	28.91	43.25	n/a	26.21	7.51
SS 017	14.03	53.37	n/a	79.21	n/a	53.90	25.81	n/a	n/a
SS 120	19.73	61.11	59.52	n/a	34.95	66.25	23.96	32.43	15.18
HG	28.57	67.19	40.14	n/a	n/a	83.90	33.30	32.49	14.52
SK	11.38	57.51	70.07	n/a	n/a	44.56	n/a	n/a	14.11
SUPA	n/a	n/a	82.11	n/a	27.00	n/a	n/a	23.14	8.99

4.2.3. Season 2016-2017

The cultivation of three genotypes (HG, SG, ss 007) during 2016-2017 was executed at Potchefstroom and the genotypes were planted in a glasshouse. Below is the chemical analysis of the bagasse that was done by the ARC: API in Pretoria (see also Appendix F).

Table 16. Compositional analysis of the bagasse of three genotypes at 0 kg ha^{-1} N fertiliser and 200 kg ha^{-1} N fertiliser applications. All values are given as wt. % on a wet basis

	ss 007		**HG**		**SG**	
	0 kg N ha^{-1}	**200 kg N ha^{-1}**	**0 kg N ha^{-1}**	**200 kg N ha^{-1}**	**0 kg N ha^{-1}**	**200 kg N ha^{-1}**
Dry matter	86.87	88.70	87.87	89.06	87.96	86.69
Moisture	13.13	11.30	12.13	10.94	12.04	13.31
Ash	7.58	6.46	10.70	8.91	7.01	4.20
Protein	5.26	7.53	7.96	3.81	5.07	4.42
Fat	0.66	0.87	0.95	1.22	0.96	1.04
Carbohydrates	73.37	73.84	68.26	75.12	74.92	77.03
NDF	57.25	64.62	58.14	61.39	61.86	50.63
ADF	36.35	42.51	35.59	34.74	34.80	28.60
ADL	8.08	11.95	6.92	6.19	7,27	10.14
Cellulose	28.27	30.56	28.67	28.55	27.53	18.46
Hemicellulose	20.90	22.11	22.55	26.65	27.06	22.03
Bagasse sugars	49.17	52.67	51.22	55.20	54.59	40.49
Residual sugars	16.12	9.22	10.12	13.73	13.06	26.40
Total sugars	**65.29**	**61.89**	**61.34**	**68.93**	**67.65**	**66.89**

The EtOH potential could be calculated from the bagasse sugars by assuming that the cellulose breakdown results in glucose as main sugar and hemicellulose yields xylose when hydrolysed. Ethanol potential from the residual sugars was calculated by assuming the total residual sugars consist of glucose. The sum of cellulose and hemicellulose yield was taken as the bagasse yield for purposes of these calculations.

The effect of N applications on biomass yield, juice yield and Brix% for three different genotypes, planted in Potchefstroom during the 2016-2017 planting season, is shown in Figure 42.

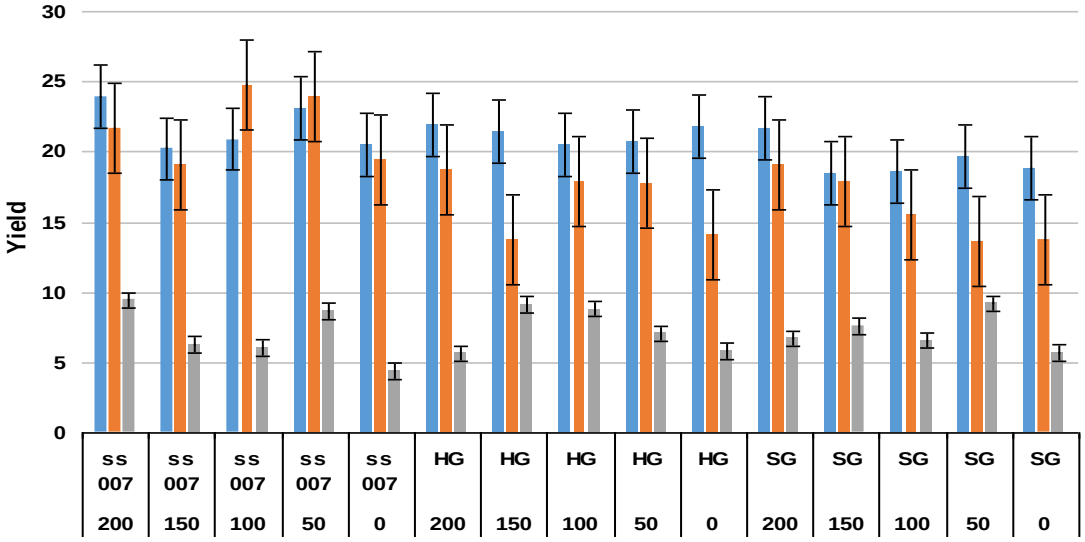

Figure 42: Effect of nitrogen application levels (0 to 200 kg ha^{-1}) on biomass yield (t ha^{-1}) (■, a), Brix index (%) (■, b) and juice yield (t ha^{-1}) (■, c) obtained from different genotypes planted at Potchefstroom in the 2016-2017 planting season

a) biomass LSD (p=0.05): 3.745
b) Brix% LSD (p=0.05): 5.351
c) juice LSD (P=0.05): 0.612

The ss 007 and HG genotypes were the best performers in the Potchefstroom trial regarding biomass yield, Brix% and juice yield. Genotype ss 007 and HG also produced well in the genotype evaluation trials (Figure 19). An outlyer is visible as indicated by the highest biomass yield, 23.94 t ha $^{-1}$ that was obtained from genotype ss 007 at a N fertiliser application level of 200 kg ha^{-1}. The genotype SG produced the lowest biomass yield (18.50 t ha^{-1}) at a 150 kg ha $^{-1}$ applied N fertiliser level. The Brix% and juice yield varied significantly, but the best Brix% (24.83%) was from genotype ss 007 at 100 kg ha^{-1} N fertiliser and the highest juice yield of 10.79 t ha $^{-1}$ was produced by genotype SG at 50 kg ha^{-1} N fertiliser. The lowest Brix% was from genotype SG (12.83%) at 100 kg ha^{-1} N fertiliser and the lowest juice yield from genotype ss 007 (4.36 t ha $^{-1}$) at 0 kg ha^{-1} N fertiliser.

Brix% is a rough estimate of the amount of total dissolved solids in juice and is an easy measurement that can be made on the farm. Brix% measurements are however based on the relative density of the juice and any components in the juice that is not fermentable sugar could affect the Brix% reading. Therefore, a more comprehensive compositional analysis of the juice produced at Potchefstroom during the 2016-2017 planting season was done and correlated with the Brix%. Data not presented here can be found in Appendix G. What is significant here is the fact that the Brix% reached higher values from ss 007 (24,83%) at 100 kg ha^{-1} N fertiliser and also

from ss 007 (23,9%) at 50 kg ha^{-1} N fertiliser, although the juice yields were low. It again shows that the most effective N application rate should be between 50 kg ha^{-1} to 100 kg ha^{-1} and it also indicates that there is no general trend regarding the effect of N fertiliser applications on the genotypes' reactions which can be presented to farmers and stakeholders. Farmers and stakeholders should therefore apply their genotype preferences on what suit them best and which genotype appeared to produce best in a specific area. Despite the variances in the performances of the genotypes a recommendation regarding the best genotypes (eg. ss 007) can be done, as was presented by this research.

A correlation matrix showing the correlation of biomass yield, juice yield, and Brix% with N application levels is given in Table 17.

Table 17. Correlation matrix for biomass yield, juice yield and Brix% with N applications for genotypes planted in Potchefstroom in the 2016/2017 planting season

	N application	**Biomass yield**	**Brix%**	**Juice yield**
N application	1	0.183(p=0.23)	0.197(p=0.194)	0.212(p=0.162)
Biomass yield	0.183(p=0.23)	1	0.254(p=0.092)	0.133(p=0.385)
Brix%	0.197(p=0.194)	0.254(p=0.092)	1	-0.120(p=0.432)
Juice yield	0.212(p=0.162)	0.133(p=0.285)	-0.120(p=0.432)	1

The correlation matrix show a very weak correlation between biomass yield, juice yield and Brix% to N application levels. Despite the randomness of the data, a general trend of an increase in biomass (up to 50 kg ha^{-1} dosage of N) can be seen for genotypes ss 007 and SG. Furthermore, juice yield increased up to a dosage of 100 kg ha^{-1} N for genotype HG and all genotypes showed an increase in Brix% up to a dosage of 50 kg ha^{-1} N. Some advantage can therefore be gained by a low dosage of N fertiliser (between 50 kg ha^{-1} to 100 kg ha^{-1}) for most of the genotypes investigated. An assumption for the absences of good correlations might be that sweet sorghum is a robust crop and therefore did not do well in the glasshouse.

The compositional analysis of the juice obtained from the different genotypes, planted in Potchefstroom during the 2016-2017 planting season at different N fertiliser application levels is given in Appedix H 1 to H 11. The reducing sugar yield, 5-carbon sugar yield, acid yield and alcohol yield was calculated from the juice yield (t ha^{-1}) and the concentration of these components in the yield as determined by the HPLC analysis. The alcohols content of the juice was mostely methanol and ethanol. These alchols are degradation products of the sugars and does

not constitute ethanol yield based on sugar content.

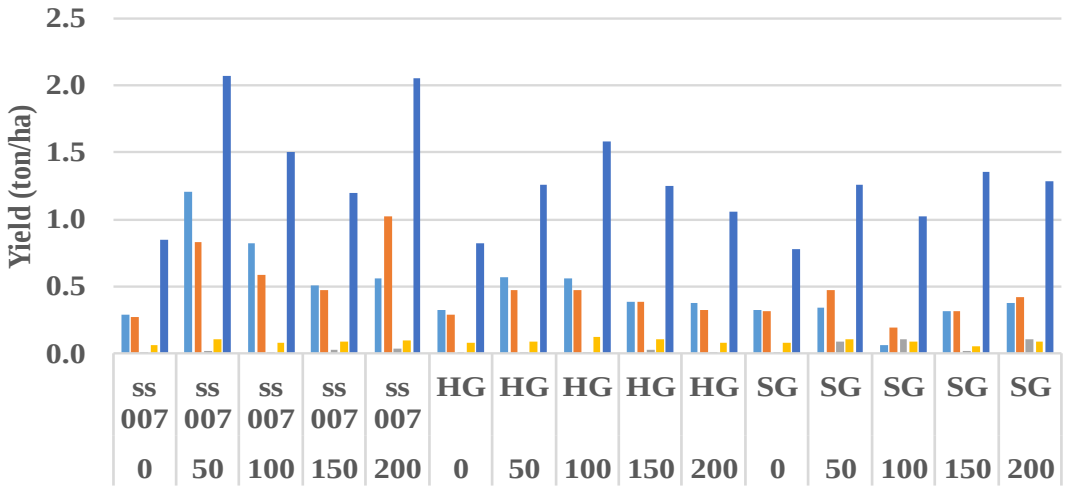

Figure 43 Effect on nitrogen application and genotype on reducing sugar yield (■), 5-carbon sugar yield (xylose) (■), alcohol yield (■), organic acid yield (■) and sugar yield based on Brix% (■) from juice

Compositional sugar analysis of the juice obtained from each genotype with different N applications showed that in all cases, the actual fermentable sugar (reducing sugar) yield of the crops were over estimated from Brix%, although the Brix% does give an indication of the relationship between fermentable sugar yield and the N application. This is mostly due to the fact that Brix% is measured from the density of the juice and the sugar content measured is the sum of all sugars present in the juice (glucose and xylose sugars). Figure 43 shows that on average, the highest fermentable sugar yield (1.14 t ha^{-1}) was obtained from the ss 007 genotype with 50 kg/ha N application. Furthermore, N application had a positive effect on fermentable sugar yield for genotypes ss 007 and HG up to a dosage of 50 kg ha^{-1}, after which increased N applications resulted in a decrease in fermentable sugar yield. Nitrogen application had no significant effect on sugar yield from the SG genotype. Xylose sugar yield (5-carbon sugar yield) was positively effected by N applications for all genotype investigated, up to a dosage of 50 kg ha^{-1} ha. Xylose cannot be readily fermented to ethanol using *Saccharomyces cerevisiae*, the organism that is most widely used for 1st generation ethanol production.

4.3. Calculated potential bio-ethanol production from sweet stem sorghum

The calculated total amount of potential total EtOH production is presented in Figure 46 (Appendix C 3) which represent the combined production from the bagasse (Figure 44, Appendix

C 1) and the juice (Figure 45, Appendix C 2) across the growing seasons and locations.

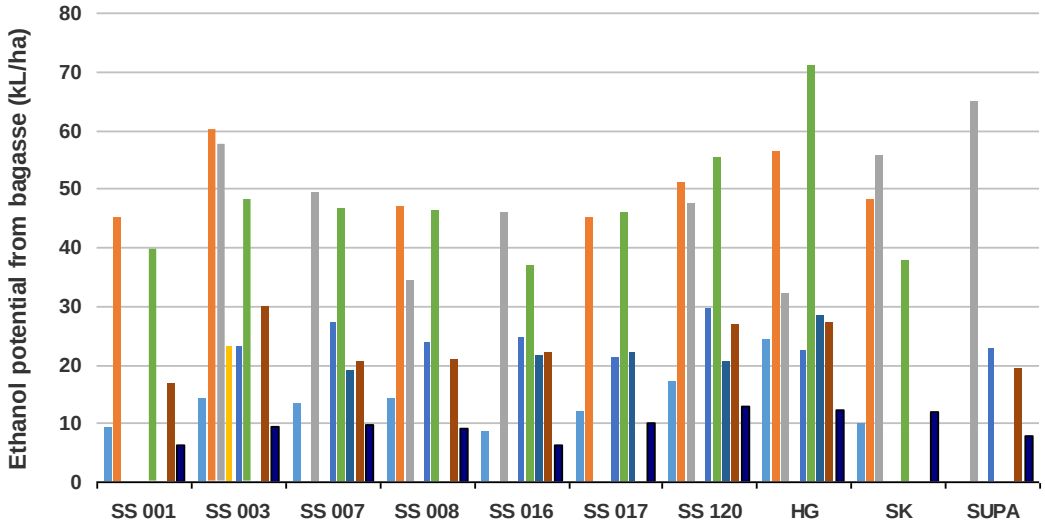

Figure 44. Gaphical representation of EtOH potential produced from bagasse from various genotypes across locations and production years. Year and location: Potchefstroom 2012(); Potchefstroom 2013(); Potchefstroom 2014(); Bethlehem 2012(); Bethlehem 2013(); Bethlehem 2014(); Rustenburg 2102(); Rustenburg 2013(); Rustenburg 2014()

The data from the genotype trials shows that there is a huge difference between the best and worst EtOH production levels. The highest amount of EtOH from bagasse was produced at Potchefstroom from HG produced 71.10 kL ha^{-1} and the lowest amount of 6.34 kL ha^{-1} from ss 001 was produced at Bethlehem. Despite the huge difference in the amounts, the EtOH produced from ss 001 is still a substantial amount.

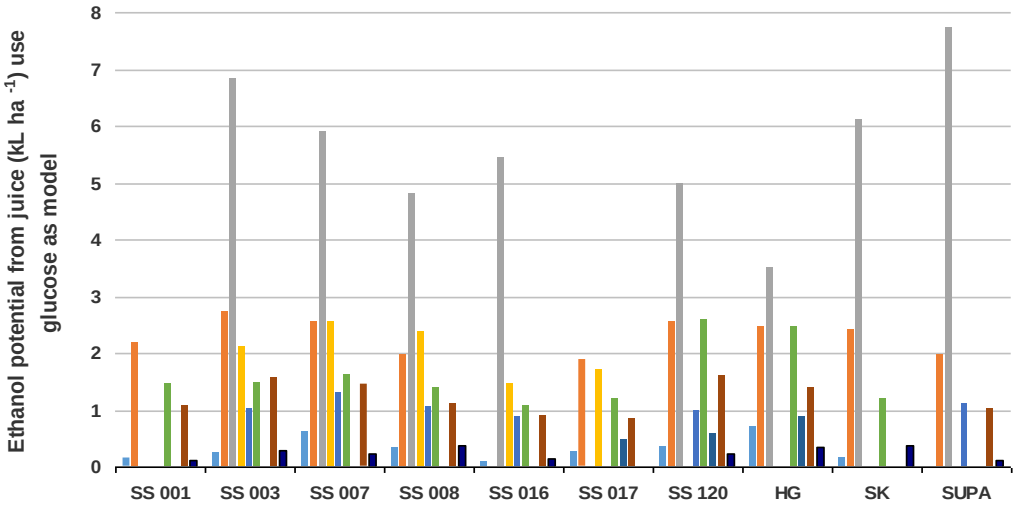

Figure 45. Graphical representation of ethanol potential from juice across locations and production years. Year and location: Potchefstroom 2012(■); Potchefstroom 2013(■); Potchefstroom 2014(■); Bethlehem 2012(■); Bethlehem 2013(■); Bethlehem 2014(■); Rustenburg 2102(■); Rustenburg 2013(■); Rustenburg 2014(■)

The calculated values as presented in Figures 44 (ethanol from bagasse) and Figure 45 (ethanol from juice) are combined in Figure 46 to give a calculated estimation of the total EtOH production by sweet sorghum.

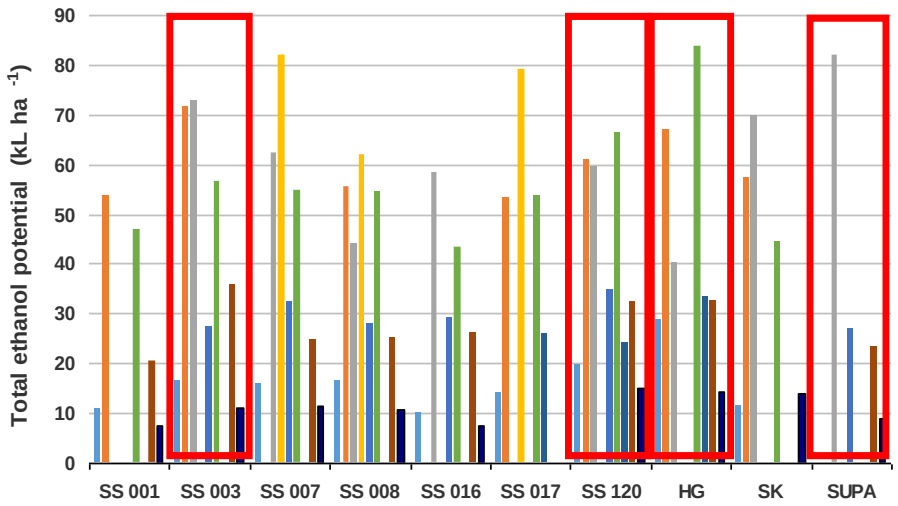

Figure 46. Graphical representation of total EtOH potential from the genotype evaluation trial across locations and production years. Year and location: Potchefstroom 2012(■); Potchefstroom 2013(■); Potchefstroom 2014(■); Bethlehem 2012(■); Bethlehem 2013(■); Bethlehem 2014(■); Rustenburg 2102(■); Rustenburg 2013(■); Rustenburg 2014(■)

The genotypes, marked with red blocks, indicated that ss 003, ss 120, HG and Supa were the most stable across locations and production years and can be recommended to stakeholders whom want

to get involve in EtOH production. All four genotypes produced more than 60 kL ha^{-1} EtOH, which is very high taken into consideration that only a standard N fertiliser application was done. However, the variations amongst all the genotypes should be taken into consideration when a choice has to be made.

Figure 47 is a representation of the calculated total EtOH potential from bagasse, where a 54% glucose and 46% xylose were assumed, in an attempt to get a standard through which the performances of the genotypes and the reactions to various N fertiliser applications can be compared. The rainfall, temperature and heat units were included in the calculations to get to a zero effect, which allows for the performance of the genotypes reaction on the N fertiliser levels to be compared. Various genotypes were tested across the four seasons. Vaalharts and Wilgeboom were dryland trials and the genotype trial in Potchefstroom was planted in a glasshouse. No trend regarding the effect of the N fertiliser levels and the potential EtOH production from bagasse could be determined. The effect of the soil types were not included, but worthwhile to note that the soil at Vaalharts is sandy and the climatic conditions is dry and hot. The soil type at Wilgeboom (small holding 8 kilometers outside Potchefstroom) is sandy-loam with a slight sandy texture and the trial was cultivated under dryland conditions. The climatic conditions are the same as was mentioned in the genotype evalutions trials. The soil in the glasshouse trail at Potchefstroom (ARC: GCI) is also sandy-loam, but with a slightly higher clay content. Irrigation water was supplied and the climatic conditions were kept humid inside the greenhouse.

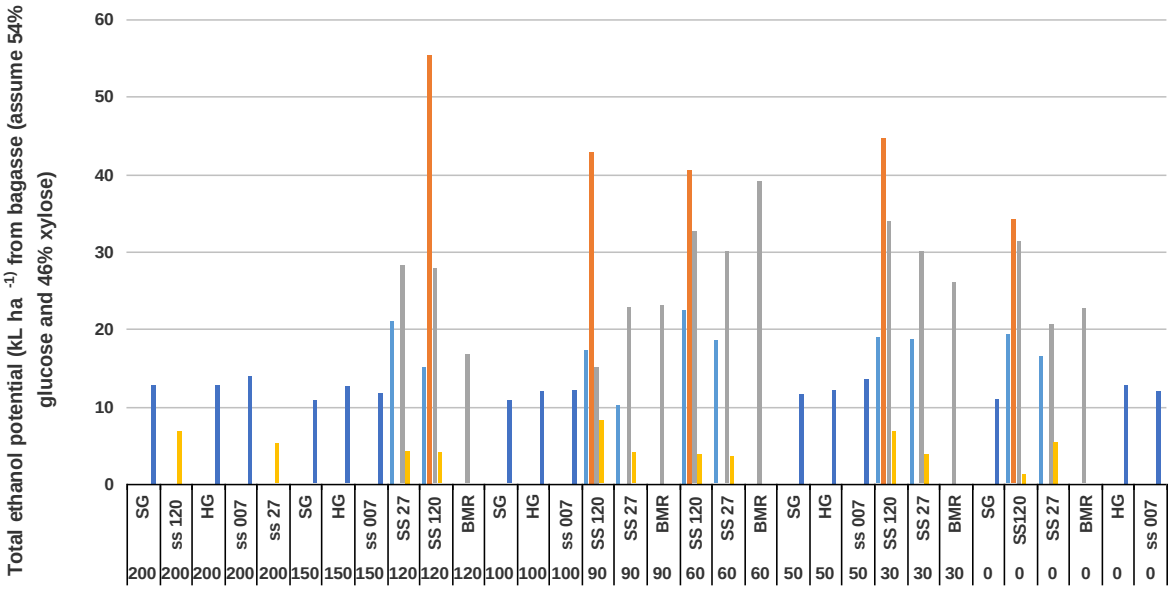

Figure 47 Illustration of the total EtOH (kL ha^{-1}) potential from bagasse with various nitrogen applications. Year and location: Vaalharts 2012 (■); Vaalharts 2013 (■);Wilgeboom 2012 (■);Wilgeboom 2014 (■); Potchefstroom 2016 (■)

The best overall potential EtOH production from the bagasse was produced by ss 120 at a 120 kg ha^{-1} N application level (55.46 kL ha^{-1}) at Vaalharts during the 2013 season. The second best calculated EtOH yield, also at Vaalharts during the 2013 season, was 44.73 kL ha^{-1} produced from ss 120 at 30 kg ha^{-1}. During the 2012 season the best production at Vaalharts was 22.55 kL ha^{-1} and at Wilgeboom it was 33.99 kL ha^{-1}. The best production was 8.13 kL ha^{-1} and a very low 1.16 kL ha^{-1} potential EtOH was produced at Wilgeboom during 2014. Apart from the other low performances, on average a better performance was put up during 2016-17 at Potchefstroom. However, the EtOH production values were low (best 13.93 kL ha^{-1}) during 2016-17 compared to the 2012 to 2014 seasons, which might show that sweet sorghum is sensitive regarding the synthesis of sugars in artificial conditions.

Figure 48 (Appendix C 4) illustrates the calculated potential EtOH yields from the extracted juice. An individual performance by BMR (5.75 kL ha^{-1}) during the 2012 season at Wilgeboom occured, but the best overall performance was by ss 120 and ss 27 covering more seasons and localities regarding good EtOH productions from the juice. Figure 47 illustrates that ss 120 also produced the highest amount of EtOH from bagasse across the locations and across the different production seasons. Although other individual genotypes produced more EtOH at various stages, the genotype ss 27 performed second best when the inclusion of the various N fertiliser levels, locations and seasons are taken into consideration.

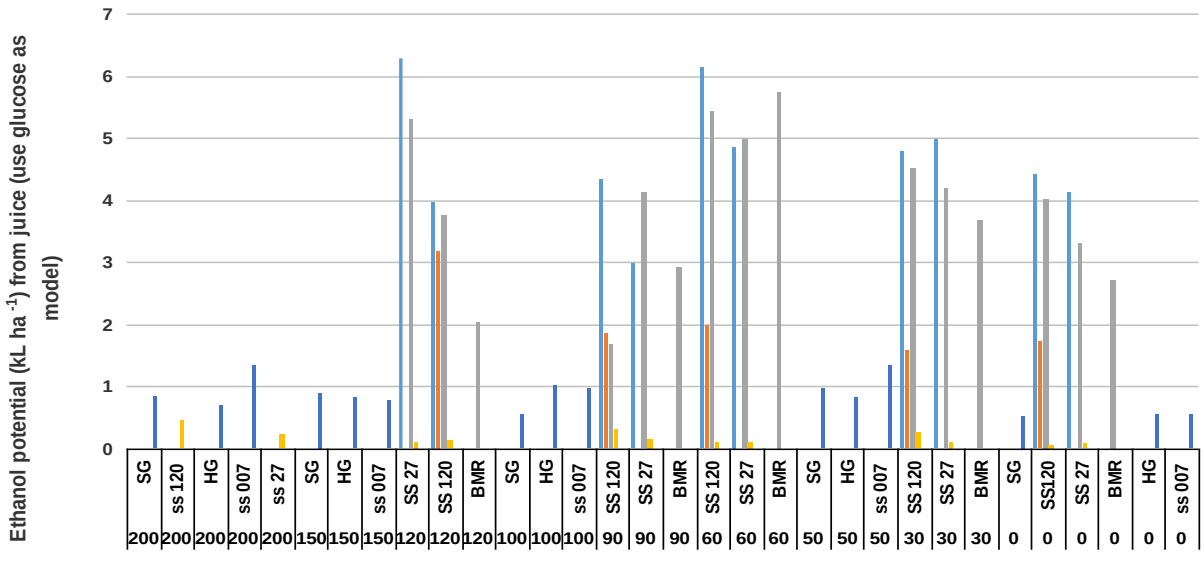

Figure 48. Illustration of the EtOH potential from the extracted sweet sorghum juice with various nitrogen applications. Year and location: Vaalharts 2012 (■); Vaalharts 2013 (■);Wilgeboom 2012 (■);Wilgeboom 2014 (■); Potchefstroom 2016 (■)

Figure 48 indicates the bad season during 2014 at Wilgeboom, indicated by the lowest EtOH production of 0.04 kL ha^{-1}. The 1.34 kL ha^{-1} EtOH production at Potchefstroom is also low compared to the other locations, excluding Wilgeboom 2014. The genotype ss 27 (6.28 kL ha^{-1}) during the 2012 season produced the best overall when the inclusion of the various N fertiliser levels, locations and seasons are taken into consideration. Even though the productions were low the best performers, eg. ss 120 and ss 007 can be recommended for EtOH production.

Figure 49 represents the calculated total EtOH potential from residual sugars (assume glucose). The irregular pattern of the performances of the genotypes continues even through the calculated EtOH productions from the residual sugars values. During 2012 in Vaalharts the best EtOH was produced by ss 120 at 60 kg ha^{-1} N fertiliser application level (3.28 kL ha^{-1}) and the lowest production was 1.48 kL ha^{-1} by ss 27 at 90 kg ha^{-1} N fertiliser application level.

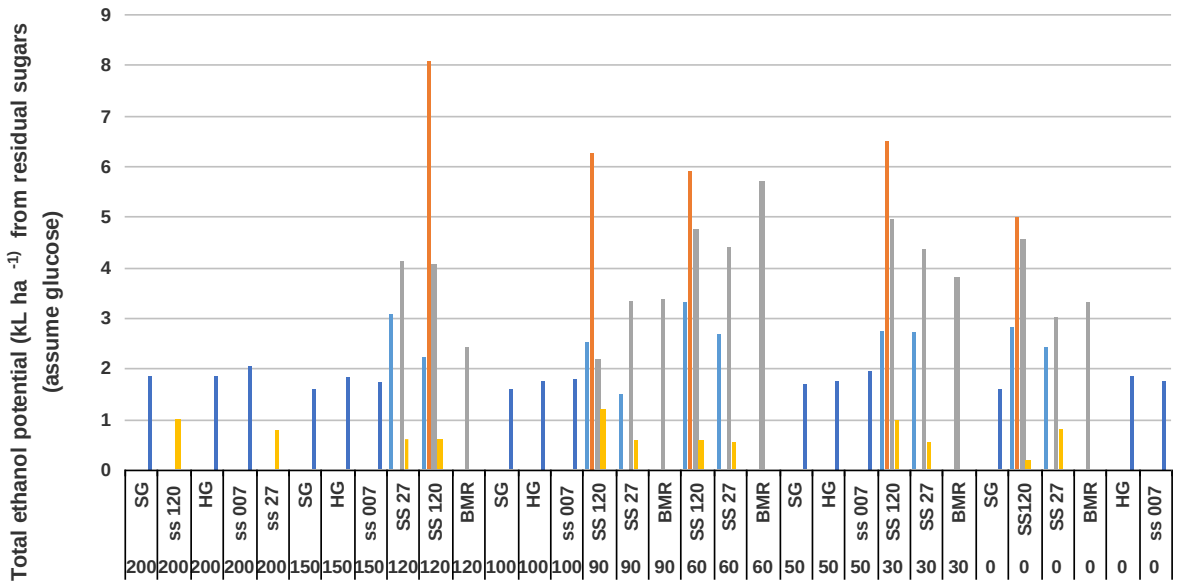

Figure 49. Illustration of the total EtOH potential from residual sugars with various nitrogen applications. Year and location: Vaalharts 2012 (■); Vaalharts 2013 (■);Wilgeboom 2012 (■);Wilgeboom 2014 (■); Potchefstroom 2016 (■)

The performance of ss 120 at 120 kg ha^{-1} (8.07 kL ha^{-1}) in Figure 49 (EtOH production from residual sugars) can be regarded as an outlyer due to the highest amount of EtOH produced from the residual sugars across genotypes, locations and seasons. Almost all other results indicate that even the 0 kg ha^{-1} N fertiliser application levels produced better results amongst the other genotypes, compared to higher N levels.

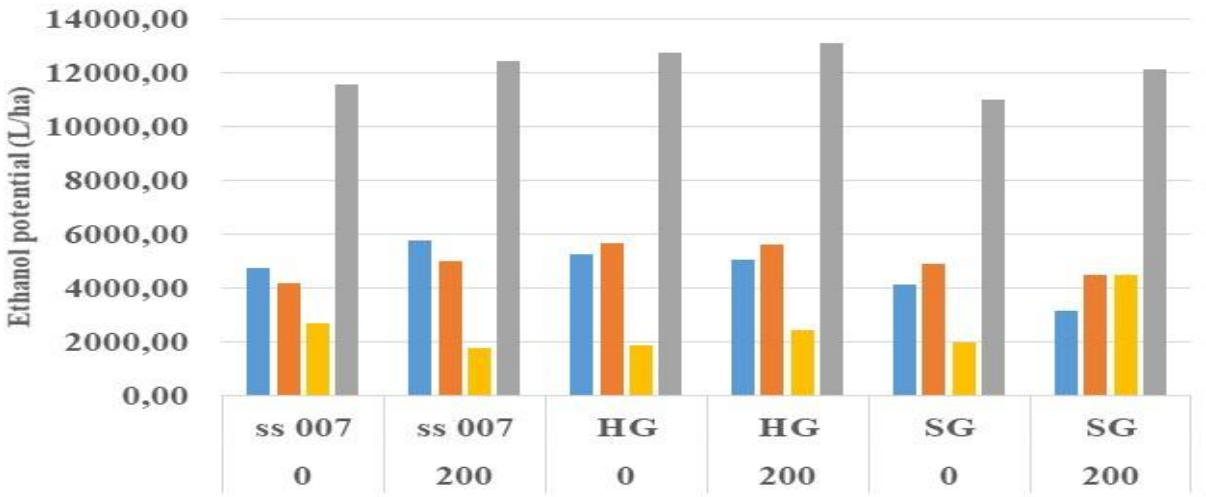

Figure 50. Effect of N application (kg ha^{-1}) and genotype on EtOH potential for genotypes planted at Potchefstroom during the 2016/2017 planting season. Ethanol yield: ■, Ethanol from cellulose sugars; ■, Ethanol from hemicellulose sugars; ■, Ethanol from residual sugars; ■, Total Ethanol

Figure 50 shows the calculated values and consolidation of all the data regarding EtOH production by the genotypes with a constant N fertiliser application and the performances where N fertiliser levels were altered. It also indicates that the genotype ss 007 produced more than 70 000 L ha^{-1} EtOH at an application rate of 50 kg ha^{-1} N fertiliser. When the total EtOH potential production is calculated by including the juice, bagasse and residual sugar values, the pattern/trend is again erratic (Appendix C 5). The only constant is ss 120 at 120 kg ha^{-1}, which again performed the best during 2013 in Vaalharts by producing a calculated value of 66.71 kL ha^{-1} EtOH. Except for the low EtOH production during 2014 in Wilgeboom (1.37 kL ha^{-1}), the second lowest EtOH production (8.19 kL ha^{-1}) by ss 120 at a 0 kg ha^{-1} applied N fertiliser level at Wilgeboom during 2014, is still a very good yield. The genotype SG reacted negatively and a decrease in production is visible between 50 kg ha^{-1} N fertiliser and 150 kg ha^{-1} N fertiliser. A constant EtOH production was illustrated by HG with a slight drop in production from 100 kg ha^{-1} N fertiliser. The production stays constant with almost no increase in EtOH from from a 150 kg ha^{-1} N fertiliser and more, indicating that sweet sorghum does not produce better at high N fertiliser levels. Genotype ss 007 produced more cellulose sugars than hemicellulose sugars while HG and SG produced more hemicellulose sugars than cellulose sugars. Cellulose hydrolyses to form glucose is much easier and more economical to convert to EtOH than hemicellulose sugars. With the exception of the SG genotype, a N application of 200 kg ha^{-1} resulted in a slight increase in both the cellulose and hemicellulose EtOH yield, compared to the case where no additional N was applied. When data sets and calculations made from data sets are applied it showed that N

applications improved the EtOH potential with between 300 and 1000 L/ha. Genotype HG showed the highest EtOH potential and would be the preferred genotype when cultivating sweet stem sorghum for 2nd generation EtOH production.

The ethanol potential from the juice (using Brix% and HPLC analysis) is compared to the ethanol potential from the bagasse in Figure 51.

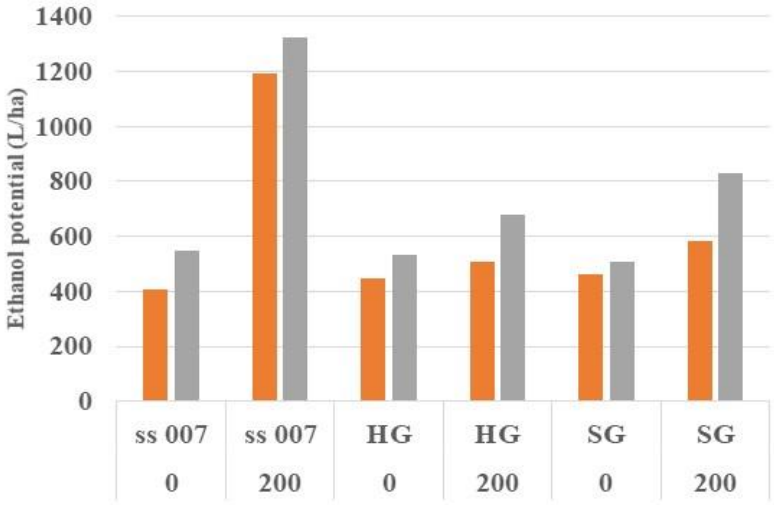

Figure 51. Comparison of EtOH production potential from the juice as calculated using either Brix% (■) or HPLC sugar analysis (■) for different genotypes at different N application levels

The Brix% slight over predicted the sugar yield and thus also the potential EtOH yield. Brix% is measured as a function of the density of the juice and since the juice also contain alcohols and acids that affects its density, the slight over estimation is expected. The Brix% is much easier and more affordable to measure than HPLC analyses and it is a good estimation tool to use to predict potential EtOH yields from an energy crop.

Bagasse is the plant material left after the juice has been pressed from the plants. The bagasse contains on average approximately 30 wt.% residual reducing sugars (glucose, sucrose and fructose) (Marx *et al*, 2014) that was deposited onto the stalks during the juice pressing process. The bagasse is a 2nd generation resources and the cellulose and hemicellulose in the stalks can also be converted to EtOH through a 2nd generation production process. The cellulose and hemicellulose content of the stalks can be calculated from the neutral determined fibre (NDF), the acid determined fibre (ADF) and the acid determined lignin (ADL) content determined from the bagasse analysis. The compositional analysis of the bagasses from the genotypes investigated for effect of N application in the 2016-2017 planting season is given in Table 16. The total potential

sugar yield can be calculated from Tabel 16 as the sum of cellulose, hemicellulose and residual sugar yield.

The residual sugar yield was calculated as the difference between total carbohydrates and the sum of the structural carboydrates (cellulose, hemicellulose and lignin). See Appendix F 2.

The xylose can however be converted to EtOH using organisms such as *Zonamonas mobilis* or *Pichia stipites* (Fu &Peiris, 2008). These results thus show that genotype ss 007 is the preferred genotype to produce a sugar rich juice that can be used for 1st and 2nd generation EtOH production. Although organic acids such as acetic acid (which is present in all juices) is a natural inhibitor for 1st generation EtOH production, the levels are well below the inhibition limit of 8 to 10 g/L (Appendix H). It is known that 1 mole of sugar will produce 2 mole of EtOH during fermentation. If it is assumed that the total sugar yield as determined from HPLC analysis is glucose, the EtOH potential for each genotype at the different N application can be calculated (see Figure 52).

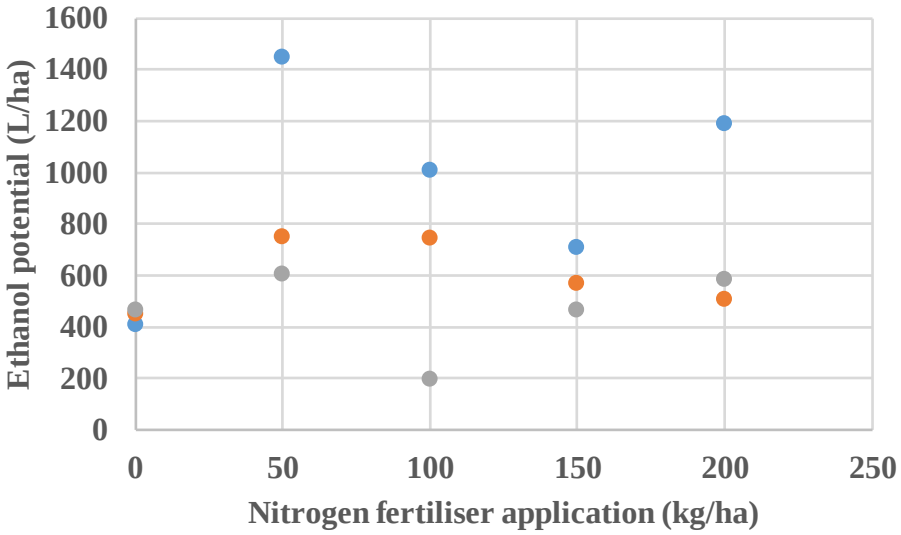

Figure 52. Effect of genotype and N application on ethanol potential from juice
Genotype: ●, ss 007; ●, HG; ●, SG

Figure 52 comfirm the positive effect of N application on EtOH potential for genotypes ss 007 and HG. Nitrogen application did not have a significant effect on the calculated EtOH potential for the SG genotype, which was also seen from the sugar yield. From these results it can thus be concluded that genotype ss 007 is the best genotype to use for 1st generation EtOH production from the juice of sweet sorghum and that a N application of just 50 kg ha^{-1} would increase the

ethanol yield almost three-fold (●; ±400 L ha^{-1} to more than 1400 L ha^{-1}). During 2016-17 in Potchefstroom (Figure 47, EtOH from bagasse) ss 007 at 200 kg ha^{-1} produced 13.93 kL ha^{-1} EtOH. The 150 kg ha^{-1} N fertilisation applications also showed no major effect on the genotypes' performances. Although there occurred drops in the ethanol yields amongst the different N application levels, all three genotypes showed an increase from 0, with the most effective N applications levels between 100 and 150 kg ha^{-1}. It can therefore be deducted that too much N will only lead to unnecessary expenses with no major benefit regarding better production by the genotypes and for higher EtOH production.

However, comparing the production of EtOH from sweet stem sorghum to other crops, it was indicated that a number of genotypes performed above average and therefore sweet stem sorghum is a very viable alternative crop for the production of renewable EtOH. The EtOH potential calculated from the sugar yields in this study compares well to reported EtOH potential from sweet stem sorghum cultivated in China in the same planting period (approx. 2000 L/ha, data adapted from Diallo *et al*, 2019 and Ho *et al*, 2014). See Table 18.

Table 18. Comparison regarding ethanol potential amongst different crops and different countries (Gupta *et al*, 2014)

Energy Crop	**Crop yield (ton/ha)**	**Ethanol potential (L/ha)**	**Country**
Sugarcane	79.5	3800	Brazil
Sugarcane	79.1	7900	South Africa
Sweet sorghum	20.84 (avg)	12000 (avg)	South Africa (this study)
Sweet sorghum	6.69	2600	China
Sugar beet	60	5000	EU
Maize	9.9	4100	USA
Cassava	13.6	137	Brazil

The results from this study (using new genotypes), obtained under dryland conditions, show much higher yields (especially for biomass yield) and EtOH potential compared to other crops.

4.4. References

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Dolciotti, I., Mambelli, S., Grandi, S. & Venturi, G. 1998. Comparison of two sorghum genotypes for sugar and fibre production. *Industrial crops products*, 7(2-3):265-272.

Fu, N., Peiris, P. 2008. Co-fermentation of a mixture of glucose and xylose to ethanol by Zymomonas mobilis and Pichia stipites. *World Journal of Microbiology and Biotechnology*. Vol 24(7):1091-1097.

Gupta, V. K., Potumarthi, R., O'Donovan, A., Kubicek, C. P., Sharma, G. D. Tuahy, M. G. 2014. Bioenergy Research: An overview on technological developments and bioresources. Science Direct. *Bioenergy Research and Applications*, Ch 2. p23-41.

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Chapter 5

5.1 Conclusion

This study was a result of trials done for the European Union funded project – "Sweetfuel", aimed at investigating sweet sorghum as a viable renewable resource for biofuel production. It is a crop that can withstand difficult climatic conditions and can be cultivated on marginal soils.

The N application trials were a follow-on to the genotype evaluation trials. There was no research done in South Africa so far, to detemine the effect of different N application levels on the performance of sweet sorghum and it's EtOH production potential, should the South African market opens for biofuel production and the blending thereof with fossil fuels. The results which are presented in the study covered five production seasons and did not supply, beyond all doubt, significant prove that high N application levels will result in higher sugar (TSS) content needed for the EtOH production process. However, N application levels have had an effect on biomass yields which results in higher juice production of specific genotypes in different locations. Although the Brix% per unit of juice seemed not to be effected highly by different N application levels, more syrup results in more TSS to be fermented. Indirectly the higher biomass yields result in higher Brix% levels and higher EtOH production.

At EMBRAPA, a Brazilian Research Institute, research done by Dr R Schaffert determined that the lowest Brix% value to produce a vaible amount of EtOH from sweet sorghum should be 16% (personal communication, 2011). Most of the genotypes' Brix% values (Figures 10 to 18) show much higher values than 16%. In cases where the Brix% values are higher than 20% the juice can be diluted which increases the EtOH production per hectare, making sweet sorghum an economical viable energy crop.

Although a very slight effect was observed, there were variations amongst Brix% readings which might be the effect of the different N application levels. High levels of stalkborer infestations did occur which might have caused some of the variations. The stalkborer damage resulted in lower juice production and sugar quality, but was not an overall problem. Variables like fertilisation, differences in maturing stages, and the time of processing after harvesting also had an effect on the sugar content and quality of the juice. Biomass production of 50 t ha^{-1} is a very good average for sweet sorghum in a dryland production system to comply with the requirements of optimum EtOH production. Due to the fact that the sugars are to be fermented, it is clear that the amount of juice and the quantity thereof will determine the success of the EtOH production. It is therefore

important to know the optimum N levels to produce the correct kind and the correct amount of sugars available for the fermentation process. From the results, it is clear that the necessary sugars (glucose and sucrose) and the optimum amount of sugars were produced by sweet sorghum genotypes for optimum EtOH production.

Various genotypes across the locations and across the production seasons performed well enough to be considered as renewable resources for EtOH production. During the 2011/12 season the genotypes ss 007, ss 017, ss 008 and BMR were stable regarding biomass yields, juice yields and Brix%. During the 2012/13 season the genotypes ss 003, BMR, HG, ss 120, SK, ss 008, ss 001, ss 017 and Supa were stable regarding biomass yields, juice yields and Brix%. During the 2013/14 season the genotypes ss 003, ss 007, p 868, E3 and Supa were stable regarding biomass yields, juice yields and Brix%. Table 20 summarises the performances of the genotypes and their adaptation to different soil types.

Table 19. Summary of performances and adaptations of genotypes to climate variations and the major soil types which occurred at the various trial cites

A		Genotype trial 2011/12			Genotype trial 2012/13			Genotype trial 2013/14		
	genotype	bio-mass	juice	Brix %	bio-mass	juice	Brix %	bio-mass	juice	Brix %
	HG	x			x		x			
sand	ss 007			x			x			
	ss 008			x						x
	ss 003			x	x		x			
	ss 120	x			x		x		x	
	HG	x	x							
	ss 017	x			x	x				
clay	ss 120			x		x				
	ss 007	x		x				x		x
	ss 003				x	x	x	x		x
	BMR				x	x	x			
	SK				x	x	x	x		x
	Supa					x		x	x	x
	ss 007	x	x	x	x		x	x	x	x
	ss 017	x	x	x						
loam	ss 003			x				x	x	x
	ss 008		x	x			x			
	BMR				x		x			
	ss 120				x			x	x	
	HG							x	x	
	ss 001							x		x

The genotypes marked in red in Table 19 is a summary of those genotypes that appeared most times in a repetitive manner across the locations and seasons, and was not necessarily listed based on yields, and can therefore be recommended as quite stable genotypes regarding the inclusion into EtOH production programmes. From the genotype evaluations, the conclusion can be drawn that although there were inconsistent patterns depicted amongst the variables under investigation,

a number of genotypes qualify for inclusion into biofuel programmes.

Table 20. The best adapted genotypes regarding sugar potential used for EtOH (1st and 2nd generation) production with rainfall and ambient temperature taken into account

	genotype	sugars for 1st generation bio-ethanol	sugars for 2nd generation bio-ethanol
sand	ss 003	x	x
	ss 007	x	
	Supa	x	
	SK		x
	ss 008		x
	HG	x	
	ss 017		x
	ss 016		x
	ss 120	x	x
clay	ss 001	x	x
	ss 007	x	
	ss 003	x	x
	ss 120	x	x
	SK	x	x
	Supa	x	
	HG		x
	ss 017		x
	ss 008		x
	ss 016	x	
loam	ss 007	x	x
	ss 003	x	
	ss 120	x	x
	ss 008	x	
	HG	x	x
	ss 016		x
	ss 017		x

The genotypes marked in red in Table 20 is also a summary of those genotypes that appeared the most in a repetitive manner regarding sugar production in reaction to RF and HU changes and the effect different soil types might have, and was not listed based on yield levels as such. The majority of the genotypes listed here correspond to the genotypes in Table 19.

Tables 21 and 22 are representing a selection of the genotypes that occurred the most regarding sugar and EtOH production.

Table 21. Best performing genotypes regarding sugar production from juice and bagasse during the genotype trial

	Juice
sand	ss 001, ss 003,ss 007, ss 008, ss 120, HG
clay	ss 003, ss 007, ss 120, SK, Supa
loam	ss 007, ss 017, ss 120, HG,
	Bagasse
sand	HG, ss 120, ss 003, ss 007, ss 016, ss 017
clay	ss 003, HG, SK, ss 007, ss 008, ss 017
loam	HG, ss 007, ss 017, ss 008, ss 120, ss 003

Table 22. Best performing genotypes regarding the calculated potential EtOH production from bagasse and juice during genotype trial

Juice
ss 003, ss 007, ss 120, HG
ss 003, ss 007, ss 120, HG, ss 016, SK, Supa
ss 003, ss 007, ss 008, HG, ss 017, ss 120
Bagasse
ss 003, ss 016, Supa, ss 120, HG
SK, Supa, ss 001, ss 003, ss 008, ss 016, ss 120
ss 003, ss 007, ss 008, HG, ss 017, ss 120

Tables 21 and 22 indicate that the genotypes in red correspond with the genotypes in red as selected in Tables 19 and 20. It can clearly be seen that the same genotypes performed well under all the conditions tested and those are the genotypes that can be recommended to be included into an EtOH production programme.

The following AMMI-byplots represented in Figures 53 to 55 incorporate the information in Tables 19 to 22 and display the genotypes and their adaptations and performances across the locations and years where they were investigated. The N application data could not be represented in AMMI-byplots due to the inconsistency in the genotypes, N application levels and years.

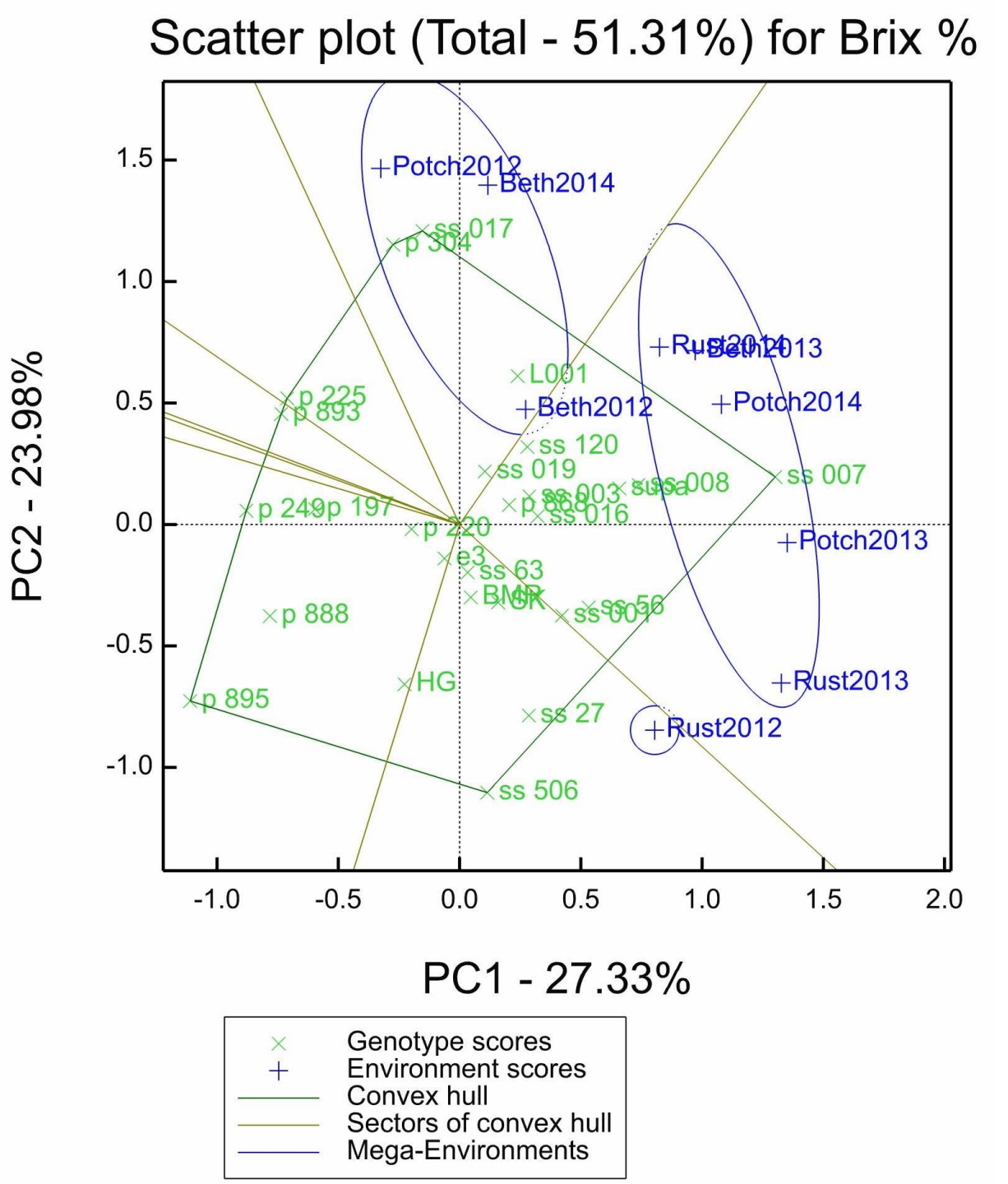

Figure 53. AMMI-byplot : Brix% representing the genotypes' performance across seasons and localities regarding the Brix index of the juice

Figure 53 represents a summary of the genotypes' performances regarding Brix% and if the stakeholder's goal is to obtain high Brix% values, genotype ss 017 can be recommendated for Potchefstroom and Bethlehem. In Rustenburg, Bethlehem and Potchefstroom the genotype ss 007 performed well. Genotype ss 27 can also be recommended for Rustenburg.

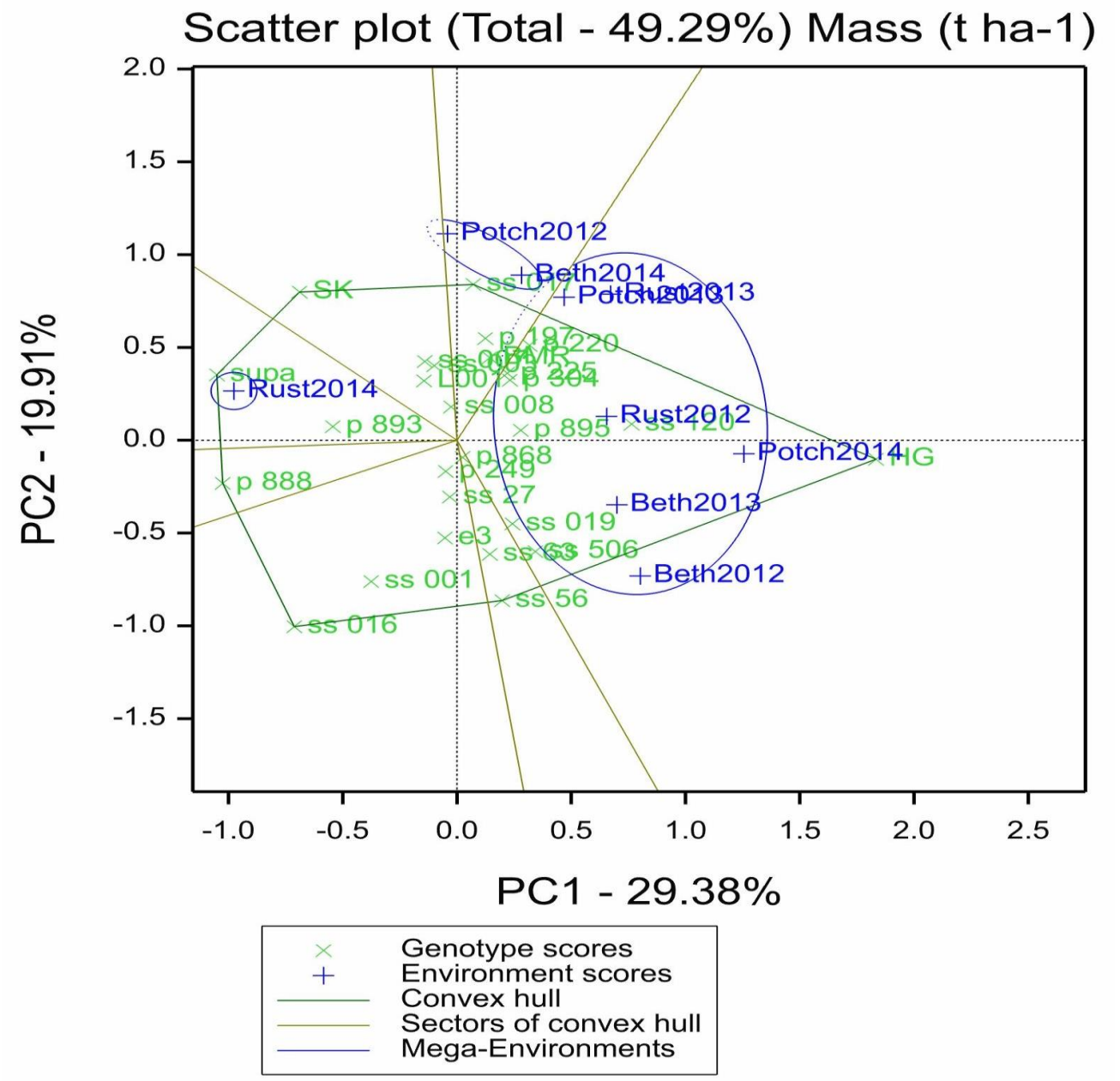

Figure 54. AMMI byplot : Mass representing the genotypes' performance across seasons and localities regarding biomass yield (t ha^{-1})

Figure 54 represents a summary of the genotypes' performances regarding biomass yield and if the stakeholder's goal is to obtain high biomass yields, genotype ss 017 can be recommendated for Potchefstroom, Rustenburg and Bethlehem. In Bethlehem ss 27 and HG in Potchefstroom also performed well. Genotype Supa can also be recommended for Rustenburg.

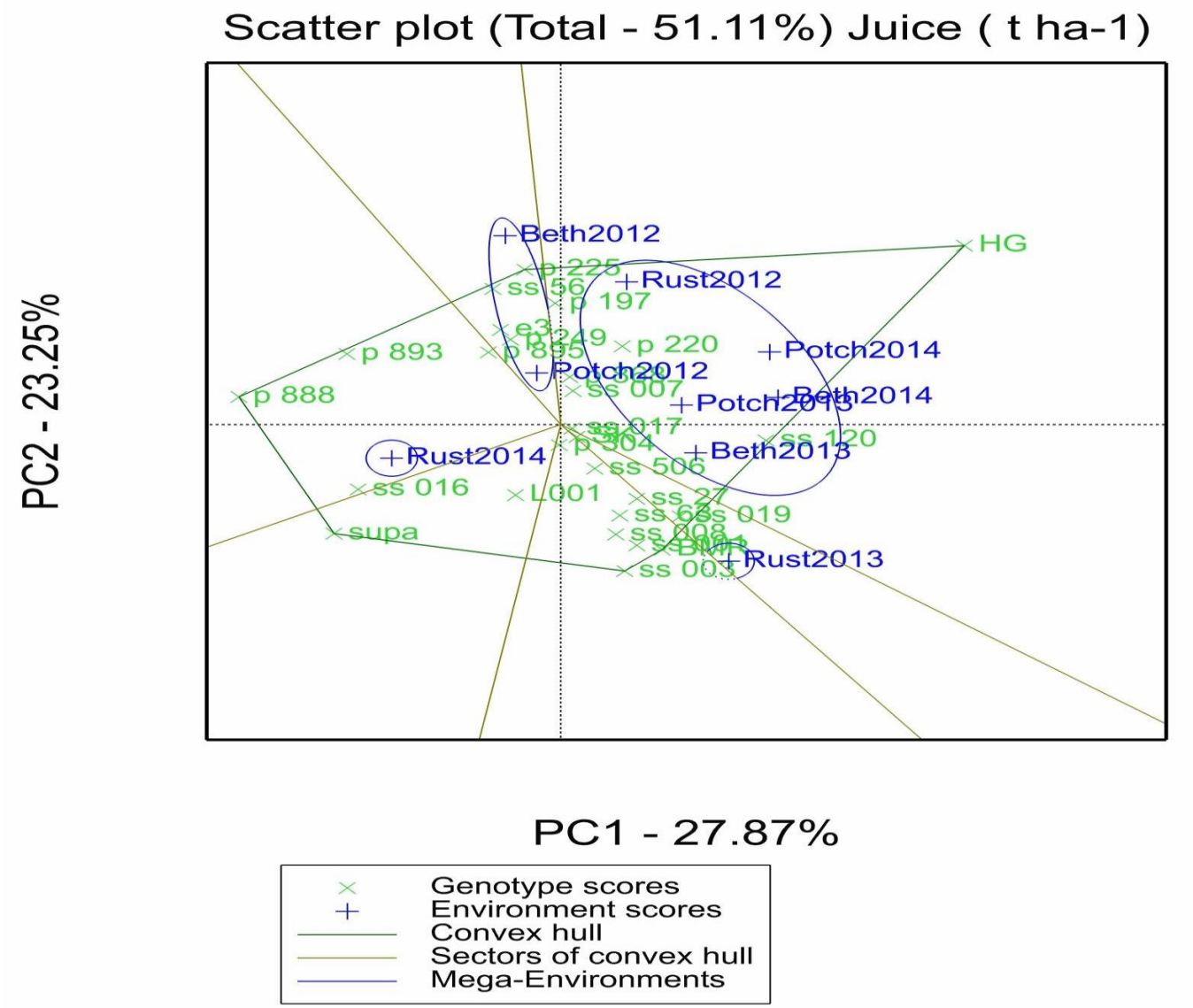

Figure 55. AMMI byplot : Juice representing the genotypes' performance across seasons and localities regarding juice production (t ha^{-1})

Figure 55 represents a summary of the genotypes' performances regarding juice yield and if the stakeholder's goal is to obtain high juice yields, genotype HG can be recommendated for Potchefstroom and Bethlehem. In Bethlehem ss 120 and in Rustenburg ss 016 performed well. For both Rustenburg and Bethlehem genotypes ss 27, ss 120, ss 27 and ss 003 can also be recommended.

From the data in Chapter 4 it was shown that there was a slight decline in the sweet sorghum's performance regarding juice and sugar production at N fertiliser levels from more than 150 kg ha^{-1} and no improvement at high 200 kg ha^{-1} N fertiliser application levels. Total EtOH productions across the locations and production years were high as shown by the results produced by the

processed raw materials and by the calculated EtOH values. References made in this study to the genotypes' performances indicated high calculated EtOH production levels from the best genotypes, viz HG 83.9 kL ha^{-1}, ss 003 72.69 kL ha^{-1} and ss 120 66.25 kL ha^{-1} as was calculated from the analysed sugars in the bagasse. The potential EtOH yield from sugars in the juice reached a total amount of 9978.23 L ha^{-1}. It is clear from the results that it is very difficult to recommend a specific genotype due to the variances amongst the genotypes, although the EtOH yields are high enough to use sweet sorghum as alternative resource for the production of biofuels.

The same scenario as depicted in Tables 19 to 21 is visible in Tables 23 to 25 indicating a summary of the response of genotypes to the various N application levels on the measured variables.

Table 23. Best performing genotypes regarding EtOH production from juice in reaction to variations in N application levels

2011/12	sand (Vaalharts)	ss 27 @ 120 kg ha^{-1}, ss 27 @ 60 kg ha^{-1}, ss 120 @ 90 kg ha^{-1}, ss 27 @ 30 kg ha^{-1}
	sand/loam (Wilgeboom)	ss 27 @ 120 kg ha^{-1}, ss 27 @ 90 kg ha^{-1}, ss 27 @ 30 kg ha^{-1}, BMR @ 60 kg ha^{-1}, ss 27 @ 60 kg ha^{-1}, ss 120 @ 30 kg ha^{-1}
2012/13/14	sand (Vaalharts)	ss 120 @ 120 kg ha^{-1}, ss 120 @ 90 kg ha^{-1}, ss 120 @ 60 kg ha^{-1}
	sand/loam (Wilgeboom)	ss 120 @ 200 kg ha^{-1}, ss 27 @ 200 kg ha^{-1}, ss 120 @ 90 kg ha^{-1}, ss 120 @ 30 kg ha^{-1}
2016/17	loam (Potchefstroom)	ss 007 @ 200 kg ha^{-1}, HG @ 100 kg ha^{-1}, ss 007 @ 100 kg ha^{-1}, SG @ 50 kg ha^{-1}, ss 007 @ 50 kg ha^{-1}

Table 24. Best performing genotypes regarding EtOH production from bagasse in reaction to variations in N application levels

2011/12	sand (Vaalharts)	ss 27 @ 120 kg ha^{-1}, ss 120 @ 60 kg ha^{-1}, ss 120 @ 30 kg ha^{-1}, ss 120 @ 0 kg ha^{-1}
	sand/loam (Wilgeboom)	ss 120 @ 60 kg ha^{-1}, BMR @ 60 kg ha^{-1}, ss 120 @ 30 kg ha^{-1}, ss 120 @ 0 kg ha^{-1}
2012/13/14	sand (Vaalharts)	ss 120 @ 120 kg ha^{-1}, ss 120 @ 90 kg ha^{-1}, ss 120 @ 30 kg ha^{-1}
	sand/loam (Wilgeboom)	ss 120 @ 200 kg ha^{-1}, ss 120 @ 90 kg ha^{-1}, ss 120 @ 30 kg ha^{-1}, ss 27 @ 0 kg ha^{-1}
2016/17	loam (Potchefstroom)	HG @ 200 kg ha^{-1}, ss 007 @ 200 kg ha^{-1}, ss 007 @ 50 kg ha^{-1}, HG @ 0 kg ha^{-1}

Table 25. Best performing genotypes regarding total EtOH production from bagasse, juice and residual sugars in reaction to variations in N application levels

F		
2011/12	sand (Vaalharts)	ss 27 @ 120 kg ha^{-1}, ss 120 @ 60 kg ha^{-1}, ss 120 @ 30 kg ha^{-1}, ss 120 @ 0 kg ha^{-1}
	sand/loam (Wilgeboom)	ss 120 @ 60 kg ha^{-1}, BMR @ 60 kg ha^{-1}, ss 120 @ 30 kg ha^{-1}, ss 120 @ 0 kg ha^{-1}
2012/13/14	sand (Vaalharts)	ss 120 @ 120 kg ha^{-1}, ss 120 @ 90 kg ha^{-1}, ss 120 @ 30 kg ha^{-1}
	sand/loam (Wilgeboom)	ss 120 @ 200 kg ha^{-1}, ss 120 @ 90 kg ha^{-1}, ss 120 @ 30 kg ha^{-1}, ss 27 @ 0 kg ha^{-1}
2016/17	loam (Potchefstroom)	SG @ 200 kg ha^{-1}, ss 007 @ 200 kg ha^{-1}, ss 007 @ 50 kg ha^{-1}, HG @ 200 kg ha^{-1}

All red marked genotypes are those who performed well under different conditions. The specific yields were not regarded in the selection, but only the repetitious character of the various genotypes. It is obvious that almost the same genotypes occur in the N application trials (Tables 23 to 25), as was the case in the genotype trials (Tables 20 to 22).

The statistical analysis indicated significant correlations between the biomass yields and the juice yields. This is an obvious correlation because more juice can be extracted from high volumes of bagasse. It should be kept in mind that when the harvesting exceeds the physiological maturity stage of the stems, it can dry off and it will cause a decline in juice yields. There was no significant correlation between N fertiliser applications levels and the increase in the production levels of the variables. No significant correlation exists between biomass yields, Brix% levels and juice yield, but rather individual correlations exist between two of the variables. A relatively strong relationship between fermentable sugars and environmental conditions was shown. A strong correlation is visible between Brix% and N application levels, a mild correlation between biomass yield and N applications, relatively low correlation between biomass yield and N applications and a mild correlation between Brix% and juice yield. A strong correlation is visible between biomass yield and juice yield. A small number of genotypes performed well in the sandy soil areas, but a better correlation was visible between genotypes and their performances in the laom and clay soils.

In an attempt to make the presented research data in this dissertation more applicable, a scenario is drawn below to indicate the effect the blending of EtOH into fossil fuels might have on the South African petroleum industry. Table 18 showed an average calculated EtOH production of

12 000 L ha^{-1} as was recorded from the data in this study.

According to the Department of Agriculture, Forestry and Fisheries (www.daff.gov.za/statistics) the national sorghum production is cultivated on approximately 50 500 ha. These mentioned values can achieve a potential production of 606 000 000 L p.a. of EtOH. According to the Department of Energy (http://www.energy.gov.za/files) during the 1st and 2nd quarter of 2019 a total of 14 057 326 655 litres of fossil fuels were consumed in South Africa, which gives a rough estimation of 28 114 653 310 L p.a. When a blend of 2% biofuel (Biofuels Industrial Stategy, 2007) is allowed by the government, an amount of 502 293 066 L p.a. of EtOH will be needed, which can be met from the calculated amount of EtOH produced from sweet stem sorghum. The current average price of petrol in South Africa is R 15.79 / L (https://www.aa.co.za/fuel-pricing) and the blend can have a cost effect of R 7 931 207 512 p.a. on the fuel market.

However, stakeholders must have access to selected genotypes on account of the adaptability of the genotypes to specific areas and the niche the genotype must fill in their farming operation regarding the production of juice and biomass for EtOH production. The future of the bio-ethanol industry in South African where sweet sorghum and/or other crops can play a role depends on the Government's commitment to open up the market for the production of bio-ethanol and the blending trhereof. Although research was done, there are opportunities for the development and selection of the best sweet sorghum that can still be part of future research. Appropriate and sustainable agricultural practices must be improved, eg. to ensure genotypes to be optimally adapted to the various soil types and to be viable and optimally productive. This can be done through breeding programmes that will allow for the best genotypes to be put forward to the industry in South Africa. These programmes and the production of bio-ethanol must be economical viable and are depending on support and funding. Only a legislated market will get investors involve in the concept of producing bio-ethanol aimed at the blending thereof with fossil fuels.

It is evident through the results in this study obtained from the genotype evaluation trials and the N application trials, that sweet sorghum is a very suitable crop to be used as a renewable resource for bio-ethanol production.

5.2 References

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APPENDICES

Appendix A. Additional crops yield data

A1. Best performing genotypes in three locations during 2011/2012

Bethlehem				Potchefstroom				Rustenburg			
genotype	**mass t ha^{-1}**	**Brix %**	**juice t ha^{-1}**	**genotype**	**mass t ha^{-1}**	**Brix %**	**Juice t ha^{-1}**	**genotype**	**mass t ha^{-1}**	**Brix %**	**juice t ha^{-1}**
HG	48.64	14.76	9.13	**ss 007**	118.43	16.5	30.37	**HG**	41.82	16.59	6.43
ss 017	38.01	14.54	4.92	**ss 017**	112.90	18.28	33.31	**p 506**	36.45	17.46	3.9
ss 016	36.58	16.9	6.31	**L001**	103.14	18.6	29.22	**p 895**	31.84	13.97	0.93
ss 120	35.08	19.85	4.54	**p 304**	97.86	18.88	24.45	**p 304**	30.11	14.52	2.98
ss 019	34.80	14.83	4.26	**ss 003**	95.87	17.1	23.01	**ss 120**	29.06	17.1	3.2
p 175	33.48	16.67	5.75	**ss 008**	89.70	17.28	20.22	**p 175**	28.90	19.1	1.7
ss 007	32.31	18.26	5.64	**sswd**	86.37	15.42	22.34	**ss 008**	24.29	19.26	2.56
p 197	28.50	15.82	2.93	**BMR**	75.90	14.23	15.78	**ss 003**	24.22	18.8	1.98
								ss 007	22.94	21.32	4.48

A 2. Best performing genotypes in three locations during 2012/2013

Bethlehem				Potchefstroom				Rustenburg			
genotype	**mass t ha^{-1}**	**Brix (%)**	**juice t ha^{-1}**	**genotype**	**mass t ha^{-1}**	**Brix %**	**juice t ha^{-1}**	**genotype**	**mass t ha^{-1}**	**Brix %**	**juice t ha^{-1}**
ss 003	51.49	16.31	14.73	**BMR**	57.54	17.96	12.76	**ss 003**	103.44	16.87	25.05
HG	46.62	14.63	14.86	**ss 120**	50.95	15.07	10.03	**BMR**	98.88	16.5	21.54
ss 120	46.24	16.82	14.68	**ss 007**	46.34	19.44	10.35	**HG**	97.06	17.61	21.72
ss 56	41.33	12.95	10.3	**supa**	38.84	17.49	9.76	**ss 120**	87.84	18.46	21.29
ss 081	38.76	13.87	10.2	**ss 016**	42.04	16.64	8.17	**p 220**	84.28	13.43	14.45
ss 016	37.99	13.74	9.97	**p 868**	40.58	17.99	9.3	**SK**	82.67	18.6	20.06
ss 008	35.76	15.68	10.73	**ss 008**	40.37	18.84	8.48	**ss 008**	80.48	13.98	21.88
ss 007	35.04	18.38	12.3	**ss 003**	39.35	17.27	9.17	**ss 001**	77.56	15.14	22.3
supa	33.17	13.77	11.5	**ss 56**	38.76	18.03	9.09	**ss 017**	77.24	13.73	21.08
ss 001	28.82	16.23	10.17					**p 868**	72.09	16.99	16.11
p 868	27.48	14.33	10.99					**supa**	70.65	16.21	18.7

A3. Best performing genotypes in three locations during 2013/14

Bethlehem				Potchefstroom				Rustenburg			
genotype	**mass t ha^{-1}**	**Brix %**	**juice t ha^{-1}**	**genotype**	**mass t ha^{-1}**	**Brix %**	**juice t ha^{-1}**	**genotype**	**mass t ha^{-1}**	**Brix (%)**	**juice t ha^{-1}**
ss 27	26.13	14.97	6.28	**HG**	122.16	14.14	26.86	**p 893**	58.78	19.78	10.06
ss 120	22.42	13.86	2.56	**ss 120**	95.51	15.5	25.68	**supa**	57.38	20.84	11.56
HG	21.26	13.07	4.1	**ss 003**	82.79	14.99	15.39	**ss 003**	56.09	18.83	9.78
SK	20.62	16.33	3.46	**p 893**	80.24	15	12.39	**p 197**	52.60	19.32	9.07
p 893	19.47	15.87	2.69	**p 868**	80.19	16.26	17.38	**E3**	51.75	19.47	10.67
ss 017	17.55	15.04	2.82	**ss 007**	79.90	16.44	15.38	**SK**	49.58	19.04	9.94
ss 007	16.78	16.72	2.05	**ss 008**	79.41	17.03	12.65	**p 249**	48.81	17.27	8.71
ss 003	16.14	15.63	2.82	**ss 017**	79.06	15.11	12.14	**p 225**	48.81	19.61	10.33
ss 008	15.75	17.1	3.5	**ss 001**	68.23	17.26	13.04	**p 895**	47.66	15.29	9.28
p 868	14.35	14.47	3.71	**SK**	65.06	15.82	11.6	**ss 016**	47.02	17.96	7.22
				ss 016	63.25	16.5	10.08	**ss 007**	46.08	19.87	8.89
				ss 081	58.29	15.02	15.28	**p 888**	43.70	17.88	9.39
				p 888	55.75	14.56	10.96				

Appendix B. Additional biomass yield, juice yield and Brix(%) data

B 1. Best biomass yield (t/ha) across locations and years

	Rustenburg			**Potchefstroom**			**Bethlehem**		
Genotype	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
SS 001	15,74	77,56				68,23		28,82	10,89
SS 003	24,22	103,44	98,78	95,87	39,35	82,79		51,49	16,14
SS 007	22,94		84,89	118,43	46,34	79,9	32,31	35,04	16,78
SS 008	24,29	80,48	58,78	89,7	40,37	79,41		35,76	15,75
SS 016	14,78		79,22	60,99	42,04	63,25	36,58	37,99	11,02
SS 017	20,64	77,24		112,9	36,45	79,06	38,01		17,55
SS 120	29,06	87,84	81,78		50,95	95,51	35,08	46,24	22,42
HG	41,82	97,06	54,94		38,4	122,16	48,64	46,62	21,26
SK	16,86	82,67	95,94			65,06			20,62
SUPA			111,56		38,84			33,17	13,32

B 2. Best juice yield (t/ha) across locations and years

	Rustenburg			Potchefstroom			Bethlehem		
Genotype	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
SS 001	1.34	22.3				13.04		10.17	1.28
SS 003	1.98	25.05	56.09	19.89	9.17	15.39		14.73	2.82
SS 007	4.48	19.76	46.08	26.26	10.35	15.38		12.3	2.05
SS 008	2.56	21.88	41.78	17.49	8.48	12.65		10.73	3.5
SS 016	0.86		47.02		8.17	10.08		9.97	1.67
SS 017	2.88	21.08		28.8		12.14	4.92	8.43	
SS 120	3.2	21.29	39.74	14.77	10.03	25.68	4.54	14.68	2.56
HG	6.43	21.72	30.03	19.17	8.97	26.86	9.13	14.86	4.1
SK	1.15	20.06	49.58			11.6		9.57	3.46
SUPA		18.7	57.38		9.76			11.5	1.28

B 3. Highest Brix index (wt.%) measures across locations and years

	Rustenburg			Potchefstroom			Bethlehem		
Genotype	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
SS 001	18.04	15.14			17.28	17.26	17.81	16.23	14.18
SS 003	18.8	16.87	18.83		17.27	14.99	16.87	16.31	15.63
SS 007	21.32	20.02	19.87	16.5	19.44	16.44	18.26	18.38	16.72
SS 008	19.26	13.98	17.79	17.28	18.84	17.03	17.41	15.68	17.1
SS 016	16.64	18.36	17.96	16.2	16.64	16.5	16.90	13.74	14.61
SS 017	14.18	13.73		18.28	15	15.11	14.54	15.36	15.04
SS 120	17.1	18.46	19.46	16.56	15.07	15.5	19.58	16.82	13.86
HG	16.59	17.61	18.06			14.14	14.76	14.63	13.07
SK	18.38	18.46	19.04	17.1	17.5	15.82	18.18		16.33
SUPA		16.21	20.84		17.49	16.42		13.77	13.32

B 4. Biomass yield per mm rain (kg/ha/mm)

	Rustenburg			Potchefstroom			Bethlehem		
Genotype	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
SS 001	30,38	172,32	0,00	0,00	0,00	108,84	0,00	41,21	15,27
SS 003	46,74	229,82	127,47	147,90	51,85	132,07	0,00	73,63	22,63
SS 007	44,27	0,00	109,54	182,70	61,06	127,46	67,75	50,11	23,53
SS 008	46,88	178,81	75,85	138,38	53,19	126,68	0,00	51,14	22,08
SS 016	28,52	0,00	102,23	94,09	55,39	100,90	76,68	54,33	15,45
SS 017	39,83	171,61	0,00	174,17	48,03	126,12	79,69	0,00	24,61
SS 120	56,08	195,16	105,53	0,00	67,13	152,36	73,55	66,13	31,43
HG	80,71	215,65	70,89	0,00	50,60	194,87	101,97	66,67	29,81
SK	32,54	183,67	123,80	0,00	0,00	103,79	0,00	0,00	28,91
SUPA	0,00	0,00	143,96	0,00	51,18	0,00	0,00	47,44	18,68

B 5. Biomass yield per HU

	Rustenburg			Potchefstroom			Bethlehem		
Genotype	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
SS 001	1,59	7,66	0,00	0,00	0,00	9,52	0,00	7,28	2,72
SS 003	2,45	10,21	10,35	12,97	5,23	11,55	0,00	13,00	4,04
SS 007	2,32	0,00	8,90	16,03	6,16	11,15	7,79	8,85	4,20
SS 008	2,46	7,94	6,16	12,14	5,37	11,08	0,00	9,03	3,94
SS 016	1,50	0,00	8,30	8,25	5,59	8,83	8,81	9,59	2,76
SS 017	2,09	7,62	0,00	15,28	4,85	11,03	9,16	0,00	4,39
SS 120	2,94	8,67	8,57	0,00	6,78	13,33	8,45	11,68	5,61
HG	4,24	9,58	5,76	0,00	5,11	17,05	11,72	11,77	5,32
SK	1,71	8,16	10,06	0,00	0,00	9,08	0,00	0,00	5,16
SUPA	0,00	0,00	11,69	0,00	5,16	0,00	0,00	8,38	3,33

B 6 Biomass yield (kg/ha/mm/ °C)

	Rustenburg			Potchefstroom			Bethlehem		
Genotype	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
SS 001	3.08	17.01	0.00	0.00	0.00	14.95	0.00	10.41	3.82
SS 003	4.74	22.69	13.36	20.01	6.89	18.14	0.00	18.59	5.66
SS 007	4.49	0.00	11.48	24.72	8.12	17.51	16.32	12.65	5.88
SS 008	4.75	17.65	7.95	18.73	7.07	17.40	0.00	12.91	5.52
SS 016	2.89	0.00	10.72	12.73	7.37	13.86	18.48	13.72	3.86
SS 017	4.04	16.94	0.00	23.57	6.39	17.32	19.20	0.00	6.15
SS 120	5.68	19.27	11.06	0.00	8.93	20.93	17.72	16.70	7.86
HG	8.18	21.29	7.43	0.00	6.73	26.77	24.57	16.84	7.45
SK	3.30	18.13	12.98	0.00	0.00	14.26	0.00	0.00	7.23
SUPA	0.00	0.00	15.09	0.00	6.81	0.00	0.00	11.98	4.67

B 7 Juice yield (kg/ha/mm/ °C)

	Rustenburg			Potchefstroom			Bethlehem		
	2012	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
SS 001	3.08	4.89	0.00	0.00	0.00	2.86	0.00	3.67	0.45
SS 003	4.74	5.49	7.59	4.22	1.61	3.37	0.00	5.32	0.99
SS 007	4.49	4.33	6.23	4.80	1.81	3.37	0.00	4.44	0.72
SS 008	4.75	4.80	5.65	4.63	1.49	2.77	0.00	3.87	1.23
SS 016	2.89	0.00	6.36	3.29	1.43	2.21	0.00	3.60	0.59
SS 017	4.04	4.62	0.00	3.57	0.00	2.66	2.49	3.04	0.00
SS 120	5.68	4.67	5.38	0.00	1.76	5.63	2.29	5.30	0.90
HG	8.18	4.76	4.06	0.00	1.57	5.89	4.61	5.37	1.44
SK	3.30	4.40	6.71	0.00	0.00	2.54	0.00	3.46	1.21
SUPA	0.00	4.10	7.76	0.00	1.71	0.00	0.00	4.15	0.45

B 8.1 Total sugar potential from juice, ton sugar/ha across locations and years

	Rustenburg			Potchefstroom			Bethlehem		
Genotype	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
SS 001	0.24	3.38	n/a	n/a	n/a	2.25	n/a	1.65	0.18
SS 003	0.37	4.23	10.56	n/a	1.58	2.31	n/a	2.40	0.44
SS 007	0.96	3.96	9.16	5.01	2.01	2.53	n/a	2.26	0.34
SS 008	0.49	3.06	7.43	3.49	1.60	2.15	n/a	1.68	0.60
SS 016	0.14	n/a	8.44	n/a	1.36	1.66	n/a	1.37	0.24
SS 017	0.41	2.89	n/a	6.09	n/a	1.83	0.72	1.29	n/a
SS 120	0.55	3.93	7.73	2.83	1.51	3.98	0.89	2.47	0.35
HG	1.07	3.82	5.42	n/a	n/a	3.80	1.35	2.17	0.54
SK	0.21	3.70	9.44	n/a	n/a	1.84	n/a	n/a	0.57
SUPA	n/a	3.03	11.96	n/a	1.71	n/a	n/a	1.58	0.17

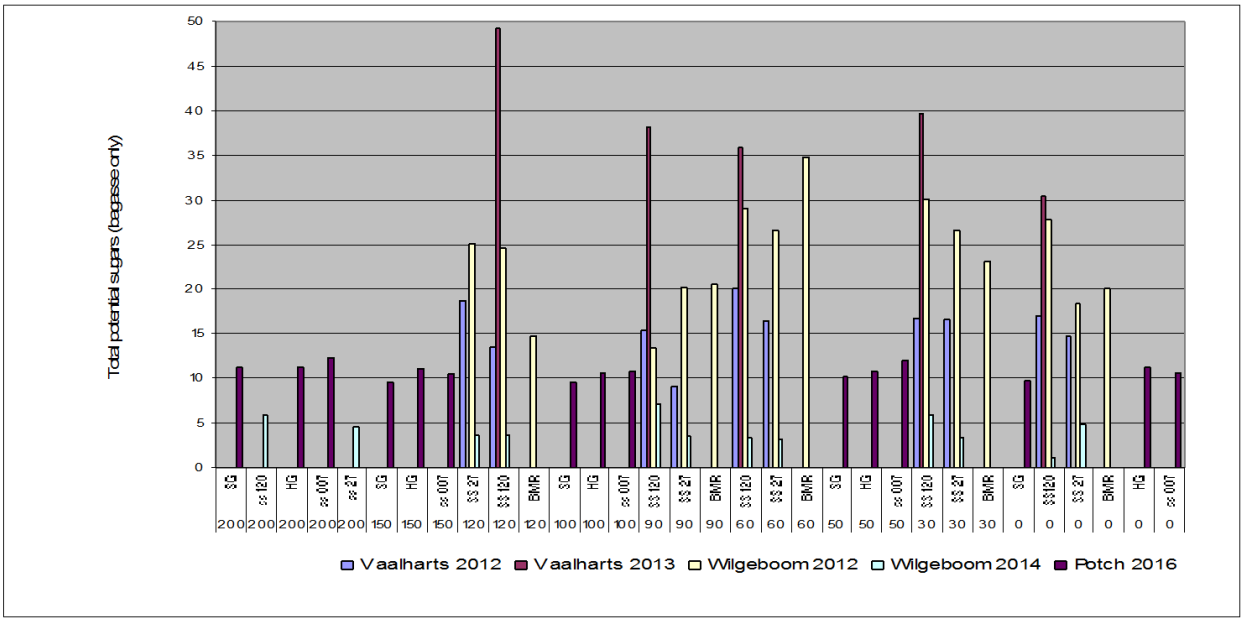
B 8.2 Total sugar potential from bagasse, ton sugar/ha across locations and years

	Rustenburg			Potchefstroom			Bethlehem		
Genotype	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
SS 001	8.13	40.07	#VALUE!	#VALUE!	#VALUE!	35.25	#VALUE!	14.89	5.63
SS 003	12.51	53.44	51.03	49.53	20.33	42.77	#VALUE!	26.60	8.34
SS 007	11.85	#VALUE!	43.85	61.18	23.94	41.28	16.69	18.10	8.67
SS 008	12.55	41.58	30.37	46.34	20.86	41.02	#VALUE!	18.47	8.14
SS 016	7.64	#VALUE!	40.93	31.51	21.72	32.67	18.89	19.63	5.69
SS 017	10.66	39.90	#VALUE!	58.32	18.83	40.84	19.64	#VALUE!	9.07
SS 120	15.01	45.38	42.25	#VALUE!	26.32	49.34	18.12	23.89	11.58
HG	21.60	50.14	28.38	#VALUE!	19.84	63.11	25.13	24.08	10.98
SK	8.71	42.71	49.56	#VALUE!	#VALUE!	33.61	#VALUE!	#VALUE!	10.65
SUPA	#VALUE!	#VALUE!	57.63	#VALUE!	20.06	#VALUE!	#VALUE!	17.14	6.88

B 9. Total potential sugars – bagasse only

		Vaalharts		Wilgeboom		Potch
N appl kg ha^{-1}	**genotype**	**2012**	**2013**	**2012**	**2014**	**2016**
200	**SG**	0.00	0.00	0.00	0.00	11.23
200	**ss 120**	0.00	0.00	0.00	6.00	0.00
200	**HG**	0.00	0.00	0.00	0.00	11.32
200	**ss 007**	0.00	0.00	0.00	0.00	12.37
200	**ss 27**	0.00	0.00	0.00	4.57	0.00
150	**SG**	0.00	0.00	0.00	0.00	9.56
150	**HG**	0.00	0.00	0.00	0.00	11.08
150	**ss 007**	0.00	0.00	0.00	0.00	10.45
120	**SS 27**	18.65	0.00	25.13	3.65	0.00
120	**SS 120**	13.49	49.23	24.64	3.59	0.00
120	**BMR**	0.00	0.00	14.77	0.00	0.00
100	**SG**	0.00	0.00	0.00	0.00	9.60
100	**HG**	0.00	0.00	0.00	0.00	10.60
100	**ss 007**	0.00	0.00	0.00	0.00	10.81
90	**SS 120**	15.30	38.14	13.35	7.22	0.00
90	**SS 27**	9.05	0.00	20.22	3.51	0.00
90	**BMR**	0.00	0.00	20.50	0.00	0.00
60	**SS 120**	20.01	35.91	29.01	3.39	0.00
60	**SS 27**	16.34	0.00	26.67	3.13	0.00
60	**BMR**	0.00	0.00	34.82	0.00	0.00
50	**SG**	0.00	0.00	0.00	0.00	10.18
50	**HG**	0.00	0.00	0.00	0.00	10.74
50	**ss 007**	0.00	0.00	0.00	0.00	11.94
30	**SS 120**	16.79	39.70	30.16	5.92	0.00
30	**SS 27**	16.49	0.00	26.59	3.31	0.00
30	**BMR**	0.00	0.00	23.07	0.00	0.00
0	**SG**	0.00	0.00	0.00	0.00	9.73
0	**SS120**	17.06	30.32	27.78	1.03	0.00
0	**SS 27**	14.70	0.00	18.37	4.79	0.00
0	**BMR**	0.00	0.00	20.07	0.00	0.00
0	**HG**	0.00	0.00	0.00	0.00	11.27
0	**ss 007**	0.00	0.00	0.00	0.00	10.58

B 10. Total potential sugars – bagasse only

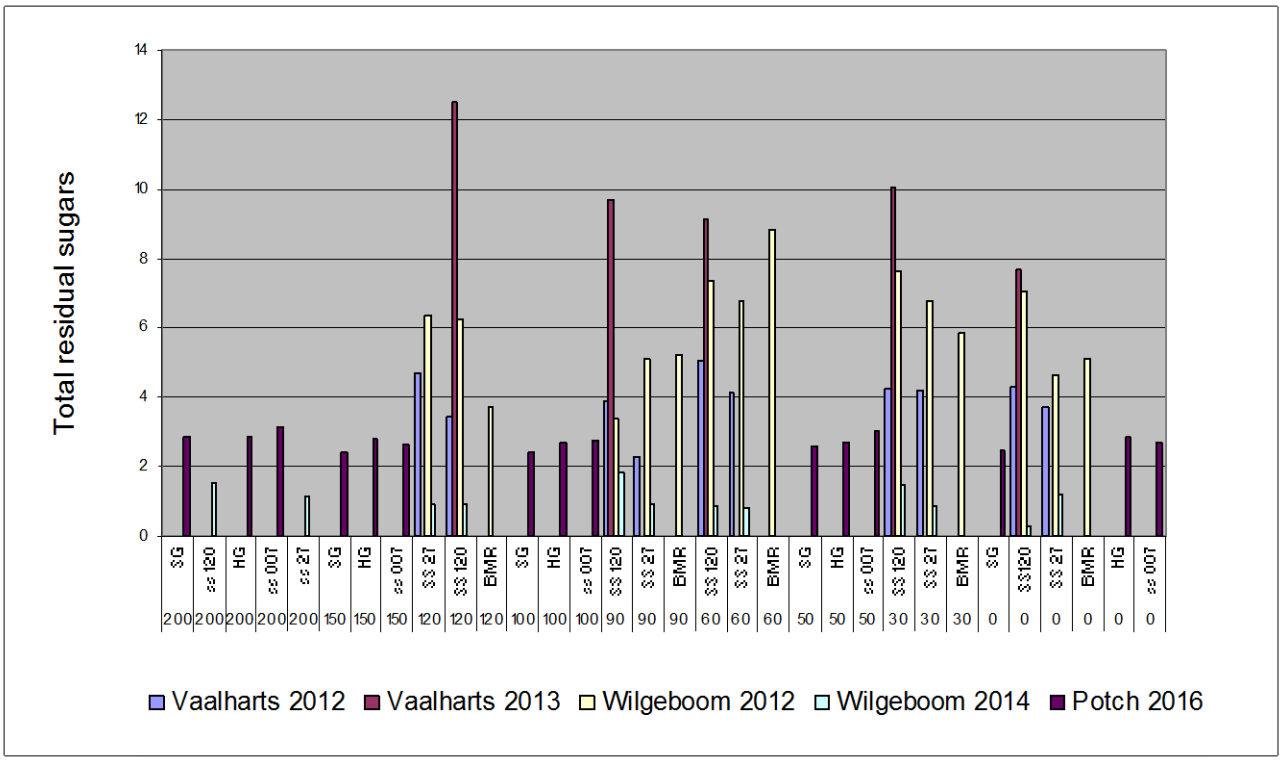


B 11. Table residual sugars

		Vaalharts		Wilgeboom		Potch
N appl kg ha^{-1}	**genotype**	**2012**	**2013**	**2012**	**2014**	**2016**
200	**SG**	0.00	0.00	0.00	0.00	2.85
200	**ss 120**	0.00	0.00	0.00	1.52	0.00
200	**HG**	0.00	0.00	0.00	0.00	2.87
200	**ss 007**	0.00	0.00	0.00	0.00	3.14
200	**ss 27**	0.00	0.00	0.00	1.16	0.00
150	**SG**	0.00	0.00	0.00	0.00	2.42
150	**HG**	0.00	0.00	0.00	0.00	2.81
150	**ss 007**	0.00	0.00	0.00	0.00	2.65
120	**SS 27**	4.73	0.00	6.37	0.92	0.00
120	**SS 120**	3.42	12.48	6.25	0.91	0.00
120	**BMR**	0.00	0.00	3.75	0.00	0.00
100	**SG**	0.00	0.00	0.00	0.00	2.43
100	**HG**	0.00	0.00	0.00	0.00	2.69
100	**ss 007**	0.00	0.00	0.00	0.00	2.74
90	**SS 120**	3.88	9.67	3.38	1.83	0.00
90	**SS 27**	2.30	0.00	5.13	0.89	0.00
90	**BMR**	0.00	0.00	5.20	0.00	0.00
60	**SS 120**	5.07	9.11	7.36	0.86	0.00
60	**SS 27**	4.14	0.00	6.76	0.79	0.00
60	**BMR**	0.00	0.00	8.83	0.00	0.00
50	**SG**	0.00	0.00	0.00	0.00	2.58
50	**HG**	0.00	0.00	0.00	0.00	2.72
50	**ss 007**	0.00	0.00	0.00	0.00	3.03
30	**SS 120**	4.26	10.07	7.65	1.50	0.00
30	**SS 27**	4.18	0.00	6.74	0.84	0.00
30	**BMR**	0.00	0.00	5.85	0.00	0.00
0	**SG**	0.00	0.00	0.00	0.00	2.47

0	**SS120**	4.33	7.69	7.05	0.26	0.00
0	**SS 27**	3.73	0.00	4.66	1.22	0.00
0	**BMR**	0.00	0.00	5.09	0.00	0.00
0	**HG**	0.00	0.00	0.00	0.00	2.86
0	**ss 007**	0.00	0.00	0.00	0.00	2.68

B 12. Total residual sugars



Appendix C. Additional bagasse yield, juice yield, sugar yield and potential ethanol production data

C 1. Total ethanol potential from bagasse (assume 54% glucose and 46% xylose) across locations and years

	Rustenburg			Potchefstroom			Bethlehem		
Genotype	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
SS 001	9.16	45.14	#VALUE!	#VALUE!	#VALUE!	39.71	#VALUE!	16.77	6.34
SS 003	14.10	60.21	57.49	55.80	22.90	48.19	#VALUE!	29.97	9.39
SS 007	13.35	#VALUE!	49.41	68.93	26.97	46.50	18.81	20.39	9.77
SS 008	14.14	46.84	34.21	52.21	23.50	46.22	#VALUE!	20.81	9.17
SS 016	8.60	#VALUE!	46.11	35.50	24.47	36.81	21.29	22.11	6.41
SS 017	12.01	44.96	#VALUE!	65.71	21.22	46.02	22.13	#VALUE!	10.21
SS 120	16.91	51.13	47.60	#VALUE!	29.65	55.59	20.42	26.91	13.05
HG	24.34	56.49	31.98	#VALUE!	22.35	71.10	28.31	27.13	12.37
SK	9.81	48.12	55.84	#VALUE!	#VALUE!	37.87	#VALUE!	#VALUE!	12.00
SUPA	#VALUE!	#VALUE!	64.93	#VALUE!	22.61	#VALUE!	#VALUE!	19.31	7.75

C 2. Ethanol potential from juice (use glucose as model)

	Rustenburg			Potchefstroom			Bethlehem		
Genotype	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
SS 001	0.16	2.18	#VALUE!	#VALUE!	#VALUE!	1.46	#VALUE!	1.07	0.12
SS 003	0.24	2.73	6.83	#VALUE!	1.02	1.49	#VALUE!	1.55	0.28
SS 007	0.62	2.56	5.92	3.24	1.30	1.63	#VALUE!	1.46	0.22
SS 008	0.32	1.98	4.81	2.26	1.03	1.39	#VALUE!	1.09	0.39
SS 016	0.09	#VALUE	5.46	#VALUE!	0.88	1.08	#VALUE!	0.89	0.16
SS 017	0.26	1.87	#VALUE!	3.94	#VALUE!	1.19	0.46	0.84	#VALU
SS 120	0.35	2.54	5.00	1.83	0.98	2.57	0.57	1.60	0.23
HG	0.69	2.47	3.51	#VALUE!	#VALUE!	2.46	0.87	1.41	0.35
SK	0.14	2.39	6.10	#VALUE!	#VALUE!	1.19	#VALUE!	#VALU	0.37
SUPA	#VALU	1.96	7.73	#VALUE!	1.10	#VALUE!	#VALUE!	1.02	0.11

C 3. Total ethanol potential from juice, bagasse and residual sugars

	Rustenburg			Potchefstroom			Bethlehem		
Genotype	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**	**2012**	**2013**	**2014**
SS 001	10.65	53.89	#VALUE!	#VALUE!	#VALUE!	46.95	#VALUE!	20.28	7.38
SS 003	16.39	71.70	72.69	#VALUE!	27.26	56.69	#VALUE!	35.88	11.05
SS 007	15.91	#VALUE!	62.52	82.20	32.20	54.91	#VALUE!	24.82	11.41
SS 008	16.51	55.64	44.00	62.06	27.95	54.34	#VALUE!	24.93	10.89
SS 016	9.95	#VALUE!	58.28	#VALUE!	28.91	43.25	#VALUE!	26.21	7.51
SS 017	14.03	53.37	#VALUE!	79.21	#VALUE!	53.90	25.81	#VALUE!	#VALUE!
SS 120	19.73	61.11	59.52	#VALUE!	34.95	66.25	23.96	32.43	15.18
HG	28.57	67.19	40.14	#VALUE!	#VALUE!	83.90	33.30	32.49	14.52
SK	11.38	57.51	70.07	#VALUE!	#VALUE!	44.56	#VALUE!	#VALUE!	14.11
SUPA	#VALUE!	#VALUE!	82.11	#VALUE!	27.00	#VALUE!	#VALUE!	23.14	8.99

C 4. Ethanol potential from juice (use glucose as model)

		Vaalharts		Wilgeboom		Potch
N appl kg ha^{-1}	**genotype**	**2012**	**2013**	**2012**	**2014**	**2016**
200	**SG**	0.00	0.00	0.00	0.00	0.83
200	**ss 120**	0.00	0.00	0.00	0.44	0.00
200	**HG**	0.00	0.00	0.00	0.00	0.68
200	**ss 007**	0.00	0.00	0.00	0.00	1.33
200	**ss 27**	0.00	0.00	0.00	0.21	0.00
150	**SG**	0.00	0.00	0.00	0.00	0.88
150	**HG**	0.00	0.00	0.00	0.00	0.81
150	**ss 007**	0.00	0.00	0.00	0.00	0.78
120	**SS 27**	6.28	0.00	5.30	0.11	0.00
120	**SS 120**	3.97	3.17	3.76	0.12	0.00
120	**BMR**	0.00	0.00	2.02	0.00	0.00
100	**SG**	0.00	0.00	0.00	0.00	0.54
100	**HG**	0.00	0.00	0.00	0.00	1.02
100	**ss 007**	0.00	0.00	0.00	0.00	0.97
90	**SS 120**	4.35	1.85	1.68	0.30	0.00
90	**SS 27**	2.98	0.00	4.12	0.16	0.00
90	**BMR**	0.00	0.00	2.91	0.00	0.00
60	**SS 120**	6.14	1.98	5.43	0.11	0.00
60	**SS 27**	4.86	0.00	4.98	0.10	0.00
60	**BMR**	0.00	0.00	5.75	0.00	0.00
50	**SG**	0.00	0.00	0.00	0.00	0.95
50	**HG**	0.00	0.00	0.00	0.00	0.81
50	**ss 007**	0.00	0.00	0.00	0.00	1.34
30	**SS 120**	4.79	1.58	4.50	0.24	0.00
30	**SS 27**	4.98	0.00	4.19	0.09	0.00
30	**BMR**	0.00	0.00	3.66	0.00	0.00
0	**SG**	0.00	0.00	0.00	0.00	0.51
0	**SS120**	4.40	1.72	4.01	0.04	0.00
0	**SS 27**	4.12	0.00	3.29	0.08	0.00
0	**BMR**	0.00	0.00	2.72	0.00	0.00
0	**HG**	0.00	0.00	0.00	0.00	0.53
0	**ss 007**	0.00	0.00	0.00	0.00	0.55

C 5. Total ethanol potential from juice, bagasse and residual sugars

		Vaalharts		Wilgeboom		Potch
N appl kg ha^{-1}	**genotype**	**2012**	**2013**	**2012**	**2014**	**2016**
200	**SG**	0.00	0.00	0.00	0.00	15.32
200	**ss 120**	0.00	0.00	0.00	8.19	0.00
200	**HG**	0.00	0.00	0.00	0.00	15.29
200	**ss 007**	0.00	0.00	0.00	0.00	17.29
200	**ss 27**	0.00	0.00	0.00	6.10	0.00
150	**SG**	0.00	0.00	0.00	0.00	13.21
150	**HG**	0.00	0.00	0.00	0.00	15.11
150	**ss 007**	0.00	0.00	0.00	0.00	14.26
120	**SS 27**	30.35	0.00	37.73	4.81	0.00
120	**SS 120**	21.38	66.71	35.56	4.75	0.00
120	**BMR**	0.00	0.00	21.08	0.00	0.00
100	**SG**	0.00	0.00	0.00	0.00	12.93
100	**HG**	0.00	0.00	0.00	0.00	14.70
100	**ss 007**	0.00	0.00	0.00	0.00	14.92
90	**SS 120**	24.09	51.07	18.90	9.61	0.00
90	**SS 27**	14.66	0.00	30.21	4.69	0.00
90	**BMR**	0.00	0.00	29.36	0.00	0.00
60	**SS 120**	31.97	48.32	42.86	4.49	0.00
60	**SS 27**	25.95	0.00	39.40	4.14	0.00
60	**BMR**	0.00	0.00	50.69	0.00	0.00
50	**SG**	0.00	0.00	0.00	0.00	14.09
50	**HG**	0.00	0.00	0.00	0.00	14.67
50	**ss 007**	0.00	0.00	0.00	0.00	16.75
30	**SS 120**	26.46	52.81	43.43	7.88	0.00
30	**SS 27**	26.26	0.00	38.52	4.36	0.00
30	**BMR**	0.00	0.00	33.43	0.00	0.00
0	**SG**	0.00	0.00	0.00	0.00	13.07
0	**SS120**	26.42	40.85	39.87	1.37	0.00
0	**SS 27**	23.10	0.00	27.01	6.27	0.00
0	**BMR**	0.00	0.00	28.62	0.00	0.00
0	**HG**	0.00	0.00	0.00	0.00	15.08
0	**ss 007**	0.00	0.00	0.00	0.00	14.20

C 6. Total ethanol potential from bagasse (assume 54% glucose and 46% xylose)

		Vaalharts		Wilgeboom		Potch
N appl kg ha^{-1}	**genotype**	**2012**	**2013**	**2012**	**2014**	**2016**
200	**SG**	0.00	0.00	0.00	0.00	12.65
200	**ss 120**	0.00	0.00	0.00	6.76	0.00
200	**HG**	0.00	0.00	0.00	0.00	12.75
200	**ss 007**	0.00	0.00	0.00	0.00	13.93
200	**ss 27**	0.00	0.00	0.00	5.15	0.00
150	**SG**	0.00	0.00	0.00	0.00	10.77
150	**HG**	0.00	0.00	0.00	0.00	12.48
150	**ss 007**	0.00	0.00	0.00	0.00	11.77
120	**SS 27**	21.01	0.00	28.31	4.11	0.00
120	**SS 120**	15.20	55.46	27.77	4.05	0.00
120	**BMR**	0.00	0.00	16.64	0.00	0.00
100	**SG**	0.00	0.00	0.00	0.00	10.81
100	**HG**	0.00	0.00	0.00	0.00	11.94
100	**ss 007**	0.00	0.00	0.00	0.00	12.18
90	**SS 120**	17.24	42.97	15.04	8.13	0.00
90	**SS 27**	10.20	0.00	22.78	3.95	0.00
90	**BMR**	0.00	0.00	23.09	0.00	0.00
60	**SS 120**	22.55	40.46	32.68	3.82	0.00
60	**SS 27**	18.41	0.00	30.05	3.52	0.00
60	**BMR**	0.00	0.00	39.23	0.00	0.00
50	**SG**	0.00	0.00	0.00	0.00	11.47
50	**HG**	0.00	0.00	0.00	0.00	12.10
50	**ss 007**	0.00	0.00	0.00	0.00	13.45
30	**SS 120**	18.91	44.73	33.99	6.66	0.00
30	**SS 27**	18.58	0.00	29.96	3.73	0.00
30	**BMR**	0.00	0.00	25.99	0.00	0.00
0	**SG**	0.00	0.00	0.00	0.00	10.97
0	**SS120**	19.22	34.16	31.30	1.16	0.00
0	**SS 27**	16.57	0.00	20.70	5.40	0.00
0	**BMR**	0.00	0.00	22.61	0.00	0.00
0	**HG**	0.00	0.00	0.00	0.00	12.69
0	**ss 007**	0.00	0.00	0.00	0.00	11.92

C 7. Total ethanol potential from residual sugars (assume glucose)

		Vaalharts		Wilgeboom		Potch
N appl kg ha^{-1}	**genotype**	**2012**	**2013**	**2012**	**2014**	**2016**
200	**SG**	0.00	0.00	0.00	0.00	1.84
200	**ss 120**	0.00	0.00	0.00	0.98	0.00
200	**HG**	0.00	0.00	0.00	0.00	1.86
200	**ss 007**	0.00	0.00	0.00	0.00	2.03
200	**ss 27**	0.00	0.00	0.00	0.75	0.00
150	**SG**	0.00	0.00	0.00	0.00	1.57
150	**HG**	0.00	0.00	0.00	0.00	1.82
150	**ss 007**	0.00	0.00	0.00	0.00	1.71
120	**SS 27**	3.06	0.00	4.12	0.60	0.00
120	**SS 120**	2.21	8.07	4.04	0.59	0.00
120	**BMR**	0.00	0.00	2.42	0.00	0.00
100	**SG**	0.00	0.00	0.00	0.00	1.57
100	**HG**	0.00	0.00	0.00	0.00	1.74
100	**ss 007**	0.00	0.00	0.00	0.00	1.77
90	**SS 120**	2.51	6.25	2.19	1.18	0.00
90	**SS 27**	1.48	0.00	3.31	0.58	0.00
90	**BMR**	0.00	0.00	3.36	0.00	0.00
60	**SS 120**	3.28	5.89	4.76	0.56	0.00
60	**SS 27**	2.68	0.00	4.37	0.51	0.00
60	**BMR**	0.00	0.00	5.71	0.00	0.00
50	**SG**	0.00	0.00	0.00	0.00	1.67
50	**HG**	0.00	0.00	0.00	0.00	1.76
50	**ss 007**	0.00	0.00	0.00	0.00	1.96
30	**SS 120**	2.75	6.51	4.95	0.97	0.00
30	**SS 27**	2.70	0.00	4.36	0.54	0.00
30	**BMR**	0.00	0.00	3.78	0.00	0.00
0	**SG**	0.00	0.00	0.00	0.00	1.60
0	**SS120**	2.80	4.97	4.56	0.17	0.00
0	**SS 27**	2.41	0.00	3.01	0.79	0.00
0	**BMR**	0.00	0.00	3.29	0.00	0.00
0	**HG**	0.00	0.00	0.00	0.00	1.85
0	**ss 007**	0.00	0.00	0.00	0.00	1.73

Appendix C 8

N appl	genotype	t/ha glucose	t/ha xylose	Residual Sugar Ethanol L/ha	glucose Ethanol L/ha	xylose Ethanol L/ha	Total Ethanol L/ha	Total juice EtoH L/ha HPLC	Total juice EtOH L/ha Brix
0	ss 007	7,30	5,39	2688,21	4713,96	4164,25	11566,42	407,8	545,74
200	ss 007	8,90	6,44	1732,68	5743,65	4969,37	12445,71	1191,3	1324,64
0	HG	8,11	7,30	1848,62	5238,47	5638,21	12725,30	447,8	532,94
200	HG	7,81	7,29	2423,40	5039,20	5626,25	13088,86	506,1	680,52
0	SG	6,41	6,30	1963,29	4135,81	4863,82	10962,92	463,9	505,28
200	SG	4,86	5,80	4489,94	3139,76	4481,98	12111,67	582,4	827,95
						ave tot EtOH ($L\ ha^{-1}$)	12150,15		

Appendix C 9

N application ($kg\ ha^{-1}$)	Genotype	6ring (g/L)	5ring (g/L)	Juice yield (L/ha)	Sugar yield from HPLC (t/ha)	Sugar yield from HPLC (kg/ha)	Juice yield (t/ha)	Sugar yield from HPLC conc (kg/ha)	6-rings from HPLC conc (t/ha)	Juice etph based on Brix (L/ha)	Juice EtOH (L/ha)
0	ss 007	62,65	62,75	4586,84	0,29	287,37	4,36	287,82	0,27	545,74	407,76
50	ss 007	131,65	95,28	9137,28	1,20	1202,92	8,68	870,60	0,83	1339,31	1448,81
100	ss 007	129,54	96,44	6370,61	0,83	825,25	6,05	614,38	0,58	970,25	1007,15
150	ss 007	76,11	75,17	6625,44	0,50	504,26	6,29	498,03	0,47	774,74	710,09
200	ss 007	56	108,43	9938,16	0,56	556,54	9,44	1077,59	1,02	1324,64	1191,35
0	HG	53,01	50,23	6133,99	0,33	325,16	5,83	308,11	0,29	532,94	447,82
50	HG	76,31	66,34	7444,52	0,57	568,09	7,07	493,87	0,47	811,16	748,08
100	HG	60,14	53,49	9282,89	0,56	558,27	8,82	496,54	0,47	1019,07	743,81
150	HG	40,55	42,54	9610,53	0,39	389,71	9,13	408,83	0,39	807,48	567,26
200	HG	62,84	57,93	5933,77	0,37	372,88	5,64	343,74	0,33	680,52	506,14
0	SG	54,22	55,31	5970,18	0,32	323,70	5,67	330,21	0,31	505,28	463,95
50	SG	35,06	51,77	9678,07	0,34	339,31	9,19	501,03	0,48	811,18	605,92
100	SG	9,09	29	6916,67	0,06	62,87	6,57	200,58	0,19	660,89	195,47
150	SG	39,68	42,18	7972,37	0,32	316,34	7,57	336,27	0,32	876,83	463,88
200	SG	53,13	62,1	7080,48	0,38	376,19	6,73	439,70	0,42	827,95	582,37

Appendix D. Additional crop data from nitrogen trials across locations and years

D 1a & 1b. The genotype performances on dififferent nitrogen levels at two locations (2011/12)

(a) VAALHARTS (2011/12)				
genotype	N kg ha^{-1}	mass t ha^{-1}	Brix %	juice t ha^{-1}
PX 174	120	25.44	22.8	2.69
PX 174	90	31.34	23.63	2.83
PX 174	60	34.27	22.55	4.18
PX 174	30	29.47	22.08	2.74
PX 174	0	27.79	19.10	2.69
ss 120	120	26.11	23.53	2.69
ss 120	90	29.62	22.70	3.55
ss 120	60	38.74	24.53	4.9
ss 120	30	32.50	22.81	3.74
ss 120	0	33.02	20.63	4.22
ss 27	120	36.10	26.93	4.37
ss 27	90	17.52	26.33	1.63
ss 27	60	31.63	23.75	4.13
ss 27	30	31.92	24.13	4.22
ss 27	0	28.46	22.38	3.5

(b) WILGEBOOM (2011/12)				
genotype	N kg ha^{-1}	mass t ha^{-1}	Brix %	juice t ha^{-1}
BMR	120	28.59	10.92	3.44
BMR	90	39.67	11.33	3.47
BMR	60	67.40	13.2	9.21
BMR	30	44.65	12.68	2.57
BMR	0	38.84	10.82	5.06
ss 120	120	47.70	12.18	3.92
ss 120	90	25.83	10.03	3.69
ss 120	60	56.15	14.95	4.23
ss 120	30	58.39	11.93	7.43
ss 120	0	53.78	11.52	4.59
ss 27	120	48.64	16.87	8.22
ss 27	90	39.13	16.28	6.62
ss 27	60	51.63	14.92	7.53
ss 27	30	51.48	12.6	6.91
ss 27	0	35.57	14.32	7.55

D 2a & 2b. The genotype performances on dififferent nitrogen levels at Vaalharts (2012/13) and Wilgeboom (2013/14)

(a) VAALHARTS (2012/13)

genotype	N kg ha^{-1}	mass t ha^{-1}	Brix %	juice t ha^{-1}
p 868	120	58.10	18.75	14.76
p 868	90	56.83	12.98	13.53
p 868	60	43.69	12.88	11.37
p 868	30	72.43	12.97	17.64
p 868	0	43.31	13.35	12.91
ss 120	120	95.30	17.43	28.17
ss 120	90	73.82	12.22	23.4
ss 120	60	69.51	14.9	20.52
ss 120	30	76.85	10.05	24.25
ss 120	0	58.70	14.13	18.87
ss 63	120	50.68	7.5	13.76
ss 63	90	43.61	5.45	11.95
ss 63	60	28.40	12.62	13.83
ss 63	30	47.23	7.5	14.56
ss 63	0	38.35	9.87	12.07

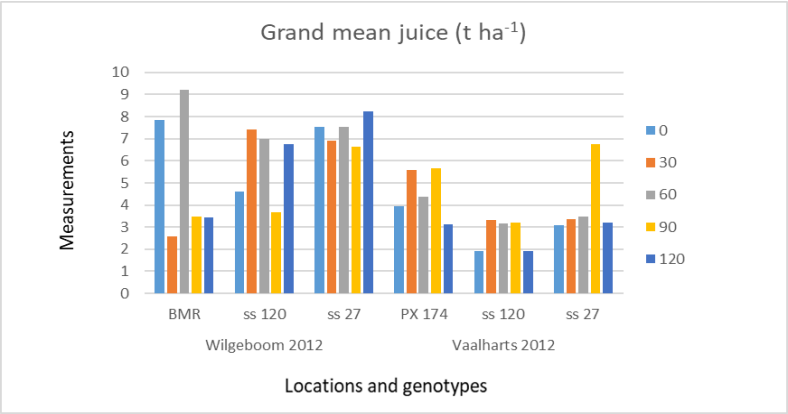
(b) WILGEBOOM (2013/14)

genotype	N kg ha^{-1}	mass t ha^{-1}	Brix %	juice t ha^{-1}
ss 27	200	8,84	16,52	1,97
ss 27	120	7,06	13,64	1,20
ss 27	90	6,79	17,46	1,40
ss 27	60	6,05	15,29	1,05
ss 27	30	6,40	16,53	0,87
ss 27	0	9,28	10,29	1,23
ss 120	200	11,62	17,21	3,95
ss 120	120	6,95	17,50	1,06
ss 120	90	13,97	14,58	3,14
ss 120	60	6,57	13,48	1,24
ss 120	30	11,45	16,90	2,24
ss 120	0	1,99	11,34	0,58
p 888	200	4,69	11,37	1,81
p 888	120	9,80	12,09	1,90
p 888	90	8,03	11,54	0,98
p 888	60	6,35	9,67	1,95
p 888	30	10,32	12,05	2,01
p 888	0	13,18	13,24	1,80

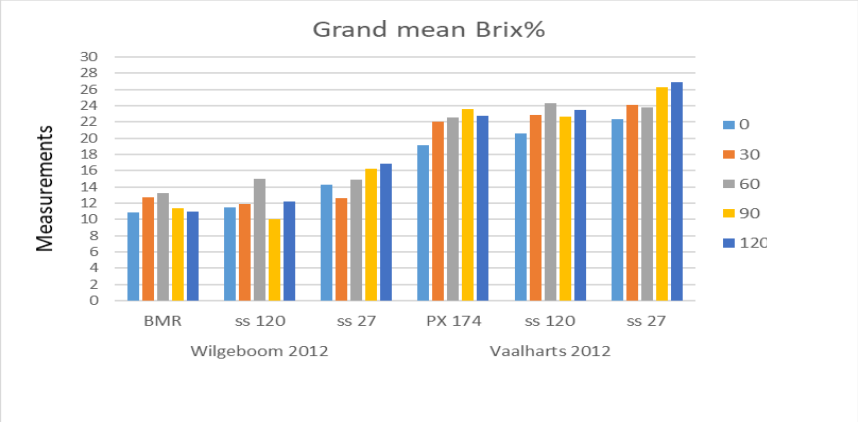
D 3. Genotypes performance at different nitrogen levels at Potchefstroom during 2016/2017

Genotype	N appl kg ha^{-1}	biomass t ha^{-1}	Brix %	juice t ha^{-1}
ss 007	200	23,94	21.73	9,44
ss 007	150	20,22	19.07	6,29
ss 007	100	20,92	24.83	6,05
ss 007	50	23,11	23.90	8,68
ss 007	0	20,48	19.40	4,36
HG	200	21,91	18.70	5,64
HG	150	21,45	13.70	9,13
HG	100	20,52	17.90	8,82
HG	50	20,79	17.77	7,07
HG	0	21,84	14.17	5,83
ss SG	200	21,73	19.07	6,73
ss SG	150	18,50	17.93	7,57
ss SG	100	18,58	12.83	6,57
ss SG	50	19,70	13.67	10,79
ss SG	0	18,84	13.80	5,67

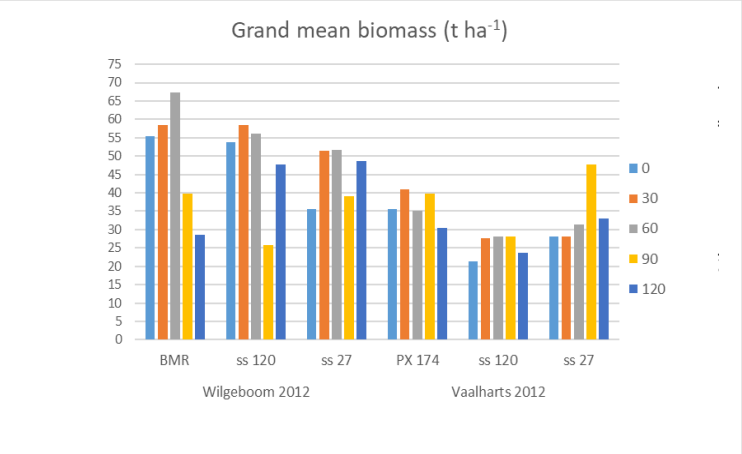
D 4. Grand mean of the juice yield at different nitrogen application levels during 2011 / 2012.



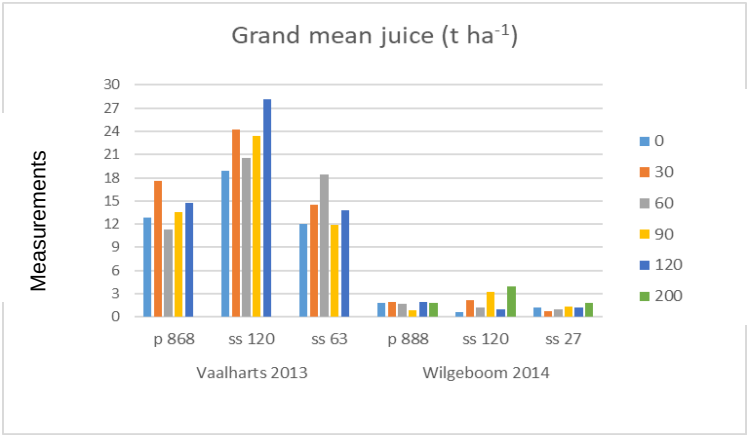
D 5. Grand mean of the Brix% at different nitrogen application levels during 2011 / 2012 at Vaalharts.



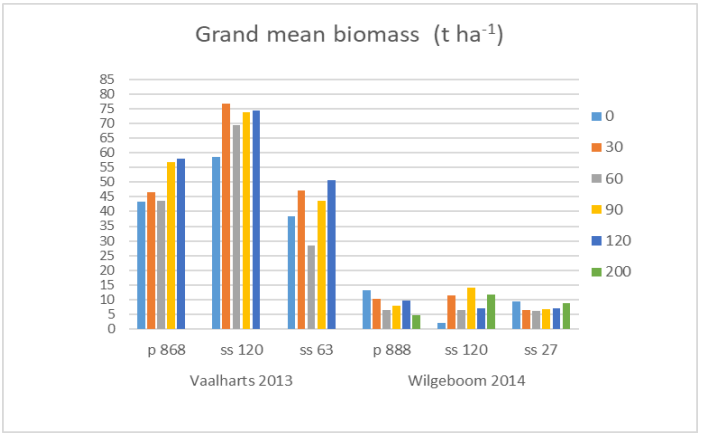
D 6. Graphical representation of the grand mean values of biomass yields at different nitrogen application levels during 2011 / 2012



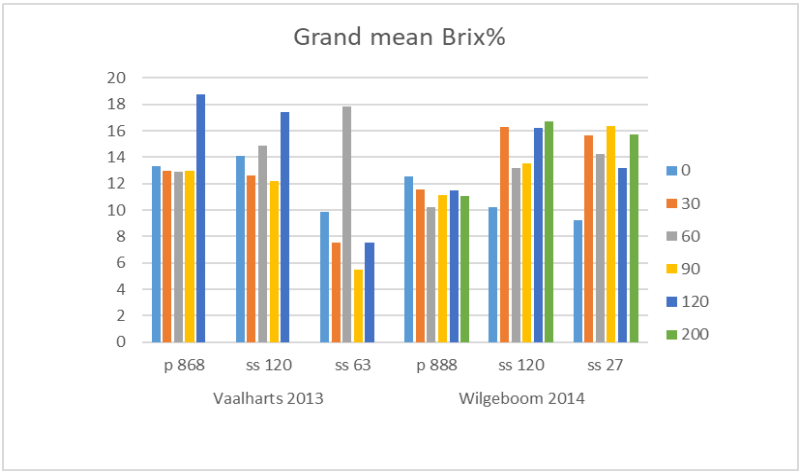
D 7. Grand mean of the juice yield at different nitrogen application levels at Vaaalharts (2013) and Wilgeboom (2014).



D 8. Grand mean of the biomass yield at different nitrogen application levels at Vaaalharts (2013) and Wilgeboom (2014).



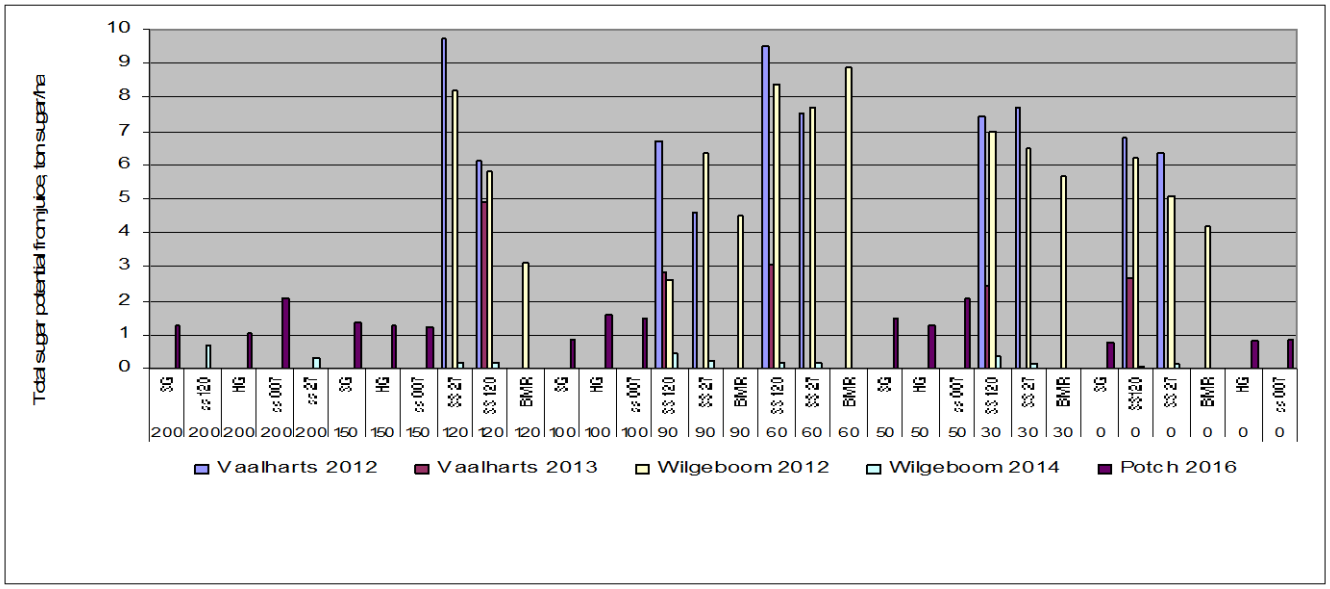
D 9. Grand mean of the Brix% as effected by different nitrogen application levels at Vaaalharts (2013) and Wilgeboom (2014).



D 10. Total sugar potential from juice, ton sugar/ha

		Vaalharts		Wilgeboom		Potch
N appl kg ha^{-1}	**genotype**	**2012**	**2013**	**2012**	**2014**	**2016**
200	**SG**	0.00	0.00	0.00	0.00	1.28
200	**ss 120**	0.00	0.00	0.00	0.68	0.00
200	**HG**	0.00	0.00	0.00	0.00	1.05
200	**ss 007**	0.00	0.00	0.00	0.00	2.05
200	**ss 27**	0.00	0.00	0.00	0.33	0.00
150	**SG**	0.00	0.00	0.00	0.00	1.36
150	**HG**	0.00	0.00	0.00	0.00	1.25
150	**ss 007**	0.00	0.00	0.00	0.00	1.20
120	**SS 27**	9.72	0.00	8.21	0.16	0.00
120	**SS 120**	6.14	4.91	5.81	0.19	0.00
120	**BMR**	0.00	0.00	3.12	0.00	0.00
100	**SG**	0.00	0.00	0.00	0.00	0.84
100	**HG**	0.00	0.00	0.00	0.00	1.58
100	**ss 007**	0.00	0.00	0.00	0.00	1.50
90	**SS 120**	6.72	2.86	2.59	0.46	0.00
90	**SS 27**	4.61	0.00	6.37	0.24	0.00
90	**BMR**	0.00	0.00	4.50	0.00	0.00
60	**SS 120**	9.50	3.06	8.39	0.17	0.00
60	**SS 27**	7.51	0.00	7.70	0.16	0.00
60	**BMR**	0.00	0.00	8.90	0.00	0.00
50	**SG**	0.00	0.00	0.00	0.00	1.47
50	**HG**	0.00	0.00	0.00	0.00	1.26
50	**ss 007**	0.00	0.00	0.00	0.00	2.07
30	**SS 120**	7.41	2.44	6.97	0.38	0.00
30	**SS 27**	7.70	0.00	6.49	0.14	0.00
30	**BMR**	0.00	0.00	5.66	0.00	0.00
0	**SG**	0.00	0.00	0.00	0.00	0.78
0	**SS120**	6.81	2.67	6.20	0.07	0.00
0	**SS 27**	6.37	0.00	5.09	0.13	0.00
0	**BMR**	0.00	0.00	4.20	0.00	0.00
0	**HG**	0.00	0.00	0.00	0.00	0.83
0	**ss 007**	0.00	0.00	0.00	0.00	0.85

D 11. Total sugar potential from juice

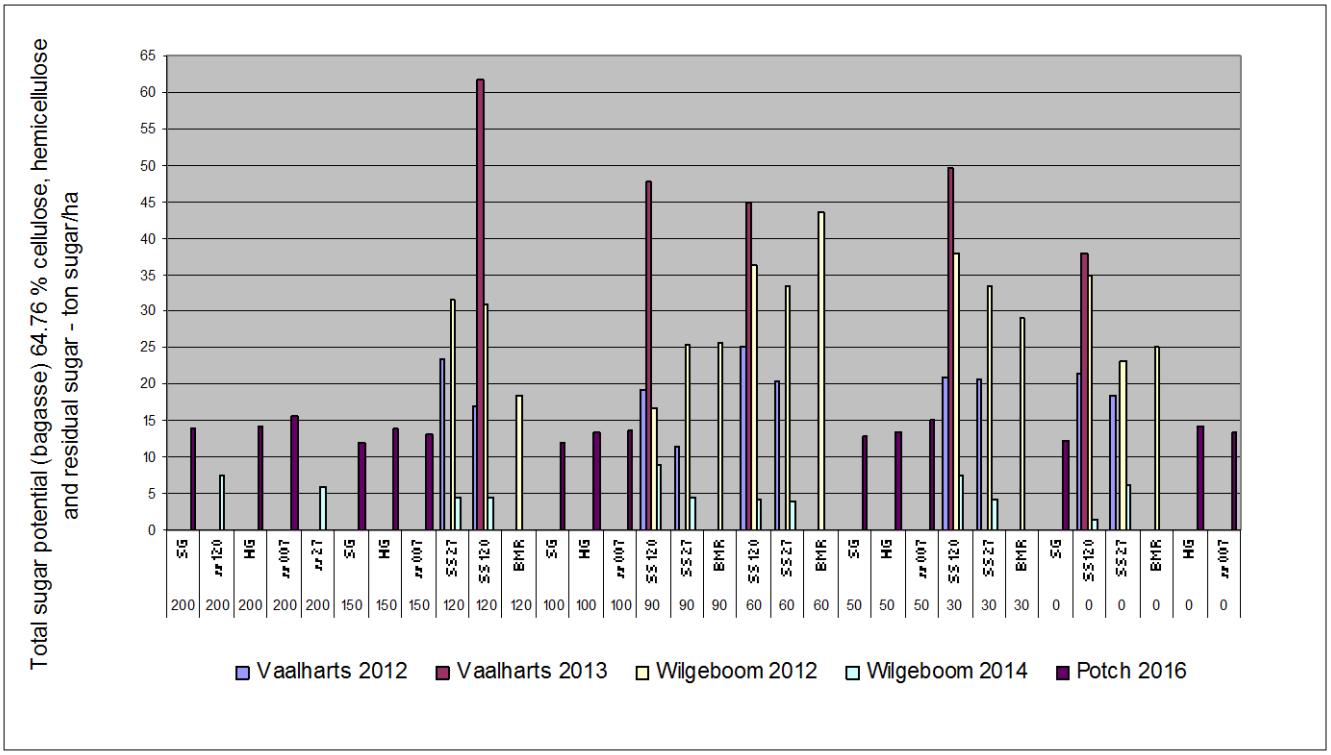


D 12. Total sugar potential (bagasse) 64.76 % cellulose, hemicellulose and residual sugar - ton sugar/ha

		Vaalharts		Wilgeboom		Potch
N appl kg ha^{-1}	**genotype**	**2012**	**2013**	**2012**	**2014**	**2016**
200	**SG**	0.00	0.00	0.00	0.00	14.07
200	**ss 120**	0.00	0.00	0.00	7.53	0.00
200	**HG**	0.00	0.00	0.00	0.00	14.19
200	**ss 007**	0.00	0.00	0.00	0.00	15.50
200	**ss 27**	0.00	0.00	0.00	5.72	0.00
150	**SG**	0.00	0.00	0.00	0.00	11.98
150	**HG**	0.00	0.00	0.00	0.00	13.89
150	**ss 007**	0.00	0.00	0.00	0.00	13.09
120	**SS 27**	23.38	0.00	31.50	4.57	0.00
120	**SS 120**	16.91	61.71	30.89	4.50	0.00
120	**BMR**	0.00	0.00	18.52	0.00	0.00
100	**SG**	0.00	0.00	0.00	0.00	12.03
100	**HG**	0.00	0.00	0.00	0.00	13.29
100	**ss 007**	0.00	0.00	0.00	0.00	13.55
90	**SS 120**	19.18	47.81	16.73	9.05	0.00
90	**SS 27**	11.35	0.00	25.34	4.40	0.00
90	**BMR**	0.00	0.00	25.69	0.00	0.00
60	**SS 120**	25.09	45.01	36.36	4.25	0.00
60	**SS 27**	20.48	0.00	33.43	3.92	0.00
60	**BMR**	0.00	0.00	43.65	0.00	0.00
50	**SG**	0.00	0.00	0.00	0.00	12.76
50	**HG**	0.00	0.00	0.00	0.00	13.46
50	**ss 007**	0.00	0.00	0.00	0.00	14.97
30	**SS 120**	21.04	49.77	37.81	7.42	0.00

30	**SS 27**	20.67	0.00	33.34	4.14	0.00
30	**BMR**	0.00	0.00	28.92	0.00	0.00
0	**SG**	0.00	0.00	0.00	0.00	12.20
0	**SS120**	21.39	38.01	34.83	1.29	0.00
0	**SS 27**	18.43	0.00	23.03	6.01	0.00
0	**BMR**	0.00	0.00	25.16	0.00	0.00
0	**HG**	0.00	0.00	0.00	0.00	14.12
0	**ss 007**	0.00	0.00	0.00	0.00	13.26

D 13. Total sugar potential



Appendix E. Additional information regarding soil analysis and fertiliser recommendations

E 1. Soil analysis: Bethlehem 2011

H J Boshoff

LNR-IGG

P/Sak X 1251

Potchefstroom 2520

2011.10.21

Grp Nr: V402

Lab Nr: V2957-V2968

Aandag: W Snijman

GRONDONTLEDINGSVERSLAG

Metodes: (pH & Weers.= Vers.waterpasta);(N - NH4+NO3 = 1:5 Eks-0.1N K2SO4); (P = 1:7.5 Eks. Bray 2); (Cl=1:2 Eks 0.1N KNO3);(Ca, Mg, K, Na = 1:10 Eks Amm.Asetaat-1N, pH7);(Zn=1:4 Eks. - 0.1N HCl);(Org.C=Walkley-Black);(Eks.Suur en Al=1:10 Eks 1N KCl);(Deeltjiegrootte-Hidrometer)

*** S-waarde = Som van ekstraheerbare Ca, Mg K en Na (c.mol(+)/kg)(me%)**

Lab.Nr:	V2957	V2958	V2959	V2960	V2961	V2962	V2963	V2964
U Beskrywing:	R						BETHLEHEM	
	A 1	B 1	C 1	A 2	B 2	C 2	1 A	1 B
pH (KCl) 1:2.5	5.29	5.28	5.51	5.26	5.19	5.33	5.54	5.30
milligram/kilogram								
N-NO3	3.40	0.90	0.25	3.00	1.50	0.50	3.40	2.50
N-NH4	2.65	1.75	1.15	1.90	2.15	1.15	1.75	1.50
P(Bray1)	7	5	2	7	7	2	52	38
K	188	113	103	210	193	105	188	185
Ca	1350	1410	1500	1340	1330	1340	638	680
Mg	1560	1620	1900	1520	1500	1690	113	128
Na	20	33	50	15	18	33	13	15
Cl								
Zn	2.04	2.00	1.28	2.12	2.08	1.32	5.12	3.52

S-(SO4)									
C %									
*** S-waarde**	**20.212**	**20.872**	**23.684**	**19.866**	**19.620**	**21.080**	**4.662**	**4.997**	
Ca %	33.4	33.8	31.7	33.7	33.9	31.8	68.4	68.0	
Mg %	63.8	64.1	66.3	63.2	63.2	66.3	20.0	21.2	
K %	2.4	1.4	1.1	2.7	2.5	1.3	10.3	9.5	
Na %	0.4	0.7	0.9	0.3	0.4	0.7	1.2	1.3	
Ekstr. suur (me%)									
Ekstr. Al (me%)									
Al (mg/kg)									
% Sand	46	47	42	44	42	42	77	74	
% Slik	10	10	9	10	10	9	3	4	
% Klei	44	43	49	46	48	49	20	22	
Lab. Nr.	V2957	V2958	V2959	V2960	V2961	V2962	V2963	V2964	0
me % Ca	6.750	7.050	7.500	6.700	6.650	6.700	3.190	3.400	0.000
Mg	12.893	13.388	15.702	12.562	12.397	13.967	0.934	1.058	0.000
K	0.482	0.290	0.264	0.538	0.495	0.269	0.482	0.474	0.000
Na	0.087	0.143	0.217	0.065	0.078	0.143	0.057	0.065	0.000
S-waarde (me%)	20.212	20.872	23.684	19.866	19.620	21.080	4.662	4.997	0.000

Metodes: (pH & Weers.= Vers.waterpasta);(N - NH4+NO3 = 1:5 Eks-0.1N K2SO4); (P = 1:7.5 Eks.
Bray 2); (Cl=1:2 Eks 0.1N KNO3);(Ca, Mg, K, Na = 1:10 Eks Amm.Asetaat-1N, pH7);(Zn=1:4 Eks.
- 0.1N HCl);(Org.C=Walkley-Black);(Eks.Suur en Al=1:10 Eks 1N KCl);(Deeltjiegrootte-Hidrometer)
*** S-waarde = Som van ekstraheerbare Ca, Mg K en Na (c.mol(+)/kg)(me%)**

Lab.Nr:	V2965	V2966	V2967	V2968				
U Beskrywing:	**B**							
	1 C	**2 A**	**2 B**	**2 C**				
pH (KCl) 1:2.5	5.28	5.75	5.37	5.21				

milligram/kilogram									
N-NO3	1.50	2.40	1.25	0.90					
N-NH4	0.90	1.65	0.65	1.00					
P(Bray1)	11	53	26	5					
K	195	185	180	163					
Ca	808	723	705	830					
Mg	173	128	148	183					
Na	20	13	15	23					
Cl									
Zn	1.60	5.72	2.96	1.16					
S-(SO4)									
C %									
*** S-waarde**	**6.057**	**5.204**	**5.275**	**6.180**					
Ca %	66.7	69.5	66.8	67.1					
Mg %	23.6	20.3	23.2	24.5					
K %	8.3	9.1	8.7	6.8					
Na %	1.4	1.1	1.2	1.6					
Ekstr. suur (me%)									
Ekstr. Al (me%)									
Al (mg/kg)									
% Sand	66	76	72	64					
% Slik	6	4	6	6					
% Klei	28	20	22	30					

Lab. Nr.	V2965	V2966	V2967	V2968	0	0	0	0	0
me % Ca	4.040	3.615	3.525	4.150	0.000	0.000	0.000	0.000	0.000
Mg	1.430	1.058	1.223	1.512	0.000	0.000	0.000	0.000	0.000
K	0.500	0.474	0.462	0.418	0.000	0.000	0.000	0.000	0.000
Na	0.087	0.057	0.065	0.100	0.000	0.000	0.000	0.000	0.000
S-waarde (me%)	6.057	5.204	5.275	6.180	0.000	0.000	0.000	0.000	0.000

E 2. Soil analysis: Wilgeboom 2014

KP Ngwato 2014.11.07

LNR-IGG Grp Nr: B339

P/Sak X1251 Lab Nr: B2951-B2962

Potchefstroom 2520 Aandag: Mnr W Snijman

GRONDONTLEDINGSVERSLAG

Metodes: (pH & Weers.= Vers.waterpasta);(N - NH4+NO3 = 1:5 Eks-0.1N K2SO4); (P = 1:7.5 Eks. Bray 2/Bray 1); (Cl=1:2 Eks 0.1N KNO3);(Ca, Mg, K, Na = 1:10 Eks Amm.Asetaat-1N, pH7);(Fe, Cu, Zn, Mn =1:4 Eks. - 0.1N HCl);S - SO4 = 1:2.5 Eks-versuurde Amm.Asetaat),(Org.C=Walkley-Black) (Eks.Suur en Al=1:10 Eks 1N KCl);(Deeltjiegrootte-Hidrometer)

*** S-waarde = Som van ekstraheerbare Ca, Mg K en Na (c.mol(+)/kg)(me%)**

Lab.Nr:	B2951	B2952	B2953	B2954	B2955	B2956	B2957	B2958
U Beskrywing:	**WBN**		**WBO**		**WBP**		**WBQ**	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
pH (KCl) 1:2.5	4.82	5.02	4.83	4.90	4.89	4.95	4.81	5.12
milligram/kilogram								
N								
P(Bray1)	13		11		8		12	
K	298	220	310	210	298	233	330	223
Ca	508	575	458	540	478	510	463	563

Mg	150	178	160	190	158	205	153	195
Na	10	10	13	10	10	5	10	8
Cl								
Fe								
Cu								
Zn	4.56		3.28		3.28		3.08	
Mn								
S-(SO4)								
C %								
*** S-waarde**	**4.587**	**4.954**	**4.464**	**4.852**	**4.503**	**4.863**	**4.469**	**5.033**
Ca %	55.4	58.0	51.3	55.6	53.1	52.4	51.8	55.9
Mg %	27.0	29.7	29.6	32.4	29.0	34.8	28.3	32.0
K %	16.7	11.4	17.8	11.1	17.0	12.3	18.9	11.4
Na %	0.9	0.9	1.3	0.9	1.0	0.4	1.0	0.7
Ekstr. suur (me%)								
Ekstr. Al (me%)								
Al (mg/kg)								
% Sand	75	72	74	73	76	72	76	72
% Slik	7	6	8	6	6	6	6	8
% Klei	18	22	18	21	18	22	18	20

Lab. Nr.	B2951	B2952	B2953	B2954	B2955	B2956	B2957	B2958	0
me % Ca	2.540	2.875	2.290	2.700	2.390	2.550	2.315	2.815	0.000
Mg	1.240	1.471	1.322	1.570	1.306	1.694	1.264	1.612	0.000
K	0.764	0.564	0.795	0.538	0.764	0.597	0.846	0.572	0.000
Na	0.043	0.043	0.057	0.043	0.043	0.022	0.043	0.035	0.000
S-waarde (me%)	4.587	4.954	4.464	4.852	4.503	4.863	4.469	5.033	0.000

GRONDONTLEDINGSVERSLAG

Metodes: (pH & Weers.= Vers.waterpasta);(N - NH4+NO3 = 1:5 Eks-0.1N K2SO4); (P = 1:7.5 Eks. Bray 2/Bray 1); (Cl=1:2 Eks 0.1N KNO3);(Ca, Mg, K, Na = 1:10 Eks Amm.Asetaat-1N, pH7);(Fe, Cu, Zn, Mn =1:4 Eks. - 0.1N HCl);S - SO4 = 1:2.5 Eks-versuurde Amm.Asetaat),(Org.C=Walkley-Black)

(Eks.Suur en Al=1:10 Eks 1N KCl);(Deeltjiegrootte-Hidrometer)

*** S-waarde = Som van ekstraheerbare Ca, Mg K en Na (c.mol(+)/kg)(me%)**

Lab.Nr:	B2959	B2960	B2961	B2962				
U Beskrywing:	**WBR**		**WBS**					
	A	**B**	**A**	**B**				
pH (KCl) 1:2.5	4.79	4.75	4.68	4.80				
milligram/kilogram								
N								
P(Bray1)	10		11					
K	210	195	318	203				
Ca	428	453	458	455				
Mg	155	160	150	168				
Na	5	8	8	5				
Cl								
Fe								
Cu								
Zn								
Mn								
S-(SO4)	2.32		2.92					
C %								
*** S-waarde**	**3.981**	**4.122**	**4.380**	**4.206**				
Ca %	53.8	54.9	52.3	54.1				
Mg %	32.2	32.1	28.3	33.0				
K %	13.5	12.1	18.6	12.4				
Na %	0.5	0.8	0.8	0.5				
Ekstr. suur (me%)								
Ekstr. Al (me%)								
Al (mg/kg)								
% Sand	76	76	76	74				
% Slik	6	4	6	6				
% Klei	18	20	18	20				

Lab. Nr.	B2959	B2960	B2961	B2962	0	0	0	0	0
me % Ca	2.140	2.265	2.290	2.275	0.000	0.000	0.000	0.000	0.000

Mg	1.281	1.322	1.240	1.388	0.000	0.000	0.000	0.000	0.000
K	0.538	0.500	0.815	0.521	0.000	0.000	0.000	0.000	0.000
Na	0.022	0.035	0.035	0.022	0.000	0.000	0.000	0.000	0.000
S-waarde (me%)	3.981	4.122	4.380	4.206	0.000	0.000	0.000	0.000	0.000

E 3. Soil analysis: Potchefstroom (ARC:GCI) 2009

ARC • LNR

INSTITUTE FOR SOIL, CLIMATE AND WATER

INSTITUUT VIR GROND, KLIMAAT EN WATER

Client : **Mr. W, Snijman**
Klient :
ARC-IGC
Potch

Tel : 018 29
Fax / Faks :

Date / Datum : 2009/01/30

RESULTS FOR REPORT No:
RESULTATE VIR VERSLAG Nr GROND 200809 4335

			1	2	3	4	5	6	7	8	9	10
T	**LabNo**	**SENDER_NR**	**Zn**	**N-NO3**	**N-NH4**	**K**	**Ca**	**Mg**	**Na**	**P**	**pH(KCl)**	**T. acid**
			mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	KCl	cmol(+)/kg
M	3674	R1 M A	24.34	4.81	4.95	157	1226	499	21.2	23.39	6.30	0
M	3675	R1 M B	11.76	3.53	4.05	93	1226	479	28.0	7.97	6.67	0
M	3676	R1 M C	1.26	2.20	5.16	62	1315	497	62.2	2.33	6.84	0
M	3677	R2 M A	23.08	1.32	2.45	165	1234	496	21.7	23.53	6.35	0
M	3678	R2 M B	6.27	1.10	2.30	80	1230	498	32.2	5.17	6.57	0
M	3679	R2 M C	4.29	1.48	2.19	72	1455	571	53.5	2.54	6.73	0
M	3680	R3 M A	27.45	6.65	1.97	122	1136	464	18.5	19.44	6.20	0
M	3681	R3 M B	13.43	6.26	1.89	70	1178	452	27.6	10.11	6.32	0
M	3682	R3 M C	3.4	2.42	2.83	75	1357	527	41.0	3.58	6.60	0
M	3683	R1 SS A	17.3	5.44	3.43	110	1229	492	21.0	15.05	6.27	0
M	3684	R1 SS B	3.47	2.44	3.56	61	1202	473	27.1	3.13	6.54	0
M	3685	R1 SS C	1.39	0.72	4.75	55	1296	495	43.6	1.66	6.76	0
M	3686	R2 SS A	14.79	5.80	2.21	109	1283	518	26.6	11.09	6.53	0
M	3687	R2 SS B	4.96	4.81	2.67	71	1291	526	37.9	3.60	6.58	0
M	3688	R2 SS C	0.85	1.72	2.70	67	1371	535	52.7	1.62	6.80	0
M	3689	R3 SS A	17.48	7.42	2.18	119	1101	468	22.3	12.36	6.33	0
M	3690	R3 SS B	13.59	5.52	2.14	69	1125	458	27.9	10.50	6.37	0
M	3691	R3 SS C	2.11	1.98	2.54	62	1336	523	47.7	2.29	6.59	0
M	3692	R1 SF A	16.19	15.40	6.67	210	1191	505	27.2	17.08	6.45	0
M	3693	R1 SF B	11.41	4.02	5.67	76	1095	425	21.2	8.98	6.49	0
M	3694	R1 SF C	2	2.45	4.80	73	1191	457	30.0	2.79	6.67	0
M	3695	R2 SF A	17.92	4.82	3.76	146	1100	466	21.0	12.29	6.37	0
M	3696	R2 SF B	9.54	3.13	3.65	74	1154	481	29.3	5.87	6.44	0
M	3697	R2 SF C	1.38	1.90	5.28	70	1339	541	42.3	1.85	6.73	0
M	3698	R3 SF A	17.53	11.12	3.78	132	1085	450	15.6	15.98	6.22	0

			1	2	3	4	5	6	7	8	9	10
T	LabNo	SENDER_NR	Zn	N-NO3	N-NH4	K	Ca	Mg	Na	P	pH(KCl)	T. acid
			mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	KCl	cmol(+)/kg
M	3699	R3 SF B	9.53	4.90	2.45	67	1080	449	25.8	6.25	6.34	0
M	3700	R3 SF C	3.65	2.55	5.29	67	1240	493	45.0	2.37	6.72	0
M	3701	Y 21 SS A	18.38	4.66	3.51	116	1099	455	18.2	14.71	6.33	0
M	3702	Y 21 SS B	7.64	1.70	2.51	78	1087	441	26.8	4.28	6.39	0
M	3703	Y 21 SS C	0.64	2.26	3.40	72	1331	529	44.2	1.51	6.67	0

METHODS USED FOR ANALYSIS :

Serial	Method
1	0.1 HCl Extract
2	KCL Extr
3	KCL Extr
4	Farmer soil analysis

Serial	Method
5	Farmer soil analysis
6	Farmer soil analysis
7	Farmer soil analysis
8	Farmer soil analysis

Serial	Method
9	Farmer soil analysis
10	Farmer soil analysis

E 4. Soil analysis: Wilgeboom & Vaalhart2 2011

LNR - INSTITUUT VIR INDUSTRIELE GEWASSE, PRIVAATSAK X82075, RUSTENBURG 0300
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Navrae: **HJ Boshoff** **2011.11.11**

LNR -IGG
P/Sak X
1251
Potchefstroom
2520

Grp Nr: **V442**
V3076-
Lab Nr. **V3114**

Aandag: **Wikus Snijman**

Lab Nr		Beskrywing		pH (KCl) 1:2.5	Anorg N N-NO3	Anorg N N-NH4	P(Bray1) mg/kg	K mg/kg	Ca mg/kg	Mg mg/kg	Na mg/kg	Zn mg/kg	Sand %	Slik %	Klei %	S-Waarde (c.mol(+)/kg) (me%)	K %	Ca %	Mg %	Na %
V	**3076**	**WB**	**A 1**	4.80	3.10	2.35	7	310	380	168	3	2.60	79	5	16	**5.439**	0.3	28.5	57.7	13.4
V	**3077**		**B 1**	4.63	1.10	1.35	2	218	400	155	3	1.68	79	3	18	**5.075**	0.1	21.5	65.1	13.3
V	**3078**		**C 1**	5.03	0.50	0.50	1	185	550	218	3	0.40	74	4	22	**6.421**	0.0	14.4	70.8	14.8
V	**3079**		**A 2**	5.01	1.60	1.60	12	290	483	165	5	4.52	78	4	18	**6.190**	0.5	23.4	64.5	11.6
V	**3080**		**B 2**	5.30	0.35	0.60	2	223	605	193	5	1.20	74	4	22	**6.959**	0.1	16.0	71.8	12.1
V	**3081**		**C 2**	5.58	0.10	0.35	1	213	640	235	10	0.56	72	5	23	**7.379**	0.0	14.4	71.7	13.8
V	**3082**		**A 3**	5.44	1.50	2.25	3	235	510	190	3	2.56	79	3	18	**6.224**	0.1	18.9	67.7	13.3
V	**3083**		**B 3**	5.38	0.60	1.10	1	138	525	193	10	1.00	78	3	19	**5.871**	0.0	11.8	73.9	14.3
V	**3084**		**C 3**	5.63	0.10	0.75	1	123	643	248	8	0.44	73	3	24	**7.010**	0.0	8.8	75.8	15.4
V	**3085**		**A 4**	6.58	1.75	2.35	52	195	1068	285	3	20	78	6	16	**11.174**	1.2	8.7	79.0	11.1
V	**3086**		**B 4**	6.87	0.60	1.35	16	83	1000	318	10	7.96	77	5	18	**10.103**	0.4	4.1	81.8	13.7
V	**3087**		**C 4**	6.93	0.25	1.00	2	53	963	353	33	0.84	73	3	24	**9.764**	0.1	2.7	81.5	15.7
V	**3088**		**A 5**	4.79	0.60	2.25	7	248	390	125	5	1.08	78	2	20	**5.025**	0.4	24.7	64.1	10.8
V	**3089**		**B 5**	4.41	0.10	1.50	2	245	343	123	8	0.56	78	2	20	**4.600**	0.1	26.6	61.6	11.6
V	**3090**		**C 5**	4.80	0.10	1.85	1	215	518	193	8	0.28	72	4	24	**6.198**	0.0	17.3	69.1	13.5
V	**3091**		**A 6**	5.27	0.35	2.10	65	165	590	183	5	4.92	80	2	18	**6.663**	2.5	12.4	73.2	11.9
V	**3092**		**B 6**	4.44	0.60	2.25	7	145	333	135	5	0.76	78	2	20	**4.082**	0.4	17.8	67.4	14.4
V	**3093**		**C 6**	4.38	0.10	1.10	1	118	415	175	13	0.36	71	3	26	**4.783**	0.1	12.3	71.7	15.9
V	**3094**	**V**	**A 1**	6.50	2.50	1.25	48	135	608	180	5	3.48	89	0	11	**6.605**	1.9	10.2	76.1	11.8
V	**3095**		**B 1**	6.66	1.85	0.85	45	108	658	190	10	2.80	89	0	11	**6.919**	1.7	7.8	78.6	11.9
V	**3096**		**C 1**	7.45	1.00	0.75	19	120	2900	313	25	0.44	84	1	15	**25.977**	0.2	2.3	92.3	5.2
V	**3097**		**A 2**	6.57	4.00	1.35	39	150	623	185	5	2.80	89	0	11	**6.803**	1.5	11.0	75.7	11.8
V	**3098**		**B 2**	6.57	3.60	1.10	22	123	660	185	5	1.16	89	0	11	**6.930**	0.8	8.9	78.7	11.6
V	**3099**		**C 2**	6.57	1.85	2.85	33	125	668	193	10	1.44	88	0	12	**7.069**	1.2	8.8	78.1	11.9

V	**3100**		**A 3**	6.08	3.00	1.10	33	165	573	195	3	2.04	89	0	11	**6.493**	1.3	12.7	72.9	13.1
V	**3101**		**B 3**	6.02	2.10	1.10	24	138	590	208	8	1.16	88	0	12	**6.532**	0.9	10.6	74.6	13.8
V	**3102**		**C3**	5.97	1.10	0.85	3	135	770	300	20	0.24	85	0	15	**8.351**	0.1	8.1	76.2	15.6
V	**3103**	**T**	**A 1**	5.26	7.10	3.00	11	205	513	130	3	1.64	88	2	10	**5.858**	0.5	17.5	72.4	9.6
V	**3104**		**B 1**	5.48	4.25	2.25	3	185	518	138	5	1.04	88	0	12	**5.814**	0.1	15.9	73.6	10.3
V	**3105**		**C 1**	5.66	2.35	1.25	1	133	485	180	10	0.24	86	0	14	**5.458**	0.0	12.2	73.4	14.3
V	**3106**		**A 2**	5.16	5.85	2.00	9	188	515	128	3	1.80	89	1	10	**5.776**	0.4	16.3	73.7	9.6
V	**3107**		**B 2**	5.54	2.25	1.50	1	168	505	143	5	1.00	88	1	11	**5.638**	0.0	14.9	74.0	11.0
V	**3108**		**C 2**	5.71	1.75	1.35	1	160	490	180	10	0.36	87	1	12	**5.635**	0.0	14.2	71.9	13.9
V	**3109**	**PY**	**A 1**	6.05	7.00	1.85	26	208	988	415	13	6.36	58	10	32	**11.076**	0.6	9.4	73.7	16.3
V	**3110**		**B 1**	6.22	5.75	1.50	10	113	1090	440	18	3.28	58	11	31	**11.512**	0.2	4.9	78.3	16.6
V	**3111**		**C 1**	6.44	3.75	1.10	3	88	1180	480	28	1.20	52	12	36	**12.287**	0.1	3.6	79.4	17.0
V	**3112**		**A 2**	6.27	16.35	1.35	19	168	1088	473	23	6.28	58	9	33	**11.937**	0.4	7.0	75.3	17.2
V	**3113**		**B 2**	6.55	5.60	1.25	8	75	1240	510	35	3.40	58	10	32	**12.861**	0.2	2.9	79.7	17.2
V	**3114**		**C 2**	6.68	2.50	1.35	1	60	1380	565	48	0.44	50	12	38	**14.164**	0.0	2.1	80.5	17.3

V	**3076**	**WB**	**A 1**	0.018	1.550	3.140	0.730	5.439
V	**3077**		**B 1**	0.005	1.090	3.306	0.674	5.075
V	**3078**		**C 1**	0.003	0.925	4.545	0.948	6.421
V	**3079**		**A 2**	0.031	1.450	3.992	0.717	6.190
V	**3080**		**B 2**	0.005	1.115	5.000	0.839	6.959
V	**3081**		**C 2**	0.003	1.065	5.289	1.022	7.379
V	**3082**		**A 3**	0.008	1.175	4.215	0.826	6.224
V	**3083**		**B 3**	0.003	0.690	4.339	0.839	5.871
V	**3084**		**C 3**	0.003	0.615	5.314	1.078	7.010
V	**3085**		**A 4**	0.133	0.975	8.826	1.239	11.174
V	**3086**		**B 4**	0.041	0.415	8.264	1.383	10.103
V	**3087**		**C 4**	0.005	0.265	7.959	1.535	9.764
V	**3088**		**A 5**	0.018	1.240	3.223	0.543	5.025
V	**3089**		**B 5**	0.005	1.225	2.835	0.535	4.600
V	**3090**		**C 5**	0.003	1.075	4.281	0.839	6.198
V	**3091**		**A 6**	0.167	0.825	4.876	0.796	6.663
V	**3092**		**B 6**	0.018	0.725	2.752	0.587	4.082
V	**3093**		**C 6**	0.003	0.590	3.430	0.761	4.783
V	**3094**	**V**	**A 1**	0.123	0.675	5.025	0.783	6.605
V	**3095**		**B 1**	0.115	0.540	5.438	0.826	6.919
V	**3096**		**C 1**	0.049	0.600	23.967	1.361	25.977
V	**3097**		**A 2**	0.100	0.750	5.149	0.804	6.803
V	**3098**		**B 2**	0.056	0.615	5.455	0.804	6.930
V	**3099**		**C 2**	0.085	0.625	5.521	0.839	7.069
V	**3100**		**A 3**	0.085	0.825	4.736	0.848	6.493
V	**3101**		**B 3**	0.062	0.690	4.876	0.904	6.532

V	**3102**		**C3**	0.008	0.675	6.364	1.304	8.351
V	**3103**	**T**	**A 1**	0.028	1.025	4.240	0.565	5.858
V	**3104**		**B 1**	0.008	0.925	4.281	0.600	5.814
V	**3105**		**C 1**	0.003	0.665	4.008	0.783	5.458
V	**3106**		**A 2**	0.023	0.940	4.256	0.557	5.776
V	**3107**		**B 2**	0.003	0.840	4.174	0.622	5.638
V	**3108**		**C 2**	0.003	0.800	4.050	0.783	5.635
V	**3109**	**PY**	**A 1**	0.067	1.040	8.165	1.804	11.076
V	**3110**		**B 1**	0.026	0.565	9.008	1.913	11.512
V	**3111**		**C 1**	0.008	0.440	9.752	2.087	12.287
V	**3112**		**A 2**	0.049	0.840	8.992	2.057	11.937
V	**3113**		**B 2**	0.021	0.375	10.248	2.217	12.861
V	**3114**		**C 2**	0.003	0.300	11.405	2.457	14.164

	Projek	M		
13067	Nr:	203/32	Bedrag:	R 9 828

E 5. Soil analysis: Rustenburg

H J Boshoff 2011.10.21

LNR-IGG Grp Nr: V402
P/Sak X 1251 Lab Nr: V2957-V2968
Potchefstroom 2520 Aandag: W Snijman

GRONDONTLEDINGSVERSLAG

Metodes: (pH & Weers.= Vers.waterpasta);(N - NH4+NO3 = 1:5 Eks-0.1N K2SO4); (P = 1:7.5 Eks. Bray 2); (Cl=1:2 Eks 0.1N KNO3);(Ca, Mg, K, Na = 1:10 Eks Amm.Asetaat-1N, pH7);(Zn=1:4 Eks. - 0.1N HCl);(Org.C=Walkley-Black);(Eks.Suur en Al=1:10 Eks 1N KCl);(Deeltjiegrootte-Hidrometer)

*** S-waarde = Som van ekstraheerbare Ca, Mg K en Na (c.mol(+)/kg)(me%)**

Lab.Nr:	V2957	V2958	V2959	V2960	V2961	V2962	V2963	V2964
U Beskrywing:	RUSTENBURG						B	
	A 1	B 1	C 1	A 2	B 2	C 2	1 A	1 B
pH (KCl) 1:2.5	5.29	5.28	5.51	5.26	5.19	5.33	5.54	5.30
milligram/kilogram								
N-NO3	3.40	0.90	0.25	3.00	1.50	0.50	3.40	2.50
N-NH4	2.65	1.75	1.15	1.90	2.15	1.15	1.75	1.50
P(Bray1)	7	5	2	7	7	2	52	38
K	188	113	103	210	193	105	188	185
Ca	1350	1410	1500	1340	1330	1340	638	680
Mg	1560	1620	1900	1520	1500	1690	113	128
Na	20	33	50	15	18	33	13	15
Cl								
Zn	2.04	2.00	1.28	2.12	2.08	1.32	5.12	3.52
S-(SO4)								
C %								

*** S-waarde**	**20.212**	**20.872**	**23.684**	**19.866**	**19.620**	**21.080**	**4.662**	**4.997**
Ca %	33.4	33.8	31.7	33.7	33.9	31.8	68.4	68.0
Mg %	63.8	64.1	66.3	63.2	63.2	66.3	20.0	21.2
K %	2.4	1.4	1.1	2.7	2.5	1.3	10.3	9.5
Na %	0.4	0.7	0.9	0.3	0.4	0.7	1.2	1.3
Ekstr. suur (me%)								
Ekstr. Al (me%)								
Al (mg/kg)								
% Sand	46	47	42	44	42	42	77	74
% Slik	10	10	9	10	10	9	3	4
% Klei	44	43	49	46	48	49	20	22

Bladsy 2/……

Lab. Nr.	V2957	V2958	V2959	V2960	V2961	V2962	V2963	V2964	0
me % Ca	6.750	7.050	7.500	6.700	6.650	6.700	3.190	3.400	0.000
Mg	12.893	13.388	15.702	12.562	12.397	13.967	0.934	1.058	0.000
K	0.482	0.290	0.264	0.538	0.495	0.269	0.482	0.474	0.000
Na	0.087	0.143	0.217	0.065	0.078	0.143	0.057	0.065	0.000
S-waarde (me%)	20.212	20.872	23.684	19.866	19.620	21.080	4.662	4.997	0.000

Bladsy 2

GRONDONTLEDINGSVERSLAG

Metodes: (pH & Weers.= Vers.waterpasta);(N - NH4+NO3 = 1:5 Eks-0.1N K2SO4); (P = 1:7.5 Eks. Bray 2); (Cl=1:2 Eks 0.1N KNO3);(Ca, Mg, K, Na = 1:10 Eks Amm.Asetaat-1N, pH7);(Zn=1:4 Eks. - 0.1N HCl);(Org.C=Walkley-Black);(Eks.Suur en Al=1:10 Eks 1N KCl);(Deeltjiegrootte-Hidrometer)

*** S-waarde = Som van ekstraheerbare Ca, Mg K en Na (c.mol(+)/kg)(me%)**

Lab.Nr:	V2965	V2966	V2967	V2968				
U Beskrywing:	**B**							
	1 C	**2 A**	**2 B**	**2 C**				
pH (KCl) 1:2.5	5.28	5.75	5.37	5.21				

milligram/kilogram									
N-NO3	1.50	2.40	1.25	0.90					
N-NH4	0.90	1.65	0.65	1.00					
P(Bray1)	11	53	26	5					
K	195	185	180	163					
Ca	808	723	705	830					
Mg	173	128	148	183					
Na	20	13	15	23					
Cl									
Zn	1.60	5.72	2.96	1.16					
S-(SO4)									
C %									
*** S-waarde**	**6.057**	**5.204**	**5.275**	**6.180**					
Ca %	66.7	69.5	66.8	67.1					
Mg %	23.6	20.3	23.2	24.5					
K %	8.3	9.1	8.7	6.8					
Na %	1.4	1.1	1.2	1.6					
Ekstr. suur (me%)									
Ekstr. Al (me%)									
Al (mg/kg)									
% Sand	66	76	72	64					
% Slik	6	4	6	6					
% Klei	28	20	22	30					

Lab. Nr.	V2965	V2966	V2967	V2968	0	0	0	0	0
me % Ca	4.040	3.615	3.525	4.150	0.000	0.000	0.000	0.000	0.000
Mg	1.430	1.058	1.223	1.512	0.000	0.000	0.000	0.000	0.000
K	0.500	0.474	0.462	0.418	0.000	0.000	0.000	0.000	0.000
Na	0.087	0.057	0.065	0.100	0.000	0.000	0.000	0.000	0.000
S-waarde (me%)	6.057	5.204	5.275	6.180	0.000	0.000	0.000	0.000	0.000
Fakt Nr:	13027	Projek Nr:	M 203/32	Bedrag:	R 3 024				

E 6. Soil analysis: Vaalharts 2013

H J Boshoff
LNR - IGG
P/Sak X1251
Potchefstroom
2520

2013.01.18
Grp Nr: X500
Lab Nr: X3166-X3169

Aandag: Mnr JL Snijman

GRONDONTLEDINGSVERSLAG

Metodes: (pH & Weers.= Vers.waterpasta);(N - NH4+NO3 = 1:5 Eks-0.1N K2SO4); (P = 1:7.5 Eks.
Bray 2/Bray 1); (Cl=1:2 Eks 0.1N KNO3);(Ca, Mg, K, Na = 1:10 Eks Amm.Asetaat-1N, pH7);(Fe, Cu, Zn, Mn =1:4 Eks.
- 0.1N HCl);S - SO4 = 1:2.5 Eks-versuurde Amm.Asetaat),(Org.C=Walkley-Black)
(Eks.Suur en Al=1:10 Eks 1N KCl);(Deeltjiegrootte-Hidrometer)
*** S-waarde = Som van ekstraheerbare Ca, Mg K en Na (c.mol(+)/kg)(me%)**

Lab.Nr:	X3166	X3167	X3168	X3169				
U Beskrywing:	**VAALHARTS**							
	A 1	**A 2**	**B 1**	**B 2**				
pH	7.20	7.26	7.21	6.98				
Weerstand	1280	1580	1460	1240				
milligram/kilogram								
N	5	5	5	11				
P(Bray2)	53	57	42	40				
P(Bray1)	46	46	36	33				
K	158	223	278	253				
Ca	468	448	498	500				
Mg	168	150	118	128				

Na	35	30	33	25				
Cl								
Fe								
Cu								
Zn	3.56	2.28	2.76	2.20				
Mn								
S-(SO4)	10	8	9	11				
C %	0.74	0.45	0.46	0.30				
*** S-waarde**	**4.286**	**4.182**	**4.322**	**4.315**				
Ca %	54.6	53.6	57.6	57.9				
Mg %	32.4	29.6	22.6	24.5				
K %	9.5	13.7	16.5	15.0				
Na %	3.6	3.1	3.3	2.5				
Ekstr. suur (me%)	0.012	0.014	0.015	0.019				
Ekstr. Al (me%)	0.000	0.000	0.000	0.000				
Al (mg/kg)	0.00	0.00	0.00	0.00				
% Sand	91	92	91	91				
% Slik	2	1	2	0				
% Klei	7	7	7	9				

Lab. Nr.	X3166	X3167	X3168	X3169	0
me % Ca	2.340	2.240	2.490	2.500	0.000
Mg	1.388	1.240	0.975	1.058	0.000
K	0.405	0.572	0.713	0.649	0.000
Na	0.152	0.130	0.143	0.109	0.000
S-waarde (me%)	4.286	4.182	4.322	4.315	0.000

Fakt Nr:	13941	Projek Nr:	M 203/32	Bedrag:	R 1 728

E 7. An example of fertiliser recommendations – Wilgeboom and Vaalharts 2011/2012

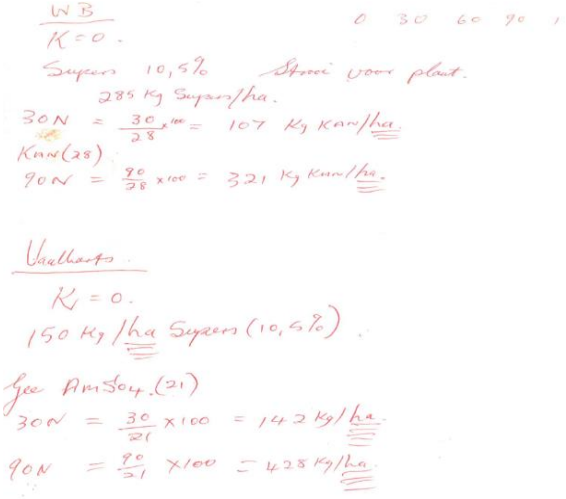
(Original Afrikaans versions below)
Recommendations 2011/12
Sorghum Nitrogen trial
Locations: Wilgeboom and Vaalharts

Wilgeboom

Fertiliser	With planting	Top dressing
Superphosphate (10,5%)	285 kg ha^{-1}	
KAN (28)		107 kg ha^{-1} for 30 kg per plot
		321 kg ha^{-1} for 90 kg per plot

Vaalharts

Fertiliser	With planting	Top dressing
Superphosphate (10,5%)	150 kg ha^{-1}	
Ammoniumsulphate (21)		142 kg ha^{-1} for 30 kg per plot
Ammoniumsulphate (21)		428 kg ha^{-1} for 90 kg per plot



E 8. An example of the fertiliser recommendations – Wilgeboom 2014

(Original Afrikaans versions below)

Recommendations Mnr W Snijman 19 November 2014

Sorghum Nitrogen trial

Plots: WBN, WBO, WBP, WBQ, WBR, WBS

Plant mixture : 200 kg NPK 2:1:0 (30) per ha…..40N2OP per plot or 200 kg NPK 2:1:0 (27) per ha…..39.6N19.8P per plot			
	Fertiliser	Application rate with plant	Top dressings
With planting	KAN	40N all plots	
Top dressing	KAN (70 kg ha^{-1})		19.6 N for 30 kg ha^{-1}
	KAN (180 kg ha^{-1})		50.4 N for 60 kg ha^{-1}
	KAN (258 kg ha^{-1})		79.8 N for 90 kg ha^{-1}
	KAN (570 kg ha^{-1})		159.6 N for 120 kg ha^{-1}

Aanbeveling mnr W Snijman 19 November 2014

Sorghum

Persele WBN, WBO, WBP, WBQ, WBR, WBS.

Plantmengsel

Plant met 200 kg 2:1:0 (30) / ha [40N20P]

Of

Plant met 200 kg 2:1:0 (27) / ha [39.6N19.8P]

Topbemesting

1) Met plant word 40N toegedien by alle persele.

2)Dien toe 70 kg KAN(28)/ha [19.6N] + [40N] = Totaal 59.6N

3)Dien toe 180 kg KAN(28)/ha [50.4N] + [40N] = Totaal 90.4N

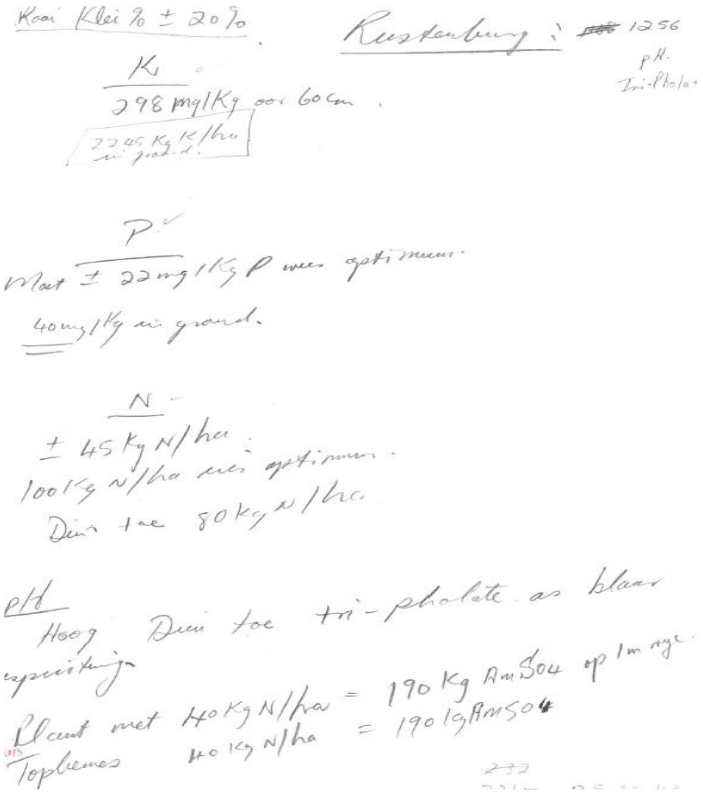
4)Dien toe 285 kg KAN(28)/ha [79.8N] + [40N] = Totaal 119.8N

5)Dien toe 570 kg KAN(28)/ha [159.6N] + [40N] = Totaal 199.6N

E 9. An example of the fertiliser recommendations – Rustenburg

(Original Afrikaans versions below)

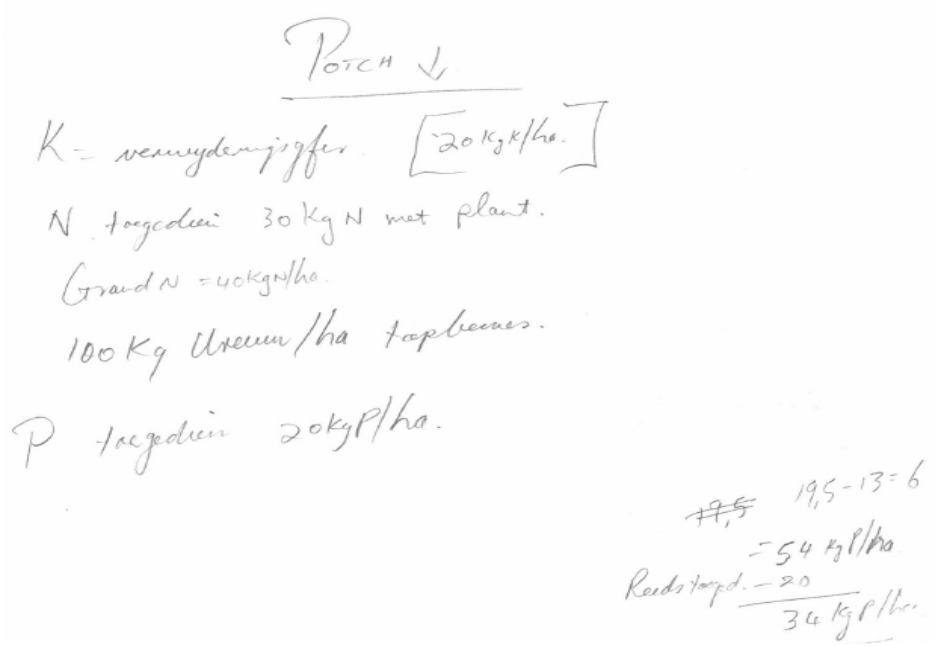
Fertiliser	With planting	Top Dressing
Ammoniumsulphate (21)	80 kg ha^{-1}	
Ammoniumsulphate (21)		190 kg ha^{-1}



E 10. An example of the fertiliser recommendations – Potchefstroom

(Original Afrikaans versions below)

Fertiliser	With planting	Top Dressing
Urea	30 kg ha^{-1}	
Urea		100 kg ha^{-1}
Phosphates	20 kg ha^{-1}	



Appendix F 1. Compositional analysis of bagasse done by the ARC: API

ARC • LNR
Excellence in Research and Development

ARC-Irene Analytical Services
LNR-Irene Analitiese Dienste

sanas
Testing Laboratory
T0053

Private Bag/Privaatsak X2, Irene, 0062 Tel: (012) 672 9294 Fax: (086) 607 7102

Enquiries: Penny Barnes
Tel: 012-672 9292/94

29/05/2017

The Manager
Univ of North West (Chemical & Mineral Engineering)
School of Chem & Mineral Engin
North West University
Private Bag X 6001
Potchefstroom
2520

Tel No: (018) 299 1377
Fax No: (018) 299 1535

Attention: Mr G van Rensburg

TEST REPORT

Date received: 11/04/2017
Date accepted: 11/04/2017
Date completed: 26/05/2017
Test report no: 2017-F-144

RESULTS OF PLANT MATERIAL (UNSPECIFIED)

Please take note that:

1. Test results relate only to the samples tested.
2. This report may not be reproduced without the written consent of the Quality Manager.
3. The samples received were thoroughly mixed before analysis.
4. Chromatogrammes, if applicable, are available on request.
5. Opinions and interpretations expressed herein are outside the scope of SANAS accreditation.

Yours sincerely

P Barnes
Technical Signatory: Chemistry

ARC-IRENE ANALYTICAL SERVICES

Physical Address: ARC-API, Old Olifantsfontein Rd, Irene

TEST REPORT 2017-F-144

This laboratory holds SANAS accreditation for analyses with an ASM number.
Results are expressed on a wet basis, therefore as samples were received.

Analysis	Method Number	Unit	Sample Number 1 : Bagasse HG 0	Sample Number 2 : Bagasse HG 200	Sample Number 3 : Bagasse SG 0	Sample Number 4 : Bagasse SG 200	Sample Number 5 : Bagasse 007/0	Sample Number 6 : Bagasse 007/200
Dry matter	ASM 013	%	86.87	88.70	87.87	89.06	87.96	86.69
Moisture	ASM 013	%	13.13	11.30	12.13	10.94	12.04	13.31
Ash	ASM 048	%	7.58	6.46	10.70	8.91	7.01	4.20
*Protein (N x 6.25)	ASM 078	%	5.26	7.53	7.96	3.81	5.07	4.42
Fat (ether extraction)	ASM 044	%	0.66	0.87	0.95	1.22	0.96	1.04
Carbohydrates (calculated)	ASM 075	%	73.37	73.84	68.26	75.12	74.92	77.03
Neutral detergent fibre	ASM 060	%	57.25	64.62	58.14	61.39	61.86	50.63
ADF	Not SANAS accredited	%	36.35	42.51	35.59	34.74	34.80	28.60
ADL	Not SANAS accredited	%	8.08	11.95	6.92	6.19	7.27	10.14

Sample	Sample type	Date analysis commenced
1-6	Plant material (unspecified)	28/04/2017

* For the conversion of nitrogen content to protein content the factor 6.25 was used.

F 2. Methods of calculations to determine potential bio-ethanol from bagasse

Value of the biomass yield/ha (dab): [mass water (calculated) and the mass ash (calculated)] minus the measured biomass weight
Bagasse/ha values of the dry bagasse are actuals as recorded when data was collected
Mass of the water component: measured bagasse/ha multiplied by analysed moisture value (Table 15) divided by 100
Mass of the ash component: measured bagasse/ha multiplied by analysed ash value divided by 100
Value of cellulose: ADF amount minus ADL amount
Value of hemi-cellulose: NDF amount minus ADF amount
Value of bagasse sugars: value of the cellulose plus value of the hemi-cellulose
Amount of residual sugars: carbohydrates minus cellulose minus hemi-cellulose minus ADL
Total sugars: bagasse sugars plus residual sugars together
Amount of sugars/ha in the bagasse: bagasse yield/ha multiplied by the total sugars in the bagasse divided by 100.
Amount of litres ethanol/ha (EtOH/ha) in the bagasse: bagasse yield/ha multiplied by 0.51 (factor) multiplied by 1000 (millilitres to litres) divided by 0.78 (factor)
Amount of he sugar/ha: the yield/ha (measured) multiplied by Brix% (measured) divided by 100
Amount of litres of EtOH/ha produced: the sugar/ha value multiplied by 0.51 (factor) multiplied by 1000 divided by 0.78 (factor).

Table F 2a. Projected ethanol production from bagasse and biomass amounts (L ha^{-1})

	BAGASSE	**HG 0 kg ha^{-1} N**	**HG 200 kg ha^{-1} N**	**SG 0 kg ha^{-1} N**	**SG 200 kg ha^{-1} N**	**ss 007 0 kg ha^{-1} N**	**SS 007 200 kg ha^{-1} N**	
Dry bagasse	**Bagasse/ha**	21,84	21,91	18,84	21,73	20,48	23,94	
	Mass	2,87	2,48	2,29	2,38	2,47	3,19	
	Mass Ash	1,66	1,42	2,02	1,94	1,44	1,01	
Biomassa	**yield/ha**	17,32	18,02	14,54	17,42	16,58	19,75	
	Sugars/ha	11,31	11,15	8,92	12,01	11,22	13,21	
	EtOH/ha	7392,53	7291,58	5831,08	7849,59	7333,14	8636,99	(L EtOH/ha)

Appendix G. Compositional sugar analysis of juice through the HPLC method by the North West University

G 1

Radebe Lehlohonono Joseph
M.Eng (Chem Eng) Student
Mobile: +27836850823
Email: 22588733@nwu.ac.za

To: Gideon Van Ransburg

Date: 22 March 2017

Subject: Interpretation of HPLC analysis (Wikus's sample)

1. In almost all of the sample, a negative peak was observed at approximately six (6) minutes and nineteen (19) minutes.
    a. The peak at 19 minutes has been observed in earlier analysis, and it was concluded that this peak was associated with the mobile phase (distilled water use in the lab).
    b. Based on 1 (a), it can be said that the peak at 6 minutes maybe associated with impurities present in the received samples.
2. It is important to note that the all peaks occurring before cellubiose (7 minutes) are associated with oligomers (xylan, arabinogalctan, arabinan, etc) and may sometimes overlap for different oligomers.
    a. Based on 2, accurate quantification and identification of these oligomers is difficult at this stage.
    b. Quantification of oligomers in aqueous solution can be performed by these oligomers to their monomers, and subsequently analysing them (NREL).
3. In the current configuration of the HPLC, mannose and xylose overlap, and the peak at approximately 9.3 minutes can represent either of the two.
    a. For the purpose of these analysis, the fore mentioned peak (3) was associated with xylose.
4. The peak at 10.78 minutes was associated with arabinose, the presence of xylitol is unexpected in these samples.
5. The peak at 7.74 minutes was associated with citric acid.
6. The Peak at 14.68 was associated with acetic acid.

X Lehlohonono Radebe

Radebe Lehlohonono
M.Eng (Chem Eng) Student

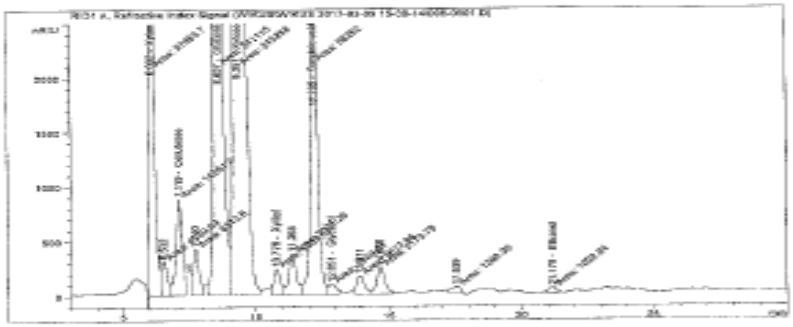
G 2

Data File C:\CHEM32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\009-0901.D
Sample Name: RS-0

Acq. Operator : Joseph
Acq. Instrument : Instrument 1
Injection date : 2017/03/10 01:51:38 AM
Seq. Line : 9
Location : Vial 8
Inj : 1
Inj Volume : 10 uL

Acq. Method : C:\Chem32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\BRIX&H HPAEL ACIDS 2016.M
Last changed : 2016/06/15 09:35:29 AM by Hestie
Analysis Method : C:\Chem32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\009-0901.D\DA.M (BRIX&H HPAEL ACIDS 2016.M)
Last changed : 2017/03/22 12:07:21 PM by Joseph (modified after loading)
Method Info : Method for use with Rainex RPX-870 column according to HPAEL method:
- Mobile phase: 0.006 M H2SO4
- Flow rate: 0.6 ml /min
- Injection volume: 10.00 µl
- Column temp = 55 °C
- RID temp: 55 °C

Sample Info : 1:24 dilution



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2016/06/31 11:06:55 AM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Instrument 1 2017/03/22 01:01:20 PM Joseph Page 1 of 2

Data File C:\CHEM32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\009-0901.D
Sample Name: RS-0

Signal 1: RRI A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	5.001		0.0000	0.00000	0.0000	Glucan
2	5.091		0.0000	0.00000	0.0000	Mannan
3	5.995		0.0000	0.00000	0.0000	Arabinan
4	6.033		0.0000	0.00000	0.0000	Arabinogalactan
5	6.092	MF	0.1387	9.70837e4	14.0410	Xylan
6	6.932	MF	0.2415	4530.48828	0.6554	?
7	7.319	MF	0.2765	1.45514e4	2.1050	Cellubiose
8	7.360		0.0000	0.00000	0.0000	Sucrose
9	7.740	MF	0.2474	6313.79932	0.9133	?
10	8.017		0.0000	0.00000	0.0000	Citric Acid
11	8.807	MF	0.3280	2.43135e5	34.8791	Glucose
12	9.336		0.0000	0.00000	0.0000	Mannose
13	9.351	MF	0.2816	2.45258e5	35.4788	Xylose
14	9.401		0.0000	0.00000	0.0000	Galactose
15	9.564		0.0000	0.00000	0.0000	Fructose
16	9.675		0.0000	0.00000	0.0000	Mannitol
17	9.909		0.0000	0.00000	0.0000	Sorbitol
18	10.254		0.0000	0.00000	0.0000	Arabitose
19	10.776	MF	0.2931	4069.03931	0.5915	Xylitol
20	11.948	MF	0.3165	7071.28394	1.0329	?
21	12.205	MF	0.2842	5.62629e4	8.4280	Succinic acid
22	12.565		0.0000	0.00000	0.0000	Lactic acid
23	12.851	MF	0.3245	1849.81250	0.2676	Glycerol
24	13.911	MF	0.3586	3617.85889	0.5259	?
25	14.680	MF	0.3507	5173.76904	0.7484	?
26	15.147		0.0000	0.00000	0.0000	Acetic acid
27	15.929		0.0000	0.00000	0.0000	Acetic acid
28	17.632		0.0000	0.00000	0.0000	1,3-Propandiol
29	17.939	MF	0.3759	1389.39441	0.1991	?
30	18.863		0.0000	0.00000	0.0000	Methanol
31	21.179	MF	0.5480	1023.84259	0.1480	Ethanol
32	23.521		0.0000	0.00000	0.0000	Butyric acid
33	23.465		0.0000	0.00000	0.0000	Acetone
34	26.283		0.0000	0.00000	0.0000	Propan-2-ol
35	29.545		0.0000	0.00000	0.0000	Butan-2-ol
36	36.668		0.0000	0.00000	0.0000	Butan-1-ol
37	20.195		0.0000	0.00000	0.0000	HMF

Totals : 6.91288e5

10 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Xylan)
Warning : Invalid calibration curve, (Cellubiose)
Warning : Invalid calibration curve, (Glucose)
Warning : Invalid calibration curve, (Xylose)
Warning : Invalid calibration curve, (Xylitol)
Warning : Invalid calibration curve, (Succinic acid)
Warning : Invalid calibration curve, (Glycerol)
Warning : Invalid calibration curve, (Ethanol)

*** End of Report ***

Instrument 1 2017/03/22 01:01:28 PM Joseph Page 2 of 2

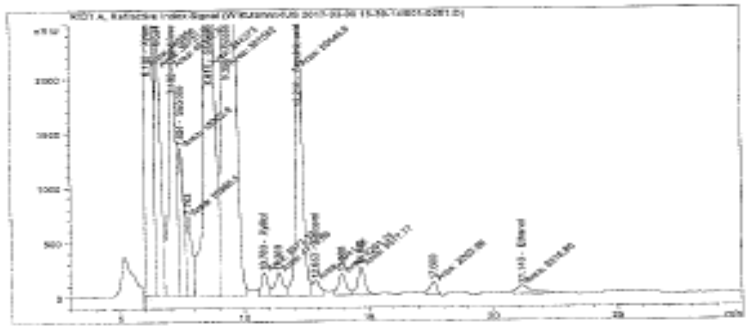
G 3

Data File C:\CHEM32\1\DATA\WINUS\WINUS 2017-03-09 15-39-14\001-0001.D
Sample Name: 007/D

Acq. Operator : Joseph
Acq. Instrument : Instrument 1
Injection Date : 2017/03/09 06:01:55 PM
Seq. Line : 2
Location : Vial 1
Inj : 1
Inj Volume : 10 µl

Acq. Method : C:\Chem32\1\DATA\WINUS\WINUS 2017-03-09 15-39-14\AMINEX HREL ACIDS 2016.M
Last changed : 2016/06/15 09:35:29 AM by Neelie
Analysis Method : C:\CHEM32\1\DATA\WINUS\WINUS 2017-03-09 15-39-14\001-0001.D\DA.M (AMINEX HREL ACIDS 2016.M)
Last changed : 2017/03/20 12:34:25 PM by Joseph (modified after loading)
Method Info : Method for use with Aminex HPX-87H column according to HREL method:
- Mobile phase: 0.005 M H2SO4
- Flow rate: 0.6 mL /min
- Injection volume: 10.00 µl
- Column temp : 55 °C
- RID temp: 55 °C

Sample Info : 1:24 dilution



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2016/09/21 11:08:55 AM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Instrument 1 2017/03/22 12:28:02 PM Joseph

Page 1 of 2

Data File C:\CHEM32\1\DATA\WINUS\WINUS 2017-03-09 15-39-14\001-0001.D
Sample Name: 007/D

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	5.801		0.0000	0.00000	0.0000	Glycan
2	5.891		0.0000	0.00000	0.0000	Mannan
3	5.995		0.0000	0.00000	0.0000	Arabinan
4	6.003		0.0000	0.00000	0.0000	Arabinogalactan
5	6.102	BV	0.1979	1.46556e6	16.3963	Xylan
6	6.536	PM	0.2503	3.67316e6	4.0995	?
7	7.132	PM	0.3069	4.63476e6	5.1884	Cellobiose
8	7.454	PM	0.2219	1.80029e6	2.0033	Sucrose
9	7.763	PM	0.2524	1.09659e6	1.2238	?
10	8.037		0.0000	0.00000	0.0000	Citric Acid
11	8.454	PM	0.2542	2.44273e6	27.3628	Glucose
12	9.336		0.0000	0.00000	0.0000	Mannose
13	9.366	PM	0.3819	3.03553e6	34.3251	Xylose
14	9.401		0.0000	0.00000	0.0000	Galactose
15	9.564		0.0000	0.00000	0.0000	Fructose
16	9.675		0.0000	0.00000	0.0000	Mannitol
17	9.809		0.0000	0.00000	0.0000	Sorbitol
18	10.264		0.0000	0.00000	0.0000	Arabinose
19	10.780	MF	0.2845	3573.16658	0.3988	Xylitol
20	11.349	PM	0.3827	4776.68988	0.5331	?
21	12.213	MF	0.2988	5.94468e4	6.6347	Succinic acid
22	12.545		0.0000	0.00000	0.0000	Lactic acid
23	12.853	MF	0.3643	3941.65820	0.3283	Glycerol
24	13.838	MF	0.3726	4291.23779	0.4788	?
25	14.681	MF	0.3262	5017.71396	0.5600	?
26	15.147		0.0000	0.00000	0.0000	Acetic acid
27	15.323		0.0000	0.00000	0.0000	Acetic acid
28	17.832		0.0000	0.00000	0.0000	1,3-Propanediol
29	17.800	MF	0.3279	2233.96094	0.2660	?
30	18.863		0.0000	0.00000	0.0000	Methanol
31	20.343	PM	0.7268	3516.86475	0.3925	Ethanol
32	23.621		0.0000	0.00000	0.0000	Butyric acid
33	23.695		0.0000	0.00000	0.0000	Acetone
34	26.383		0.0000	0.00000	0.0000	Propan-2-ol
35	29.565		0.0000	0.00000	0.0000	Butan-2-ol
36	36.656		0.0000	0.00000	0.0000	Butan-1-ol
37	38.195		0.0000	0.00000	0.0000	HMF
Totals :				8.95888e6		

11 Warnings or Errors (10 first messages follow) :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Xylan)
Warning : Invalid calibration curve, (Cellubiose)
Warning : Invalid calibration curve, (Sucrose)
Warning : Invalid calibration curve, (Glucose)
Warning : Invalid calibration curve, (Xylose)
Warning : Invalid calibration curve, (Xylitol)
Warning : Invalid calibration curve, (Succinic acid)
Warning : Invalid calibration curve, (Glycerol)

*** End of Report ***

Instrument 1 2017/03/22 12:28:02 PM Joseph

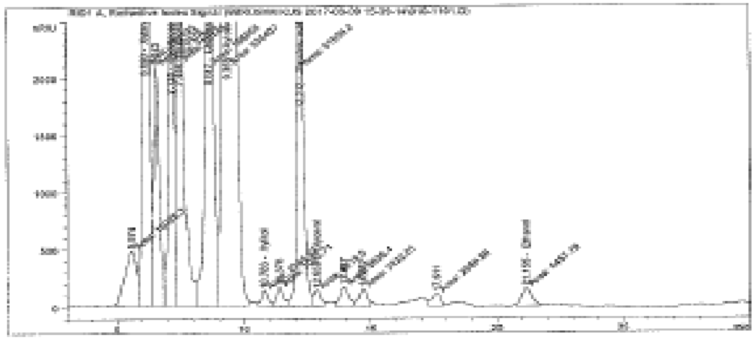
Page 2 of 2

G 4

Data File C:\CHEM32\1\DATA\MIXUS\MIXUS 2017-03-09 15-39-14\010-1101.D
Sample Name: 007/208

Acq. Operator : Joseph
Acq. Instrument : Instrument 1
Injection Date : 2017/03/10 03:54:31 AM
Seq. Line : 11
Location : Vial 18
Inj : 1
Inj Volume : 10 µl
Acq. Method : C:\Chem32\1\DATA\MIXUS\MIXUS 2017-03-09 15-39-14\AMINEX HPX. ACIDS 2016.M
Last changed : 2016/06/13 09:35:29 AM by Mestie
Analysis Method : C:\CHEM32\1\DATA\MIXUS\MIXUS 2017-03-09 15-39-14\010-1101.D\DA.M (AMINEX HPXL ACIDS 2016.M)
Last changed : 2017/03/22 01:06:23 PM by Joseph
(modified after loading)
Method Info : Method for use with Aminex HPX-87H column according to NREL method:
- Mobile phase: 0.005 M H2SO4
- Flow rate: 0.6 ml /min
- Injection volume: 10.00 µl
- Column temp : 55 °C
- RID temp: 55 °C

Sample Info : 1:24 dilution



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2015/10/31 11:08:55 AM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Instrument 1 2017/03/22 01:08:05 PM Joseph   Page 1 of 2

Data File C:\CHEM32\1\DATA\MIXUS\MIXUS 2017-03-09 15-39-14\010-1101.D
Sample Name: 007/208

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	5.574	BV	0.5241	1.50290e4	1.2184	?
2	5.801		0.0000	0.00000	0.0000	Glucan
3	5.891		0.0000	0.00000	0.0000	Mannan
4	5.995		0.0000	0.00000	0.0000	Arabinan
5	6.003		0.0000	0.00000	0.0000	Arabinogalactan
6	6.080	VV	0.1693	2.95135e5	23.9334	Xylan
7	6.562	VV	0.2426	3.04760e4	2.4709	?
8	7.135	VV	0.2520	3.78860e4	3.0715	Cellobiose
9	7.466	VV	0.2556	7.91450e4	6.4168	Sucrose
10	8.317		0.0000	0.00000	0.0000	Citric Acid
11	8.612	VV	0.2390	1.66858e5	13.5139	Glucose
12	9.326		0.0000	0.00000	0.0000	Mannose
13	9.356	VV	0.2620	5.34403e5	43.3270	Xylose
14	9.481		0.0000	0.00000	0.0000	Galactose
15	9.564		0.0000	0.00000	0.0000	Fructose
16	9.676		0.0000	0.00000	0.0000	Mannitol
17	9.809		0.0000	0.00000	0.0000	Sorbitol
18	10.254		0.0000	0.00000	0.0000	Arabinose
19	10.783	VV	0.2912	2344.61523	0.1901	Xylitol
20	11.375	VV	0.3183	3092.78558	0.2507	?
21	12.332	VV	0.2859	5.19352e4	4.2110	Succinic acid
22	12.545		0.0000	0.00000	0.0000	Lactic acid
23	12.856	VV	0.3400	2819.49658	0.2286	Glycerol
24	13.927	VV	0.3861	3936.40454	0.3191	?
25	14.682	VV	0.3813	3432.00659	0.2783	?
26	15.147		0.0000	0.00000	0.0000	Acetic acid
27	15.323		0.0000	0.00000	0.0000	Acetic acid
29	17.032		0.0000	0.00000	0.0000	1,3-Propandiol
29	17.611	VP	0.4011	2599.87549	0.2108	?
30	18.093		0.0000	0.00000	0.0000	Methanol
31	21.155	BV	0.4689	4457.76906	0.3614	Ethanol
32	22.521		0.0000	0.00000	0.0000	Butyric acid
33	23.465		0.0000	0.00000	0.0000	Acetone
34	26.393		0.0000	0.00000	0.0000	Propan-2-ol
35	29.566		0.0000	0.00000	0.0000	Butan-2-ol
36	36.666		0.0000	0.00000	0.0000	Butan-1-ol
37	38.195		0.0000	0.00000	0.0000	HMF

Totals : 1.23340e6

11 Warnings or Errors (10 first messages follow) :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, [Xylan]
Warning : Invalid calibration curve, [Cellobiose]
Warning : Invalid calibration curve, [Sucrose]
Warning : Invalid calibration curve, [Glucose]
Warning : Invalid calibration curve, [Xylose]
Warning : Invalid calibration curve, [Xylitol]
Warning : Invalid calibration curve, [Succinic acid]
Warning : Invalid calibration curve, [Glycerol]

*** End of Report ***

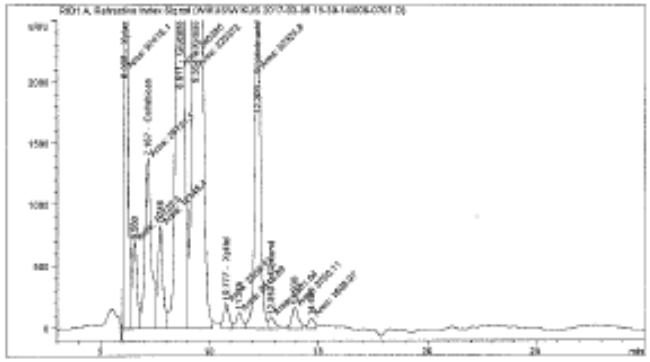
Instrument 1 2017/03/22 01:08:05 PM Joseph   Page 2 of 2

G 5

Data File C:\CHEM32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\006-0701.D
Sample Name: BG-50

Acq. Operator : Joseph Seq. Line : 7
Acq. Instrument : Instrument 1 Location : Vial 6
Injection Date : 2017/03/09 11:46:48 PM Inj : 1
Inj Volume : 10 µl
Acq. Method : C:\Chem32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\AMINEX HPEL ACIDS 2016.M
Last changed : 2016/06/15 06:35:29 AM by Heatile
Analysis Method : C:\CHEM32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\006-0701.D\DA.M (AMINEX HPEL ACIDS 2016.M)
Last changed : 2017/03/22 12:51:16 PM by Joseph
(modified after loading)
Method Info : Method for use with Aminex HPX-87H column according to NREL method:
- mobile gaase: 0.005 M H2SO4
- Flow rate: 0.6 ml /min
- Injection volume: 10.00 µl
- Column temp : 55 °C
- RID temp: 35 °C

Sample Info : 1:24 dilution



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2015/08/31 11:06:55 AM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	5.881		0.0000	0.00000	0.0000	Glucan
2	5.891		0.0000	0.00000	0.0000	Mannan
3	5.895		0.0000	0.00000	0.0000	Arabinan
4	6.063		0.0000	0.00000	0.0000	Arabinogalactan
5	6.098	PM	0.1819	9.74181e4	11.0732	Xylan
6	6.360	PM	0.2839	1.02273e4	1.1629	?
7	7.187	PM	0.3668	2.87211e4	3.2646	Cellobiose
8	7.360		0.0000	0.00000	0.0000	Sucrose
9	7.756	PM	0.2665	1.21484e4	1.3809	?
10	9.037		0.0000	0.00000	0.0000	Citric Acid
11	9.421	PM	0.2293	3.40385e5	38.6804	Glucose
12	9.326		0.0000	0.00000	0.0000	Mannose
13	9.354	PM	0.2634	3.25372e5	36.9841	Xylose
14	9.481		0.0000	0.00000	0.0000	Galactose
15	9.564		0.0000	0.00000	0.0000	Fructose
16	9.675		0.0000	0.00000	0.0000	Mannitol
17	9.809		0.0000	0.00000	0.0000	Sorbitol
18	10.254		0.0000	0.00000	0.0000	Arabinose
19	10.777	PM	0.2831	3506.41167	0.3985	Xylitol
20	11.376	PM	0.3230	2549.88367	0.2898	?
21	12.209	PM	0.2888	5.23268e4	5.9476	Succinic acid
22	12.565		0.0000	0.00000	0.0000	Lactic acid
23	12.850	MF	0.3200	1801.83816	0.2048	Glycerol
24	13.930	MF	0.3590	3700.11133	0.4206	?
25	14.694	MF	0.3697	1600.97131	0.1819	?
26	15.147		0.0000	0.00000	0.0000	Acetic acid
27	15.323		0.0000	0.00000	0.0000	Acetic acid
28	17.032		0.0000	0.00000	0.0000	1,3-Propandiol
29	18.863		0.0000	0.00000	0.0000	Methanol
30	20.728		0.0000	0.00000	0.0000	Ethanol
31	22.521		0.0000	0.00000	0.0000	Butyric acid
32	23.465		0.0000	0.00000	0.0000	Acetone
33	26.393		0.0000	0.00000	0.0000	Propan-2-ol
34	29.565		0.0000	0.00000	0.0000	Butan-2-ol
35	36.668		0.0000	0.00000	0.0000	Butan-1-ol
36	38.195		0.0000	0.00000	0.0000	HMF

Totals : 0.78764e5

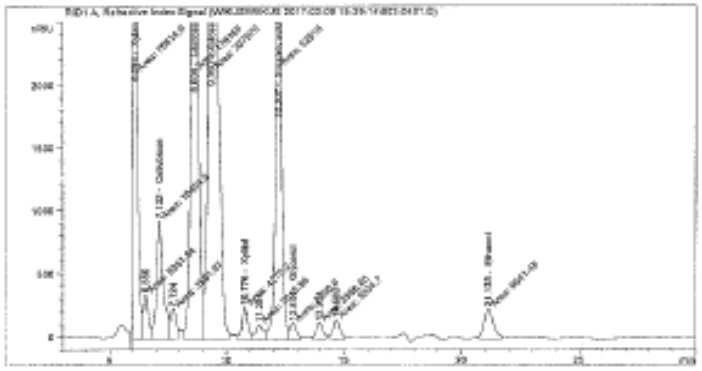
9 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Xylan)
Warning : Invalid calibration curve, (Cellubiose)
Warning : Invalid calibration curve, (Glucose)
Warning : Invalid calibration curve, (Xylose)
Warning : Invalid calibration curve, (Xylitol)
Warning : Invalid calibration curve, (Succinic acid)
Warning : Invalid calibration curve, (Glycerol)

*** End of Report ***

Data File C:\CHEM32\1\DATA\WINOS\WINOS 2017-03-09 15-39-14\003-0401.D
Sample Name: RS-150

Acq. Operator	: Joseph	Seq. Line	: 4
Acq. Instrument	: Instrument 1	Location	: Vial 3
Injection Date	: 2017/03/09 08:44:39 AM	ID)	: 1
		Inj Volume	: 30 µl
Acq. Method	: C:\Chem32\1\DATA\WINOS\WINOS 2017-03-09 15-39-14\ANIMSS NREL ACIDS 2016.M		
Last Changed	: 2016/06/15 09:35:29 AM by Laurie		
Analysis Method	: C:\CHEM32\1\DATA\WINOS\WINOS 2017-03-09 15-39-14\003-0401.D\DA.M (ANIMSS NREL ACIDS 2016.M)		
Last changed	: 2017/03/22 12:41:18 PM by Joseph (modified after loading)		
Method Info	: Method for use with Animss HPX-87H column according to NREL method - Mobile phase: 0.005 M H2SO4 - Flow rate: 0.6 ml /min - Injection volume: 10.00 µl - Column temp : 55 °C - RID temp: 55 °C		
Sample Info	: 1:20 dilution		



Area Percent Report

Sorted By	:	Signal
Calib. Data Modified	:	2015/08/31 11:08:55 AM
Multiplier	:	1.0000
Dilution	:	1.0000

Use Multiplier & Dilution Factor with ISTDs

Data File C:\CHEM32\1\DATA\WINOS\WINOS 2017-03-09 15-39-14\003-0401.D
Sample Name: RS-150

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	5.991		0.0000	0.00000	0.0000	Glucan
2	5.991		0.0000	0.00000	0.0000	Mannan
3	5.995		0.0000	0.00000	0.0000	Arabinan
4	6.002		0.0000	0.00000	0.0000	Arabinogalactan
5	6.093	MF	0.1403	7.68140e4	13.9767	Xylan
6	6.556	PM	0.3530	5363.53613	0.9511	?
7	7.133	PM	0.3265	1.84044e4	3.2649	Cellubiose
8	7.388		0.0000	0.00000	0.0000	Sucrose
9	7.724	PM	0.2473	3591.97383	0.6372	?
10	8.017		0.0000	0.00000	0.0000	Citric Acid
11	8.604	MF	0.2209	1.78160e5	31.6064	Glucose
12	9.326		0.0000	0.00000	0.0000	Mannose
13	9.590	MF	0.2784	2.07070e5	36.7336	Xylose
14	9.402		0.0000	0.00000	0.0000	Galactose
15	9.564		0.0000	0.00000	0.0000	Fructose
16	9.675		0.0000	0.00000	0.0000	Mannitol
17	9.906		0.0000	0.00000	0.0000	Sorbitol
18	10.354		0.0000	0.00000	0.0000	Arabinose
19	10.778	MF	0.2500	4275.19824	0.7584	Xylitol
20	11.394	MF	0.3801	2610.55664	0.4631	?
21	12.207	MF	0.2861	5.29160e4	9.3875	Succinic acid
22	12.545		0.0000	0.00000	0.0000	Lactic acid
23	13.644	MM	0.3100	2350.60156	0.4170	Glycerol
24	13.964	MF	0.3343	2566.65137	0.4553	?
25	14.688	MF	0.3339	5034.70093	0.5383	?
26	15.147		0.0000	0.00000	0.0000	Acetic acid
27	15.323		0.0000	0.00000	0.0000	Acetic acid
28	17.032		0.0000	0.00000	0.0000	1,3-Propanediol
29	18.963		0.0000	0.00000	0.0000	Methanol
30	21.138	MM	0.4572	6541.47607	1.1604	Ethanol
31	22.931		0.0000	0.00000	0.0000	Methyric acid
32	23.465		0.0000	0.00000	0.0000	Acetone
33	26.383		0.0000	0.00000	0.0000	Propan-2-ol
34	28.545		0.0000	0.00000	0.0000	Butan-2-ol
35	36.666		0.0000	0.00000	0.0000	Butan-1-ol
36	38.196		0.0000	0.00000	0.0000	HMF

Totals : 5.63706e5

10 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Xylan)
Warning : Invalid calibration curve, (Cellubiose)
Warning : Invalid calibration curve, (Glucose)
Warning : Invalid calibration curve, (Xylose)
Warning : Invalid calibration curve, (Xylitol)
Warning : Invalid calibration curve, (Succinic acid)
Warning : Invalid calibration curve, (Glycerol)
Warning : Invalid calibration curve, (Ethanol)

*** End of Report ***

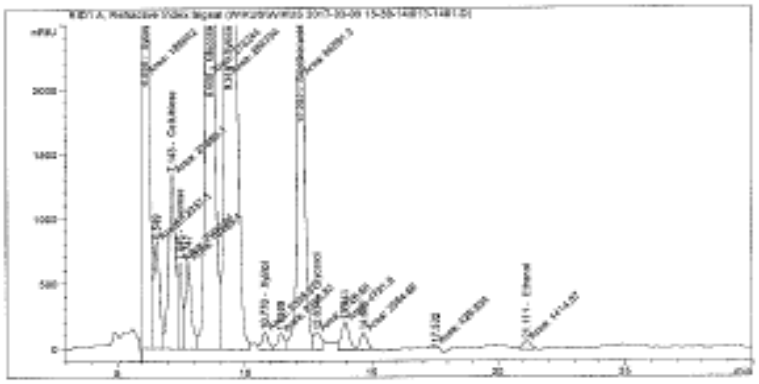
G 7

Data File C:\CHEMSTATION\1\DATA\KIEBER\WIKUS 2017-03-09 15-39-14\013-1401.D
Sample Name: BG-200

Acq. Operator : Joseph
Acq. Instrument : Instrument 1
Injection Date : 2017/03/10 06:58:42 AM
Seq. Line : 14
Location : Vial 13
Inj : 1
Inj Volume : 10 µl

Acq. Method : C:\CHEMSTATION\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\WIKUS NREL ACIDS 2016.M
Last changed : 2016/06/15 09:31:29 AM by Beckie
Analysis Method : C:\CHEMSTATION\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\013-1401.D\DA.M (NREL ACIDS 2016.M)
Last changed : 2017/03/22 01:14:50 PM by Joseph
(modified after loading)
Method Info : Method for use with Aminex HPX-87H column according to NREL method:
- Mobile phase: 0.005 M H2SO4
- Flow rate: 0.6 ml /min
- Injection volume: 10.00 µl
- Column temp : 55 °C
- RID temp: 55 °C

Sample Info : 1:24 dilution



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2015/08/31 11:08:55 AM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Instrument 1 2017/03/22 01:16:30 PM Joseph Page 1 of 2

Data File C:\CHEMSTATION\1\DATA\KIEBER\WIKUS 2017-03-09 15-39-14\013-1401.D
Sample Name: BG-200

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	5.801		0.0800	0.00000	0.0000	Glucan
2	5.891		0.0800	0.00000	0.0000	Mannan
3	5.996		0.0800	0.00000	0.0000	Arabinan
4	6.093		0.0800	0.00000	0.0000	Arabinogalactan
5	6.098	PM	0.1469	1.28642e5	15.6034	Xylan
6	6.549	PM	0.2491	1.23371e4	1.4964	?
7	7.143	PM	0.2969	2.38901e4	2.8977	Cellubiose
8	7.445	PM	0.2967	7988.64016	0.9693	Sucrose
9	7.747	PM	0.2614	1.04854e4	1.2718	?
10	8.017		0.0800	0.00000	0.0000	Citric Acid
11	8.600	PM	0.2380	2.72244e5	33.1626	Glucose
12	9.326		0.0800	0.00000	0.0000	Mannose
13	9.346	PM	0.2635	2.84704e5	34.5326	Xylose
14	9.401		0.0800	0.00000	0.0000	Galactose
15	9.564		0.0800	0.00000	0.0000	Fructose
16	9.675		0.0800	0.00000	0.0000	Mannitol
17	9.809		0.0800	0.00000	0.0000	Sorbitol
18	10.254		0.0800	0.00000	0.0000	Arabinose
19	10.770	MM	0.3866	2339.67385	0.2838	Xylitol
20	11.389	MM	0.3140	2366.01689	0.2871	?
21	12.202	MM	0.3905	6.65912e4	8.0771	Succinic acid
22	12.545		0.0000	0.00000	0.0000	Lactic acid
23	12.834	PM	0.3428	2728.69773	0.3310	Glycerol
24	13.941	PM	0.3701	4791.79941	0.5812	?
25	14.692	PM	0.3179	2584.68284	0.3135	?
26	15.167		0.0000	0.00000	0.0000	Acetic acid
27	15.823		0.0000	0.00000	0.0000	Acetic acid
28	17.032		0.0000	0.00000	0.0000	1,3-Propanediol
29	17.532	PM	0.3278	438.93417	0.0520	?
30	18.883		0.0000	0.00000	0.0000	Methanol
31	21.111	PM	0.3735	1414.07361	0.1715	Ethanol
32	22.521		0.0000	0.00000	0.0000	Butyric acid
33	23.445		0.0000	0.00000	0.0000	Acetone
34	26.383		0.0000	0.00000	0.0000	Propan-2-ol
35	29.545		0.0000	0.00000	0.0000	Butan-2-ol
36	30.686		0.0000	0.00000	0.0000	Butan-1-ol
37	30.185		0.0000	0.00000	0.0000	HMF

Totals : 8.24468e5

11 warnings or Errors (10 first messages follow) :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Xylan)
Warning : Invalid calibration curve, (Cellubiose)
Warning : Invalid calibration curve, (Sucrose)
Warning : Invalid calibration curve, (Glucose)
Warning : Invalid calibration curve, (Xylose)
Warning : Invalid calibration curve, (Xylitol)
Warning : Invalid calibration curve, (Succinic acid)
Warning : Invalid calibration curve, (Glycerol)

*** End of Report ***

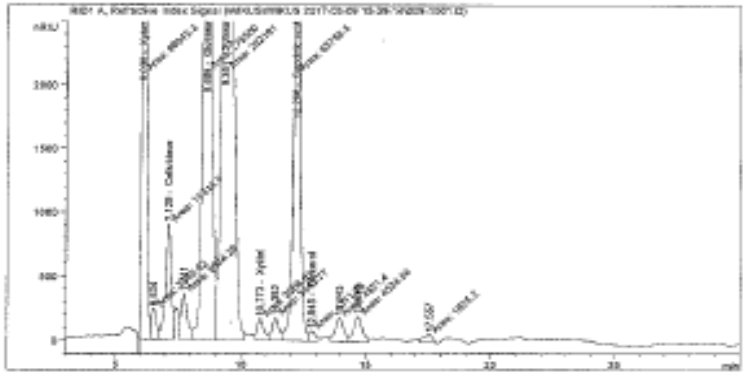
Instrument 1 2017/03/22 01:16:30 PM Joseph Page 2 of 2

G 8

Data File C:\CHEM32\1\DATA\MIKUS\WIKUS 2017-03-09 15-39-14\009-1001.D
Sample Name: MD-100

Acq. Operator : Joseph
Acq. Instrument : Instrument 1
Injection Date : 2017/03/10 03:53:04 AM
Acq. Method : C:\CHEM32\1\DATA\MIKUS\WIKUS 2017-03-09 15-39-14\AMINEX HPEL ACIDS 2016.M
Last changed : 2015/06/15 09:35:29 AM by Martin
Analysis Method : C:\CHEM32\1\DATA\MIKUS\WIKUS 2017-03-09 15-39-14\009-1001.D\DA.M (AMINEX HPEL ACIDS 2016.M)
Last changed : 2017/03/22 01:02:34 PM by Joseph (modified after loading)
Method Info : Method for use with Aminex HPX-87H column according to NREL method:
- Mobile phase: 0.005 M H2SO4
- Flow rate: 0.6 ml /min
- Injection volume: 10.00 µl
- Column temp : 55 °C
- RID temp: 55 °C

Sample Info : 1:24 dilution



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2015/08/31 11:08:55 AM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Instrument 1 2017/03/22 01:04:14 PM Joseph Page 1 of 2

Data File C:\CHEM32\1\DATA\MIKUS\WIKUS 2017-03-09 15-39-14\009-1001.D
Sample Name: MD-100

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	5.003		0.0000	0.00000	0.0000	Glucan
2	5.892		0.0000	0.00000	0.0000	Xylan
3	6.098		0.0000	0.00000	0.0000	Arabinan
4	6.003		0.0000	0.00000	0.0000	Arabinogalactan
5	6.098	MF	0.1303	9.89423e4	13.3358	Xylan
6	6.534	MF	0.2349	3340.62476	0.4503	?
7	7.125	MF	0.2646	1.43145e4	1.9286	Cellulaisge
8	7.360		0.0000	0.00000	0.0000	Sucrose
9	7.741	MF	0.2611	5634.25635	0.7594	?
10	8.017		0.0000	0.00000	0.0000	Citric Acid
11	8.606	MF	0.3279	2.75560e5	37.1338	Glucose
12	9.326		0.0000	0.00000	0.0000	Mannose
13	9.351	MF	0.2687	2.62191e5	35.3392	Xylose
14	9.401		0.0000	0.00000	0.0000	Galactose
15	9.564		0.0000	0.00000	0.0000	Fructose
16	9.675		0.0000	0.00000	0.0000	Mannitol
17	9.809		0.0000	0.00000	0.0000	Sorbitol
18	10.314		0.0000	0.00000	0.0000	Arabinose
19	10.773	MF	0.2959	3005.90991	0.4056	Xylitol
20	11.382	MF	0.3162	3443.76733	0.4642	?
21	12.288	MF	0.2837	6.37585e4	8.5936	Succinic acid
22	12.545		0.0000	0.00000	0.0000	Lactic acid
23	12.845	MF	0.2774	1311.33340	0.1767	Glycerol
24	13.863	MF	0.3963	4921.92312	0.6635	?
25	14.680	MF	0.3882	4634.56494	0.6112	?
26	15.167		0.0000	0.00000	0.0000	Acetic acid
27	15.323		0.0000	0.00000	0.0000	Acetic acid
28	17.032		0.0000	0.00000	0.0000	1,3-Propandiol
29	17.557	MF	0.4519	1626.19568	0.2192	?
30	20.063		0.0000	0.00000	0.0000	Methanol
31	20.736		0.0000	0.00000	0.0000	Ethanol
32	22.521		0.0000	0.00000	0.0000	Butyric acid
33	23.465		0.0000	0.00000	0.0000	Acetone
34	26.383		0.0000	0.00000	0.0000	Propan-2-ol
35	29.545		0.0000	0.00000	0.0000	Butan-2-ol
36	36.666		0.0000	0.00000	0.0000	Butan-2-ol
37	38.195		0.0000	0.00000	0.0000	MMF

Totals : 7.41927e5

6 Warnings or Errors :

Warning : Calibration Warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Xylan)
Warning : Invalid calibration curve, (Cellobiose)
Warning : Invalid calibration curve, (Glucose)
Warning : Invalid calibration curve, (Xylose)
Warning : Invalid calibration curve, (Xylitol)
Warning : Invalid calibration curve, (Succinic acid)
Warning : Invalid calibration curve, (Glycerol)

*** End of Report ***

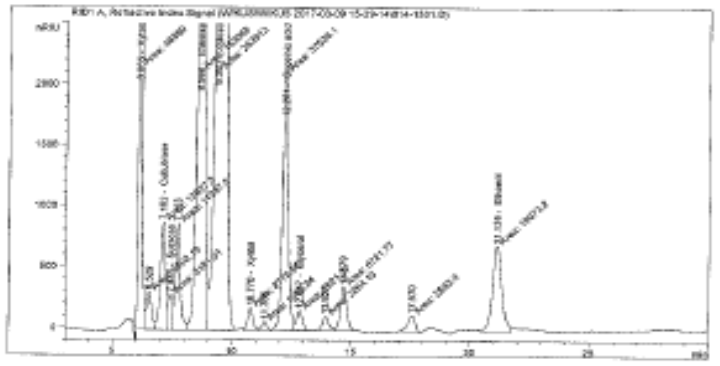
Instrument 1 2017/03/22 01:04:14 PM Joseph Page 2 of 2

G 9

Data File C:\CHEM32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\014-1501.D
Sample Name: SG-10

Acq. Operator : Joseph
Acq. Instrument : Instrument 1
Injection Date : 2017/03/10 08:56:07 AM
Seq. Line : 15
Location : Vial 14
Inj : 1
Inj Volume : 10 µl

Acq. Method : C:\chem32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\WHIBEX WHEL ACIDS 2016.M
Last changed : 2016/06/15 09:35:29 AM by Beatie
Analysis Method : C:\CHEM32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\014-1501.D\DA.M (WHIMEX WHEL ACIDS 2016.M)
Last changed : 2017/03/22 01:17:32 PM by Joseph
(modified after loading)
Method Info : Method for use with Aminex HPX-87H column according to WHEL method:
- Mobile phase: 0.005 M H2SO4
- Flow rate: 0.6 ml /min
- Injection volume: 10.00 µl
- Column temp : 55 °C
- RID temp: 55 °C



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2015/08/31 11:08:56 AM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Data File C:\CHEM32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\014-1501.D
Sample Name: SG-10

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	5.801		0.0000	0.00000	0.0000	Glucan
2	5.891		0.0000	0.00000	0.0000	Mannan
3	5.995		0.0000	0.00000	0.0000	Arabinan
4	6.083		0.0000	0.00000	0.0000	Arabinogalactan
5	6.085	VM	0.1388	4.60998e4	9.3538	Xylan
6	6.529	PM	0.2672	4602.18241	0.9167	?
7	7.102	PM	0.2608	1.30273e4	2.6427	Cellubiose
8	7.441	PM	0.1833	3181.01132	0.5885	Sucrose
9	7.723	PM	0.2178	1.13879e4	2.0281	?
10	8.017		0.0000	0.00000	0.0000	Citric Acid
11	8.598	VB	0.2290	1.53088e5	27.2654	Glucose
12	9.326		0.0000	0.00000	0.0000	Mannose
13	9.350	PM	0.3666	2.53913e5	45.2225	Xylose
14	9.601		0.0000	0.00000	0.0000	Galactose
15	9.564		0.0000	0.00000	0.0000	Fructose
16	9.675		0.0000	0.00000	0.0000	Mannitol
17	9.889		0.0000	0.00000	0.0000	Sorbitol
18	10.254		0.0000	0.00000	0.0000	Arabinose
19	10.730	PM	0.2896	2778.96345	0.4949	Xylitol
20	11.366	PM	0.2936	3092.24003	0.1945	?
21	12.201	PM	0.2758	3.70281e4	6.5948	Succinic acid
22	12.548		0.0000	0.00000	0.0000	Lactic acid
23	12.647	PM	0.2983	2821.37134	0.5025	Glycerol
24	13.927	PM	0.3220	2264.13333	0.0332	?
25	14.679	PM	0.3106	6721.76553	1.1972	?
26	15.347		0.0000	0.00000	0.0000	Acetic acid
27	15.333		0.0000	0.00000	0.0000	Acetic acid
28	17.832		0.0000	0.00000	0.0000	2,3-Propanediol
29	17.570	PM	0.3519	2683.90259	0.4780	?
30	18.863		0.0000	0.00000	0.0000	Methanol
31	21.136	VM	0.4522	1.98332e4	3.5970	Ethanol
32	22.221		0.0000	0.00000	0.0000	Butyric acid
33	23.465		0.0000	0.00000	0.0000	Acetone
34	26.383		0.0000	0.00000	0.0000	Propan-2-ol
35	29.605		0.0000	0.00000	0.0000	Butan-2-ol
36	36.606		0.0000	0.00000	0.0000	Butan-1-ol
37	38.195		0.0000	0.00000	0.0000	DMF
38	58.756	VB	0.3104	113.17132	0.0202	?

Totals : 5.61475e5

---

21 Warnings or Errors (10 first messages follow) :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Xylan)
Warning : Invalid calibration curve, (Cellubiose)
Warning : Invalid calibration curve, (Sucrose)
Warning : Invalid calibration curve, (Glucose)
Warning : Invalid calibration curve, (Xylose)
Warning : Invalid calibration curve, (Xylitol)
Warning : Invalid calibration curve, (Succinic acid)
Warning : Invalid calibration curve, (Glycerol)

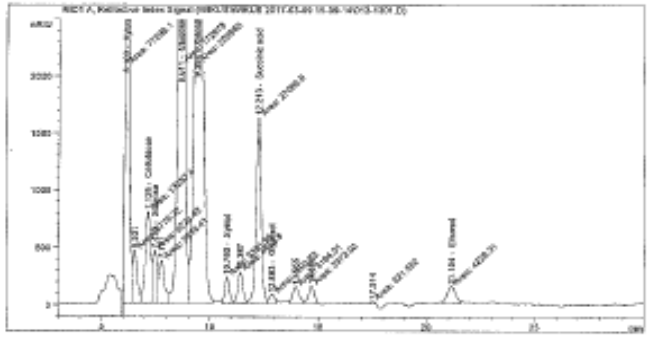
*** End of Report ***

G 10

Data File C:\CHEM32\1\DATA\MIKE\WIKUS 2017-03-08 15-39-14\012-1301.D
Sample Name: SG-150

Acq. Operator : Joseph
Acq. Instrument : Instrument 1
Injection Date : 2017/03/13 05:57:21 AM
Seq. Line : 12
Location : Vial 12
Inj : 1
Inj Volume : 10 µl
Acq. Method : G:\Chem32\1\DATA\MIKE\WIKUS 2017-03-08 15-39-14\AMINEX HPX-87H ACIDS 2016.M
Last changed : 2016/06/15 08:35:29 AM by Heatle
Analysis Method : C:\CHEM32\1\DATA\MIKE\WIKUS 2017-03-08 15-39-14\012-1301.D\DA.M (AMINEX HPX-87H ACIDS 2016.m)
Last changed : 2017/03/22 01:11:36 PM by Joseph
(Modified after loading)
Method Info : Method for use with Aminex HPX-87H column according to NREL method:
- Mobile phase: 0.005 M H2SO4
- Flow rate: 0.6 ml /min
- Injection volume: 10.00 µl
- Column temp : 55 °C
- RID temp: 55 °C

Sample Info : 1:34 dilution



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2015/08/31 11:08:55 AM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Data File C:\CHEM32\1\DATA\MIKE\WIKUS 2017-03-08 15-39-14\012-1301.D
Sample Name: SG-150

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [mRIU*s]	Area %	Name
1	5.801		0.0000	0.00000	0.0000	Glucan
2	5.891		0.0000	0.00000	0.0000	Mannan
3	5.995		0.0000	0.00000	0.0000	Arabinan
4	6.003		0.0000	0.00000	0.0000	Arabinogalactan
5	6.100	MF	0.1470	7.73981e4	14.4337	Xylan
6	6.631	MF	0.2462	6776.32324	1.2653	?
7	7.126	MF	0.2800	1.33574e4	2.4942	Cellobiose
8	7.455	MF	0.2372	6236.42236	1.1636	Sucrose
9	7.761	MF	0.2662	5816.40918	1.1018	?
10	8.817		0.0000	0.00000	0.0000	Citric Acid
11	9.411	MF	0.2297	1.72979e5	32.2995	Glucose
12	9.326		0.0000	0.00000	0.0000	Mannose
13	9.356	MF	0.2661	2.05985e5	38.4630	Xylose
14	9.481		0.0000	0.00000	0.0000	Galactose
15	9.564		0.0000	0.00000	0.0000	Fructose
16	9.675		0.0000	0.00000	0.0000	Mannitol
17	9.909		0.0000	0.00000	0.0000	Sorbitol
18	10.254		0.0000	0.00000	0.0000	Arabinose
19	10.798	MF	0.2823	3891.99705	0.6334	Xylitol
20	11.387	MF	0.2736	4456.55961	0.8321	?
21	12.213	MF	0.2773	2.70885e4	5.0540	Succinic acid
22	12.585		0.0000	0.00000	0.0000	Lactic acid
23	12.863	MF	0.2933	1400.71716	0.2616	Glycerol
24	13.955	MF	0.3612	3166.02783	0.5911	?
25	14.696	MF	0.3191	2972.03437	0.5550	?
26	15.147		0.0000	0.00000	0.0000	Acetic acid
27	15.359		0.0000	0.00000	0.0000	Acetic acid
28	17.092		0.0000	0.00000	0.0000	1,3-Propandiol
29	17.534	BB	0.2329	321.55178	0.0600	?
30	18.863		0.0000	0.00000	0.0000	Methanol
31	21.154	BB	0.4664	4238.31665	0.7915	Ethanol
32	22.521		0.0000	0.00000	0.0000	Butyric acid
33	23.465		0.0000	0.00000	0.0000	Acetone
34	24.385		0.0000	0.00000	0.0000	Propan-2-ol
35	29.545		0.0000	0.00000	0.0000	Butan-2-ol
36	30.866		0.0000	0.00000	0.0000	Butan-1-ol
37	38.195		0.0000	0.00000	0.0000	MEF
Totals :				5.35541e5		

11 Warnings or Errors (10 first messages follow) :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Xylan)
Warning : Invalid calibration curve, (Cellubiose)
Warning : Invalid calibration curve, (Sucrose)
Warning : Invalid calibration curve, (Glucose)
Warning : Invalid calibration curve, (Xylose)
Warning : Invalid calibration curve, (Xylitol)
Warning : Invalid calibration curve, (Succinic acid)
Warning : Invalid calibration curve, (Glycerol)

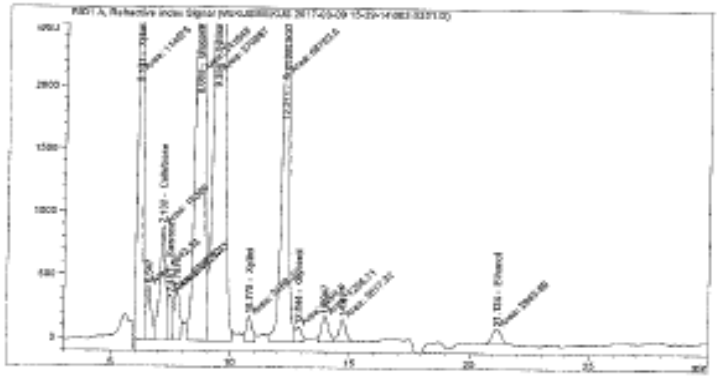
*** End of Report ***

G 11

Data File C:\CHEM32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\002-0301.D
Sample Name: SG-0

Acq. Operator : Joseph
Acq. Instrument : Instrument 1
Injection Date : 2017/03/09 07:43:16 PM
Seq. Line : 3
Location : Vial 2
Inj : 1
Inj Volume : 10 µl
Acq. Method : C:\Chem32\1\DATA\WIKUS\AMINEX 2017-03-09 15-39-14\AMINEX MN01 ACIDS 2016.M
Last changed : 2016/05/15 09:35:28 AM by Hestia
Analysis Method : C:\CHEM32\1\DATA\Wikus\WIKUS 2017-03-09 15-39-14\002-0301.D\DA.M (AMINEX MN01 ACIDS 2016.M)
Last changed : 2017/03/22 13:34:16 PM by Joseph
(modified after loading)
Method Info : Method for use with Amines HPX-87H column according to MN01 method:
- Mobile phase: 5.005 M H2SO4
- Flow rate: 0.6 ml /min
- Injection volume: 10.00 µl
- Column temp : 55 °C
- RID temp: 55 °C

Sample Info : 1:26 dilution



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2015/05/31 11:08:55 AM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Instrument 1 2017/03/22 13:37:00 PM Joseph Page 1 of 2

Data File C:\CHEM32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\002-0301.D
Sample Name: SG-0

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	5.031		0.0000	0.00000	0.0000	Glucan
2	5.091		0.0000	0.00000	0.0000	Mannan
3	5.995		0.0000	0.00000	0.0000	Arabinan
4	6.003		0.0000	0.00000	0.0000	Arabinogalactan
5	6.301	PM	0.1260	1.24975e5	11.3078	Xylan
6	6.547	BM	0.3560	6662.33105	0.6913	?
7	7.132	PM	0.2816	1.53590e4	2.0643	Cellubiose
8	7.481	BM	0.2465	5337.11802	0.7162	Sucrose
9	7.757	PM	0.2525	6120.33203	0.8213	?
10	8.017		0.0000	0.00000	0.0000	Citric Acid
11	8.439	PM	0.2313	3.41688e5	32.4270	Glucose
12	9.326		0.0000	0.00000	0.0000	Mannose
13	9.358	PM	0.2714	2.70997e5	36.3907	Xylose
14	9.401		0.0000	0.00000	0.0000	Galactose
15	9.564		0.0000	0.00000	0.0000	Fructose
16	9.675		0.0000	0.00000	0.0000	Mannitol
17	9.809		0.0000	0.00000	0.0000	Sorbitol
18	10.314		0.0000	0.00000	0.0000	Arabinose
19	10.719	PM	0.2885	3468.32925	0.4654	Xylitol
20	12.211	PM	0.2854	6.97838e4	9.3702	Succinic Acid
21	12.565		0.0000	0.00000	0.0000	Lactic Acid
22	12.844	PM	0.3019	2094.90332	0.2813	Glycerol
23	13.947	MF	0.3458	4236.20752	0.5711	?
24	14.091	MF	0.3255	3617.32446	0.4856	?
25	15.147		0.0000	0.00000	0.0000	Acetic acid
26	15.323		0.0000	0.00000	0.0000	Acetic acid
27	17.052		0.0000	0.00000	0.0000	1,3-Propanediol
28	18.863		0.0000	0.00000	0.0000	Methanol
29	21.234	MF	0.3948	2965.85913	0.3980	Ethanol
30	22.521		0.0000	0.00000	0.0000	Butyric acid
31	23.465		0.0000	0.00000	0.0000	Acetone
32	26.383		0.0000	0.00000	0.0000	Propan-2-ol
33	29.505		0.0000	0.00000	0.0000	Butan-2-ol
34	36.666		0.0000	0.00000	0.0000	Butan-1-ol
35	38.195		0.0000	0.00000	0.0000	HMF

Totals : 7.45206e5

11 Warnings or Errors (10 first messages follow) :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Xylan)
Warning : Invalid calibration curve, (Cellubiose)
Warning : Invalid calibration curve, (Sucrose)
Warning : Invalid calibration curve, (Glucose)
Warning : Invalid calibration curve, (Xylose)
Warning : Invalid calibration curve, (Xylitol)
Warning : Invalid calibration curve, (Succinic acid)
Warning : Invalid calibration curve, (Glycerol)

*** End of Report ***

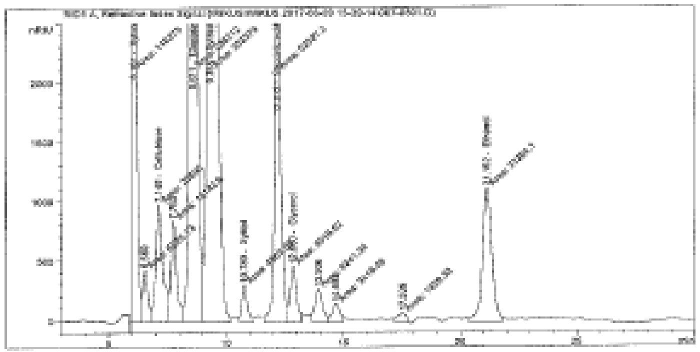
Instrument 1 2017/03/22 13:37:00 PM Joseph Page 2 of 2

G 12

Data File C:\CHEM32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\007-0801.D
Sample Name: SG-208

Acq. Operator : Joseph Seq. Line : 8
Acq. Instrument : Instrument 1 Location : Vial 3
Injection Date : 2017/03/10 12:50:13 AM Inj : 1
Inj Volume : 10 ul
Acq. Method : C:\Chem32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\AMINEX NREL ACIDS 2016.M
Last changed : 2016/06/15 09:35:29 AM by Hestie
Analysis Method : C:\CHEM32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\007-0801.D\DA.M (AMINEX NREL ACIDS 2016.M)
Last changed : 2017/03/22 12:53:59 PM by Joseph
(modified after loading)
Method Info : Method for use with Aminex HPX-87H column according to NREL method:
- Mobile phase: 0.005 M H2SO4
- Flow rate: 0.6 ml /min
- Injection volume: 10.00 ul
- Column temp : 55 °C
- RID temp: 55 °C

Sample Info : 1:24 dilution



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2015/08/31 11:08:55 AM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Data File C:\CHEM32\1\DATA\WIKUS\WIKUS 2017-03-09 15-39-14\007-0801.D
Sample Name: SG-208

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	5.801		0.0000	0.00000	0.0000	Glucan
2	5.891		0.0000	0.00000	0.0000	Mannan
3	5.895		0.0000	0.00000	0.0000	Arabinan
4	6.002		0.0000	0.00000	0.0000	Arabinogalactan
5	6.101	MF	0.1351	1.16275e5	14.4448	Xylan
6	6.560	FM	0.2416	6225.12895	0.7733	?
7	7.166	MF	0.2613	2.08950e4	2.5958	Cellubiose
8	7.380		0.0000	0.00000	0.0000	Sucrose
9	7.763	MF	0.2803	1.31426e4	1.6327	?
10	8.017		0.0000	0.00000	0.0000	Citric Acid
11	8.613	MF	0.2294	2.36212e5	29.3444	Glucose
12	9.326		0.0000	0.00000	0.0000	Mannose
13	9.355	MF	0.2686	3.03379e5	37.6885	Xylose
14	9.401		0.0000	0.00000	0.0000	Galactose
15	9.564		0.0000	0.00000	0.0000	Fructose
16	9.676		0.0000	0.00000	0.0000	Mannitol
17	9.809		0.0000	0.00000	0.0000	Sorbitol
18	10.254		0.0000	0.00000	0.0000	Arabinose
19	10.780	MF	0.2646	4863.57959	0.6042	Xylitol
20	12.313	MF	0.2876	5.25273e4	6.5254	Succinic acid
21	12.646		0.0000	0.00000	0.0000	Lactic acid
22	12.860	MF	0.2855	8518.62012	1.0583	Glycerol
23	13.526	MF	0.3782	6241.33691	0.7754	?
24	14.664	MF	0.3741	3418.08105	0.4246	?
25	15.147		0.0000	0.00000	0.0000	Acetic acid
26	15.323		0.0000	0.00000	0.0000	Acetic acid
27	17.032		0.0000	0.00000	0.0000	1,3-Propandiol
28	17.539	MF	0.4380	1929.33472	0.2397	?
29	18.043		0.0000	0.00000	0.0000	Methanol
30	21.152	MF	0.4626	3.12641e4	3.8839	Ethanol
31	22.521		0.0000	0.00000	0.0000	Butyric acid
32	23.465		0.0000	0.00000	0.0000	Acetone
33	26.303		0.0000	0.00000	0.0000	Propan-2-ol
34	29.545		0.0000	0.00000	0.0000	Butan-2-ol
35	36.666		0.0000	0.00000	0.0000	Butan-1-ol
36	38.195		0.0000	0.00000	0.0000	HMF
37	59.038	VBA	0.1339	12.30164	0.002e-3	?

Totals : 8.04963e5

10 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Xylan)
Warning : Invalid calibration curve, (Cellubiose)
Warning : Invalid calibration curve, (Glucose)
Warning : Invalid calibration curve, (Xylose)
Warning : Invalid calibration curve, (Xylitol)
Warning : Invalid calibration curve, (Succinic acid)
Warning : Invalid calibration curve, (Glycerol)
Warning : Invalid calibration curve, (Ethanol)

*** End of Report ***

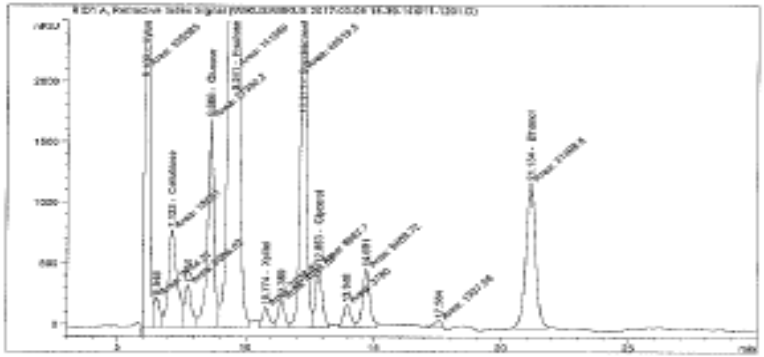
G 13

Data File c:\CEM32\1\DATA\MIKES\MIKES 2017-03-09 15-39-14\011-1281.D
Sample Name: SD-100

Acq. Operator : Joseph
Acq. Instrument : Instrument 1
Injection Date : 3/17/03/10 04:55:55 AM
Acq. Method : C:\CEM32\1\DATA\MIKES\MIKES 2017-03-09 15-39-14\AMINEX HPX-87H ACIDS 2016.M
Last changed : 2016/08/15 09:35:28 AM by Austin
Analysis Method : C:\CEM32\1\DATA\MIKES\MIKES 2017-03-09 15-39-14\011-1281.D\DA.M (AMINEX HPX-87H ACIDS 2016.M)
Last changed : 2017/03/22 01:09:13 PM by Joseph (modified after loading)
Method Info : Method for use with Amtrex HPX-87H column according to NREL method:
- Mobile phase: 0.005 M H2SO4
- Flow rate: 0.6 ml /min
- Injection volume: 10.00 µl
- Column temp : 60 °C
- RID temp: 55 °C

Rev. Line : 32
Location : Vial 11
Inj : 1
Inj Volume : 10 µl

Sample Info : 1:34 dilution



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2016/08/31 11:08:55 AM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Data File C:\CEM32\1\DATA\MIKES\MIKES 2017-03-09 15-39-14\011-1301.D
Sample Name: SD-100

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	5.893		0.0000	0.00000	0.0000	Glucan
2	5.893		0.0000	0.00000	0.0000	Mannan
3	5.893		0.0000	0.00000	0.0000	Arabinan
4	6.003		0.0000	0.00000	0.0000	Acetylgalactan
5	6.108	PM	0.1378	1.03585e5	25.3177	Xylan
6	6.568	MM	0.2946	3744.28782	0.9152	?
7	7.123	MM	0.3902	1.85810e4	4.5415	Cellubiose
8	7.360		0.0000	0.00000	0.0000	Sucrose
9	7.754	BM	0.2949	6886.03244	1.6835	?
10	8.017		0.0000	0.00000	0.0000	Citric Acid
11	8.606	MM	0.2602	2.73507e4	6.6848	Glucose
12	9.329		0.0000	0.00000	0.0000	Mannose
13	9.354		0.0000	0.00000	0.0000	Xylose
14	9.401		0.0000	0.00000	0.0000	Galactose
15	9.541	MM	0.4138	1.41089e5	34.4868	Fructose
16	9.676		0.0000	0.00000	0.0000	Mannitol
17	9.809		0.0000	0.00000	0.0000	Sorbitol
18	10.254		0.0000	0.00000	0.0000	Arabinose
19	10.774	PM	0.3858	3038.60859	0.7427	Xylitol
20	11.389	PM	0.3850	4041.62710	0.9878	?
21	12.211	PM	0.2788	4.45198e4	11.3702	Succinic acid
22	12.949		0.0000	0.00000	0.0000	Lactic acid
23	13.893	PM	0.2855	6987.69824	2.1967	Glycerol
24	13.968	PM	0.3333	3790.30464	0.9239	?
25	14.891	PM	0.3286	9409.73266	2.3194	?
26	15.147		0.0000	0.00000	0.0000	Acetic acid
27	15.323		0.0000	0.00000	0.0000	Acetic acid
28	17.033		0.0000	0.00000	0.0000	1,3-Propanediol
29	17.564	PM	0.3733	1557.57544	0.3169	?
30	18.883		0.0000	0.00000	0.0000	Methanol
31	21.134	PM	0.4389	3.14698e4	7.6835	Ethanol
32	22.521		0.0000	0.00000	0.0000	Butyric acid
33	23.465		0.0000	0.00000	0.0000	Acetone
34	24.383		0.0000	0.00000	0.0000	Propan-2-ol
35	28.545		0.0000	0.00000	0.0000	Butan-2-ol
36	36.666		0.0000	0.00000	0.0000	Butan-1-ol
37	38.195		0.0000	0.00000	0.0000	HMF

Totals : 4.09139e5

10 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Xylan)
Warning : Invalid calibration curve, (Cellubiose)
Warning : Invalid calibration curve, (Glucose)
Warning : Invalid calibration curve, (Fructose)
Warning : Invalid calibration curve, (Xylitol)
Warning : Invalid calibration curve, (Succinic acid)
Warning : Invalid calibration curve, (Glycerol)
Warning : Invalid calibration curve, (Ethanol)

*** End of Report ***

Appendix H. Compositional content of analysed sugars

Table H 1. A summary of the HPLC Analysis of juice (NWU, 2017)

Run #	Discription: genotype & N appl	Sucrose	Xylose	Arabinose	Succinic acid	Glycerol	Acetic acid	Methanol	Ethanol
#1	**ss 007-0**	6.415E+04	3.076E+05	3573	5.94E+04	2941	5017	2203	3516
#2	**ss 007-50**	1.671E+05	4.688E+05	3135	6.61E+04	2438	2404	3433	1554
#3	**ss 007-100**	6.836E+04	4.729E+05	5214	5.75E+04	2221	1006	2373	1704
#4	**ss 007-150**	1.938E+04	3.687E+05	3955	4.92E+04	3677	13916	1994	6796
#5	**ss 007-200**	1.170E+05	5.344E+05	2344	5.19E+04	2819	3432	1994	6796
#6	**HG-0**	1.455E+04	2.453E+05	4089	5.83E+04	1849	5173	1369	1022
#7	**HG-50**	2.872E+04	3.254E+05	3506	5.23E+04	1801	1608		
#8	**HG-100**	1.431E+04	2.622E+05	3008	6.38E+04	1311	4534	1626	
#9	**HG-150**	1.840E+04	2.071E+05	4275	5.30E+04	2350	3034		6541
#10	**HG-200**	3.179E+04	2.847E+05	2339	6.66E+04	2728	2584		1414
#11	**SG-0**	2.065E+04	2.709E+05	3468	6.88E+04	2094	3617		2965
#12	**SG-50**	1.701E+04	2.539E+05	2778	3.70E+04	2821	6721	2683	19073
#13	**SG-100**	1.858E+04	1.411E+05	3038	4.65E+04	8987	9489	1337	31469
#14	**SG-150**	1.958E+04	2.060E+05	3391	2.71E+04	1400	2972		4228
#15	**SG-200**	2.090E+04	3.034E+05	4863	5.25E+04	8518	3418	1929	31264

Table H 2. TSS contents of genotypes at different nitrogen fertiliser levels - data for figures 39 – 45/ Appendices 54 - 61

Run #	Naam	Sucrose	Citric acid	Glucose	Xylose	Arabinose	Succinic acid	Glycerol	Acetic acid	Methanol	Ethanol
1	**007/0**	11,62	1,91	51,03	62,19	0,56	10,53	0,5	2,02	0,95	1,52
2	**007/50**	30,27	0	101,38	94,79	0,49	11,7	0,41	0,97	1,48	0,67
3	**007/100**	12,39	3,08	117,15	95,63	0,81	10,18	0,38	0,41	1,02	0,74
4	**007/150**	3,51	0	72,6	74,55	0,62	8,72	0,62	5,62	0,86	2,93
5	**007/200**	21,2	0	34,8	108,06	0,37	9,2	0,48	1,38	0,86	2,93
6	**HG-0**	2,64	1,1	50,37	49,59	0,64	10,32	0,31	2,09	0,59	0,44
7	**HG-50**	5,2	2,12	71,11	65,79	0,55	9,27	0,3	0,65	0	0
8	**HG-100**	2,59	0,98	57,55	53,02	0,47	11,29	0,22	1,83	0,7	0
9	**HG-150**	3,33	0,63	37,22	41,87	0,67	9,38	0,4	1,22	0	2,82
10	**HG-200**	5,76	1,83	57,08	57,57	0,36	11,79	0,46	1,04	0	0,61
11	**SG-0**	3,74	1,07	50,48	54,77	0,54	12,18	0,35	1,46	0	1,28
12	**SG-50**	3,08	1,99	31,98	51,34	0,43	6,56	0,48	2,71	1,16	8,23
13	**SG-100**	3,37	1,06	5,72	28,53	0,47	8,24	1,52	3,83	0,58	13,57
14	**SG-150**	3,55	1,03	36,13	41,65	0,53	4,79	0,24	1,2	0	1,82
15	**SG-200**	3,79	2,29	49,34	61,34	0,76	9,3	1,44	1,38	0,83	13,49

Table H 3. Breakdown of TSS of different genotypes at different nitrogen fertiliser levels - data for figures 39 – 45 / Appendices 54 - 61

															etanol/ha	4,183434985	
genotype	**N_2 added**	**Sucrose**	**Citric acid**	**Glu-cose**	**Xylose**	**Arabi-nose**	**Succinic acid**	**Glycerol**	**Acetic acid**	**Methanol**	**Ethanol**	**Fermentable (g/L)**	**juice yield (ton/ha)**	**juice yield (kg/ha)**	**ferm sugar yield (kg/ha)**	**(kg/ha)**	**L EtOH/ha**	**Bagasse sugars (g/L)**
ss 007	0	11,62	1,91	51,03	62,19	0,56	10,53	0,5	2,02	0,95	1,52	62,65	4,36	4360	273	139,61	176,72	62,75
	50	30,27	0	101,38	94,79	0,49	11,7	0,41	0,97	1,48	0,67	131,65	8,68	8680	1143	584,06	739,31	95,28
	100	12,39	3,08	117,15	95,63	0,81	10,18	0,38	0,41	1,02	0,74	129,54	6,05	6050	784	400,57	507,05	96,44
	150	3,51	0	72,6	74,55	0,62	8,72	0,62	5,62	0,86	2,93	76,11	6,29	6290	479	244,69	309,73	75,17
	200	21,2	0	34,8	108,06	0,37	9,2	0,48	1,38	0,86	2,93	56	9,44	9440	529	270,19	342,02	108,43
																etanol/ha	1,745719682	
genotype	**N_2 added**	**Sucrose**	**Citric acid**	**Glu-cose**	**Xylose**	**Arabi-nose**	**Succinic acid**	**Glyce--rol**	**Acetic acid**	**Methanol**	**Etha-nol**	**Fermen-table (g/L)**	**juice yield (ton/ha)**	**juice yield (kg/ha)**	**ferm sugar yield (kg/ha)**	**(kg/ha)**	**L EtOH/ha**	**Bagasse sugars (g/L)**
HG	0	2,64	1,1	50,37	49,59	0,64	10,32	0,31	2,09	0,59	0,44	53,01	5,83	5830	309	157,96	199,95	50,23
	50	5,2	2,12	71,11	65,79	0,55	9,27	0,3	0,65	0	0	76,31	7,07	7070	540	275,75	349,05	66,34
	100	2,59	0,98	57,55	53,02	0,47	11,29	0,22	1,83	0,7	0	60,14	8,82	8820	530	271,11	343,18	53,49
	150	3,33	0,63	37,22	41,87	0,67	9,38	0,4	1,22	0	2,82	40,55	9,13	9130	370	189,22	239,52	42,54
	200	5,76	1,83	57,08	57,57	0,36	11,79	0,46	1,04	0	0,61	62,84	5,64	5640	354	181,15	229,30	57,93
																etanol/ha	0,15786865	
genotype	**N_2 added**	**Sucrose**	**Citric acid**	**Glu-cose**	**Xylose**	**Arabi-nose**	**Succinic acid**	**Glyce-rol**	**Acetic acid**	**Methanol**	**Ethanol**	**Fermen-table (g/L)**	**juice yield (ton/ha)**	**juice yield (kg/ha)**	**ferm sugar yield (kg/ha)**	**(kg/ha)**	**L EtOH/ha**	**Bagasse sugars (g/L)**
SG	0	3,74	1,07	50,48	54,77	0,54	12,18	0,35	1,46	0	1,28	54,22	5,67	5670	307	157,13	198,90	55,31
	50	3,08	1,99	31,98	51,34	0,43	6,56	0,48	2,71	1,16	8,23	35,06	10,79	10790	378	193,35	244,75	51,77
	100	3,37	1,06	5,72	28,53	0,47	8,24	1,52	3,83	0,58	13,57	9,09	6,57	6570	60	30,52	38,64	29
	150	3,55	1,03	36,13	41,65	0,53	4,79	0,24	1,2	0	1,82	39,68	7,57	7570	300	153,53	194,34	42,18
	200	3,79	2,29	49,34	61,34	0,76	9,3	1,44	1,38	0,83	13,49	53,13	6,73	6730	358	182,76	231,34	62,1

Figure H 4. Grahical representation of xylose levels of three genotypes at five nitrogen levels

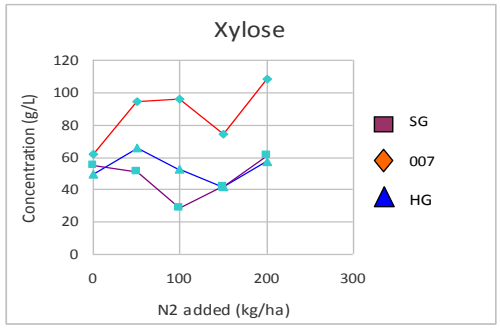


Figure H 5. Graphical representation of arabinose of three genotypes at five levels nitrogen levels

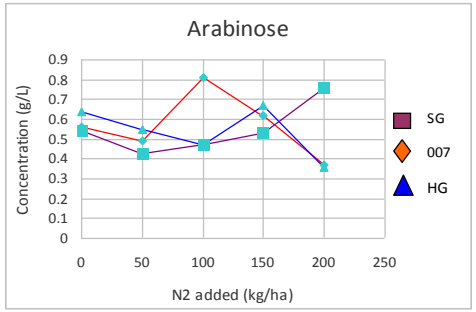


Figure H 6. Graphical representation of glycerol of three genotypes at five nitrogen levels

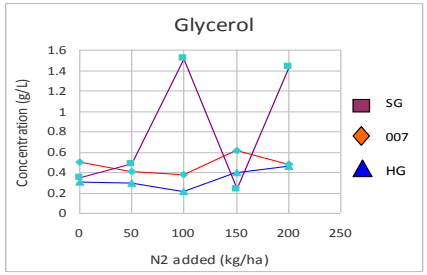


Figure H 7. Graphical representation of succinic acid of three genotypes at five nitrogen levels

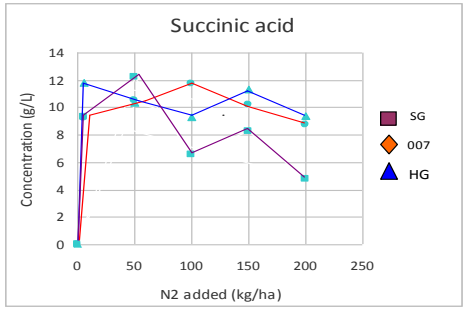


Figure H 8. Graphical representation of citric acid of three genotypes at five nitrogen levels

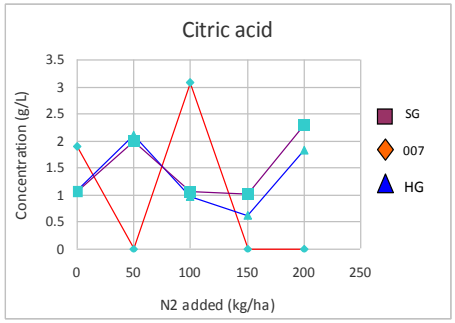


Figure H 9. Graphical representation of acetic acid of three genotypes at five nitrogen levels

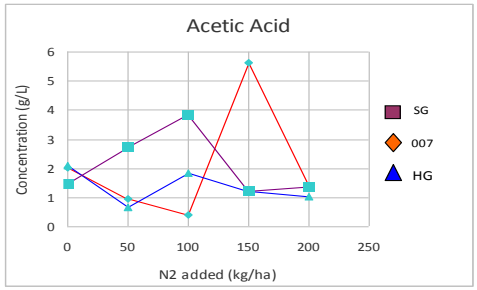


Figure H 10. Graphical representation of methanol of three genotypes at five nitrogen levels

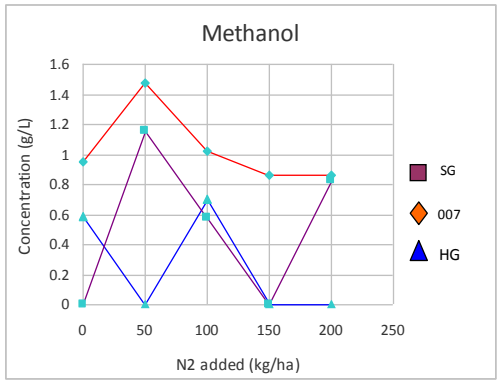
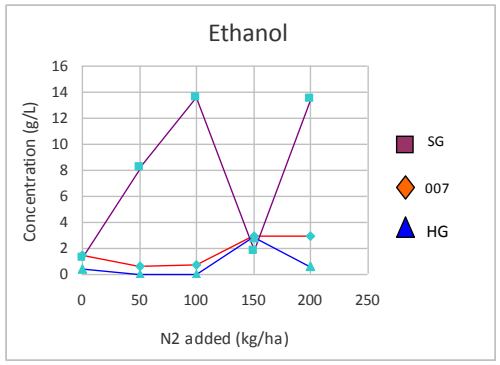


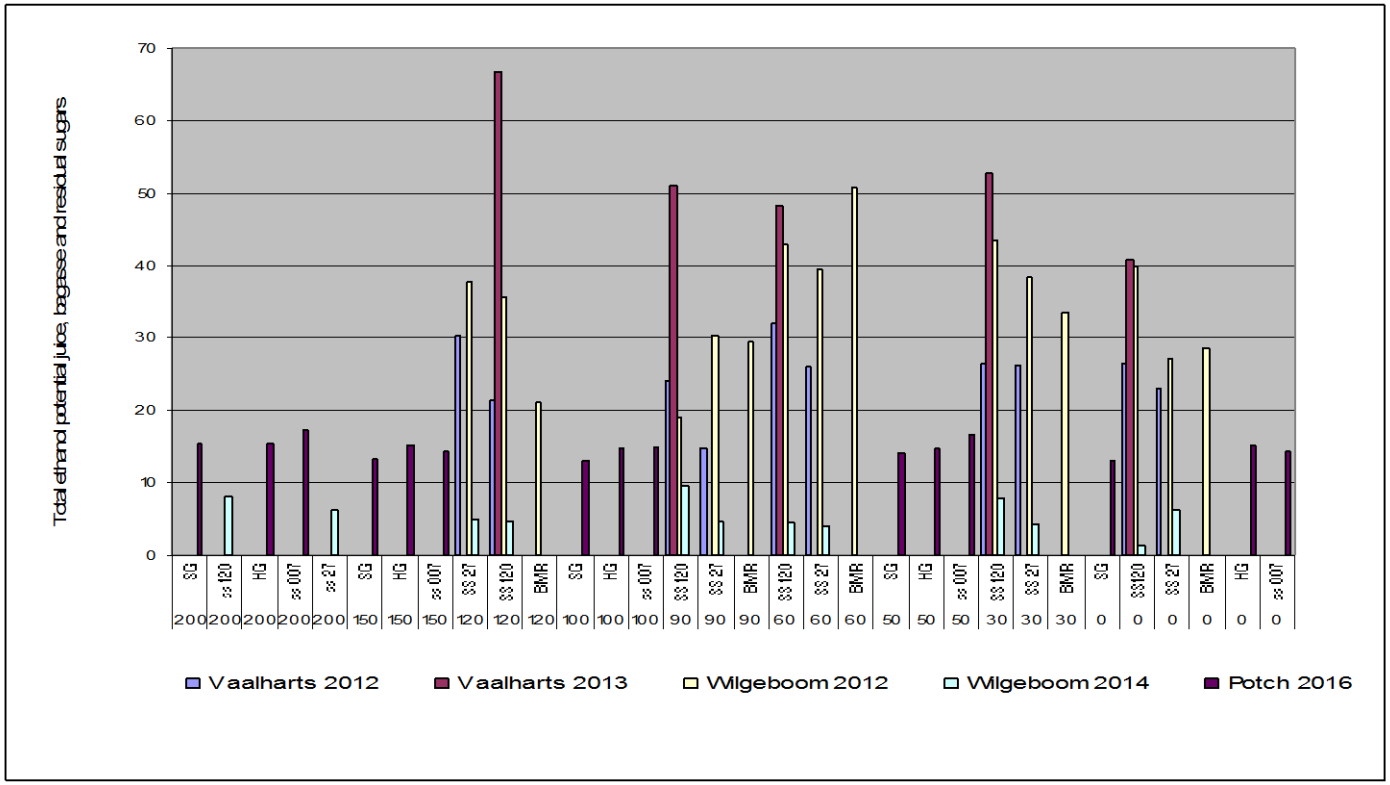
Figure H 11. Graphical representation of ethanoll of three genotypes at five nitrogen levels



Appendix I. Calculated ethanol potential

Total ethanol potential from juice, bagasse and sugars obtained during N application trial, 2011/12 to 2016/17

Figure I a. Ethanol potential from genotype trial produced from bagasse, juice and residual sugars



I b. Methods of calculations to determine potential bio-ethanol (EtOH) from the sugars in the juice and the sugars in the bagasse

Total sugars (ton/ha ~ t/ha~t ha^{-1}): total of bagasse produced plus the total of the juice produced.
Amount of ethanol (tonnes EtOH/ha) produced: total sugars (t/ha) multiplied by 0.51 (factor) multiplied by 1000 = amount of the ethanol as kg EtOH/ha.
Total amount of bio-ethanol (L EtOH/ha): juice produced plus bagasse produced divided by the amount of ethanol (kg EtOH/ha) by 0.78 (factor).
The total production of bio-ethanol (L EtOH/ha): EtOH from sugars in the juice plus EtOH from sugars in the bagasse

Table I b1. Calculated total production of bio-ethanol (L EtOH/ha) from the sugars in the juice and the sugars in the bagasse

	HG 0 kg ha^{-1} N	**HG 200 kg ha^{-1} N**	**SG 0 kg ha^{-1} N**	**SG 200 kg ha^{-1} N**	**ss 007 0 kg ha^{-1} N**	**ss 007 200 kg ha^{-1} N**
ton/ha	12,13	12,21	9,70	13,29	12,05	15,26
ton	6,19	6,23	4,95	6,78	6,14	7,78
kg	6187,49	6225,32	4947,29	6777,22	6143,23	7783,02
L	7932,68	7981,18	6342,69	8688,75	7875,93	9978,23

Appendix J. Statistical analysis: Anova's

Appendix J 1 Genotype evaluation

2011-2012

Anova Bethlehem 2011-2012

```
Genstat 64-bit Release 18.1 ( PC/Windows 8) 28 September 2017 18:04:03
Copyright 2015, VSN International Ltd.
Registered to: ARC-Grain Crops Institute

                  ____________________________________

                  Genstat Eighteenth Edition
                  Genstat Procedure Library Release PL26.1
                  ____________________________________

 1                                                SET                                [WORKINGDIRECTORY='C:/Users/mavunganidzez/Documents']
 2                                "Data                taken                from                file:                '\
-3                                C:/Users/mavunganidzez/Documents/Wikus/2012                BH                cult                coll.xls'"
 4                             DELETE                [REDEFINE=yes]                _stitle_:                TEXT                _stitle_
 5                                READ                [PRINT=*;                SETNVALUES=yes]                _stitle_
 9                                PRINT                [IPRINT=*]                _stitle_;                JUST=left

Data imported from Excel file: C:\Users\mavunganidzez\Documents\Wikus\2012 BH cult coll.xls
 on: 28-Sep-2017 18:04:36
 taken from sheet "stats data", cells A4:F69

 10                                DELETE                [REDEFINE=yes]                rep,entry,genotype,mass_t_ha,brix_%,juice_t_ha
 11                                UNITS                [NVALUES=*]
 12                FACTOR                [MODIFY=no;                NVALUES=66;                LEVELS=3;                LABELS=*;                REFERENCE=1]                rep
 13                                READ                rep;                FREPRESENTATION=ordinal

            Identifier      Values     Missing      Levels
                   rep          66           0           3

 16                FACTOR                [MODIFY=no;                NVALUES=66;                LEVELS=22;                LABELS=*;                REFERENCE=1]                entry
 17                                READ                entry;                FREPRESENTATION=ordinal

            Identifier      Values     Missing      Levels
                 entry          66           0          22
```

```
21  FACTOR [MODIFY=no; NVALUES=66; LEVELS=22; LABELS=!t('BMR','HG','L001',\
22  'p 175','p 304','p 40197','p 40220','p 893','p 895','SK','ss 001','ss 003',\
23  'ss 007','ss 008','ss 016','ss 017','ss 019','ss 020','ss 120','ss 27',\
24  'ss 506','ss 63'); REFERENCE=1] genotype
25  READ genotype; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
genotype	66	0	22

```
29  VARIATE [NVALUES=66] mass_t_ha
30  READ mass_t_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
mass_t_ha	11.29	30.99	67.56	66	0

```
37  VARIATE [NVALUES=66] brix_%
38  READ brix_%
```

Identifier	Minimum	Mean	Maximum	Values	Missing
brix_%	6.433	15.61	23.63	66	0

```
51  VARIATE [NVALUES=66] juice_t_ha
52  READ juice_t_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
juice_t_ha	0.3320	3.926	14.44	66	0

```
60
61  %PostMessage 1129; 0; 100001 "Sheet Update Completed"
62  "General Analysis of Variance"
63  BLOCK rep
64  TREATMENTS genotype
65  COVARIATE "No Covariate"
66  ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
67  PSE=diff,lsd,means; LSDLEVEL=5] brix_%
```

Analysis of variance

Variate: brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	4.22	2.11	0.16	
rep.*Units* stratum					
genotype	21	392.90	18.71	1.44	0.156
Residual	42	546.95	13.02		
Total	65	944.07			

Message: the following units have large residuals.

rep 1 *units* 15 -6.64 s.e. 2.88
rep 2 *units* 15 10.38 s.e. 2.88

Tables of means
Variate: brix_%
Grand mean 15.61

genotype	BMR	HG	L001	p 175	p 304	p 40197	p 40220
	13.54	14.76	16.62	16.67	13.27	15.82	9.43
genotype	p 893	p 895	SK	ss 001	ss 003	ss 007	ss 008
	13.77	17.41	18.18	17.81	16.87	18.26	17.41
genotype	ss 016	ss 017	ss 019	ss 020	ss 120	ss 27	ss 506
	16.90	14.54	14.83	18.80	19.58	12.97	12.52
genotype	ss 63						
	13.51						

Standard errors of means
Table genotype

rep.	3
d.f.	42
e.s.e.	2.083

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42
s.e.d.	2.946

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	5.946

Stratum standard errors and coefficients of variation
Variate: brix_%

Stratum	d.f.	s.e.	cv%
rep	2	0.310	2.0
rep.*Units*	42	3.609	23.1

```
68 "General Analysis of Variance"
69 BLOCK rep
70 TREATMENTS genotype
71 COVARIATE "No Covariate"
72 ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
73 PSE=diff,lsd,means; LSDLEVEL=5] juice_t_ha
```

Analysis of variance

Variate: juice_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

rep stratum	2	91.737	45.869	4.66	
rep.*Units* stratum					
genotype	21	220.567	10.503	1.07	0.416
Residual	42	413.548	9.846		
Total	65	725.852			

Message: the following units have large residuals.

rep 1 *units* 2	5.74	s.e.	2.50
rep 3 *units* 7	6.63	s.e.	2.50
rep 3 *units* 9	6.14	s.e.	2.50
rep 3 *units* 14	7.02	s.e.	2.50

Tables of means

Variate: juice_t_ha

Grand mean 3.93

genotype	BMR	HG	L001	p 175	p 304	p 40197	p 40220
	1.99	9.13	3.49	5.75	3.98	2.93	5.20

genotype	p 893	p 895	SK	ss 001	ss 003	ss 007	ss 008
	5.37	2.71	2.05	2.66	2.32	5.64	1.83

genotype	ss 016	ss 017	ss 019	ss 020	ss 120	ss 27	ss 506
	6.31	4.92	4.26	0.89	4.54	4.09	2.77

genotype	ss 63
	3.54

Standard errors of means

Table	genotype
rep.	3
d.f.	42
e.s.e.	1.812

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42
s.e.d.	2.562

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	5.170

Stratum standard errors and coefficients of variation

Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	1.444	36.8
rep.*Units*	42	3.138	79.9

```
"General Analysis of Variance"
BLOCK rep
TREATMENTS genotype
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
      PSE=diff,lsd,means; LSDLEVEL=5] mass_t_ha
```

Analysis of variance

Variate: mass_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	668.7	334.4	1.86	
rep.*Units* stratum					
genotype	21	4406.6	209.8	1.17	0.325
Residual	42	7541.9	179.6		
Total	65	12617.2			

Message: the following units have large residuals.

rep 3 *units* 9 26.5 s.e. 10.7

Tables of means

Variate: mass_t_ha

Grand mean 31.0

genotype	BMR	HG	L001	p 175	p 304	p 40197	p 40220
	17.0	48.6	28.2	33.5	31.7	28.5	47.3

genotype	p 893	p 895	SK	ss 001	ss 003	ss 007	ss 008
	41.0	29.8	21.9	25.9	22.5	32.3	23.0

genotype	ss 016	ss 017	ss 019	ss 020	ss 120	ss 27	ss 506
	36.6	34.0	34.8	15.3	35.1	29.5	32.0

genotype	ss 63
	33.4

Standard errors of means

Table	genotype
rep.	3
d.f.	42
e.s.e.	7.74

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42
s.e.d.	10.94

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	22.08

Stratum standard errors and coefficients of variation

Variate: mass_t_ha

Stratum	d.f.	s.e.	cv%

rep	2	3.90	12.6
rep.*Units*	42	13.40	43.2

Anova Potchefstroom 2011-2012

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-81                                                  C:/Users/mavunganidzez/Documents/Wikus/2012                Potch                cultdata.xls'"
 82                                          DELETE              [REDEFINE=yes]              _stitle_:              TEXT              _stitle_
 83                                            READ              [PRINT=*;              SETNVALUES=yes]              _stitle_
 87                                           PRINT              [IPRINT=*]              _stitle_;              JUST=left
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on: 28-Sep-2017 18:10:09
taken from sheet "stats data", cells A4:F69
 88                                                  DELETE              [REDEFINE=yes]              Rep_1,ave_brix_%
 89                                                  UNITS              [NVALUES=*]
 90                                  FACTOR              [MODIFY=no;              NVALUES=66;              LEVELS=3;              LABELS=*;              REFERENCE=1]              Rep_1
 91                                                  READ              Rep_1;              FREPRESENTATION=ordinal
    Identifier   Values     Missing     Levels
         Rep_1       66           0          3
 94                                          FACTOR              [MODIFY=yes;              NVALUES=66;              LEVELS=!(1,2,5,7,8,9,10,12,14,16,17,18,21,\
 95                                  22,23,24,25,26,27,30,31,32);              LABELS=*;              REFERENCE=1]              entry
 96                                                  READ              entry;              FREPRESENTATION=ordinal
    Identifier       Values     Missing     Levels
         entry       66           0          22
100                                 FACTOR              [MODIFY=yes;              NVALUES=66;              LEVELS=22;              LABELS=!t('BMR','HG','L001',\
101                                   'p              178','p              179','p              304','p              40220','p              40225','p              40249','p              506','P001',\
102                                  'ss              003','ss              007','ss              008','ss              016','ss              017','ss              019','ss              120','ss              27',\
103                                                  'ss              56','ss              63','sswd');              REFERENCE=1]              genotype
104                                                  READ              genotype;              FREPRESENTATION=ordinal
    Identifier       Values     Missing     Levels
      genotype       66           0          22
108                                          VARIATE              [NVALUES=66]              mass_t_ha
109                                                  READ              mass_t_ha
    Identifier      Minimum         Mean      Maximum       Values      Missing
     mass_t_ha        28.13        82.54        148.6           66            0
117                                          VARIATE              [NVALUES=66]              ave_brix_%
118                                                  READ              ave_brix_%
 Identifier      Minimum         Mean      Maximum       Values      Missing
     ave_brix_%        8.533        15.62        30.73           66            0
```

```
131                                                    VARIATE                    [NVALUES=66]                    juice_t_ha
132                                                                    READ                                        juice_t_ha
 Identifier      Minimum        Mean     Maximum      Values     Missing
          juice_t_ha         2.304       19.14       47.23          66           0
 139
140                          %PostMessage          1129;          0;          100001          "Sheet          Update          Completed"
141                                        "General                Analysis                of                Variance"
142                                                                BLOCK                                        rep
143                                                                TREATMENTS                                   genotype
144                                                    COVARIATE                    "No                         Covariate"
145                    ANOVA          [PRINT=aovtable,information,means,%cv;          FACT=32;          CONTRASTS=7;          PCONTRASTS=7;          FPROB=yes;\
146                                                    PSE=diff,lsd,means;          LSDLEVEL=5]                    ave_brix_%
```

Analysis of variance

Variate: ave_brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	12.66	6.33	0.46	
rep.*Units* stratum					
genotype	21	392.39	18.69	1.37	0.191
Residual	42	574.57	13.68		
Total	65	979.62			

Message: the following units have large residuals.

rep 1 *units* 21	-9.62	s.e.	2.95
rep 2 *units* 21	8.96	s.e.	2.95
rep 3 *units* 4	8.54	s.e.	2.95

Tables of means

Variate: ave_brix_%

Grand mean 15.62

genotype	BMR	HG	L001	p 178	p 179	p 304	p 40220
	14.23	12.89	18.06	12.86	22.22	18.88	13.72

genotype	p 40225	p 40249	p 506	P001	ss 003	ss 007	ss 008
	12.20	15.04	11.71	14.04	17.10	16.50	17.28

genotype	ss 016	ss 017	ss 019	ss 120	ss 27	ss 56	ss 63
	16.20	18.28	16.29	16.56	16.10	13.48	14.64

genotype	sswd
	15.42

Standard errors of means

Table	genotype
rep.	3
d.f.	42
e.s.e.	2.135

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42

s.e.d. 3.020

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	6.095

Stratum standard errors and coefficients of variation

Variate: ave_brix_%

Stratum	d.f.	s.e.	cv%
rep	2	0.536	3.4
rep.*Units*	42	3.699	23.7

```
147                                  "General                    Analysis                    of                  Variance"
148                                                        BLOCK                                                  rep
149                                                   TREATMENTS                                                genotype
150                                  COVARIATE                                  "No                           Covariate"
151          ANOVA  [PRINT=aovtable,information,means,%cv;  FACT=32;  CONTRASTS=7;  PCONTRASTS=7;  FPROB=yes;\
152                                  PSE=diff,lsd,means;  LSDLEVEL=5]                                            brix_%
```

Analysis of variance

Variate: brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	4.22	2.11	0.16	
rep.*Units* stratum					
genotype	21	392.90	18.71	1.44	0.156
Residual	42	546.95	13.02		
Total	65	944.07			

Message: the following units have large residuals.

rep 1 *units* 15	-6.64	s.e.	2.88
rep 2 *units* 15	10.38	s.e.	2.88

Tables of means

Variate: brix_%

Grand mean 15.61

genotype	BMR	HG	L001	p 178	p 179	p 304	p 40220
	14.54	9.43	16.62	14.76	13.77	18.18	13.54

genotype	p 40225	p 40249	p 506	P001	ss 003	ss 007	ss 008
	15.82	19.58	12.52	14.83	16.90	16.87	17.41

genotype	ss 016	ss 017	ss 019	ss 120	ss 27	ss 56	ss 63
	12.97	18.80	18.26	13.51	17.41	16.67	13.27

genotype	sswd
	17.81

Standard errors of means

Table	genotype
rep.	3
d.f.	42
e.s.e.	2.083

Standard errors of differences of means

Table	genotype
rep.	3

d.f.	42
s.e.d.	2.946

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	5.946

Stratum standard errors and coefficients of variation

Variate: brix_%

Stratum	d.f.	s.e.	cv%
rep	2	0.310	2.0
rep.*Units*	42	3.609	23.1

```
153                                  "General                    Analysis                    of                  Variance"
154                                                    BLOCK                                                      rep
155                                                    TREATMENTS                                                 genotype
156                                  COVARIATE                                 "No                              Covariate"
157          ANOVA    [PRINT=aovtable,information,means,%cv;    FACT=32;    CONTRASTS=7;    PCONTRASTS=7;    FPROB=yes;\
158                                  PSE=diff,lsd,means;                     LSDLEVEL=5]                      juice_t_ha
```

Analysis of variance

Variate: juice_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	302.04	151.02	2.31	
rep.*Units* stratum					
genotype	21	2673.50	127.31	1.95	0.033
Residual	42	2747.82	65.42		
Total	65	5723.36			

Tables of means

Variate: juice_t_ha

Grand mean 19.14

genotype	BMR	HG	L001	p 178	p 179	p 304	p 40220
	15.78	22.18	29.22	13.12	25.66	24.45	17.06

genotype	p 40225	p 40249	p 506	P001	ss 003	ss 007	ss 008
	11.17	14.11	9.50	18.21	23.01	30.37	20.22

genotype	ss 016	ss 017	ss 019	ss 120	ss 27	ss 56	ss 63
	13.63	33.31	14.78	17.09	18.14	11.39	16.42

genotype	sswd
	22.34

Standard errors of means

Table	genotype
rep.	3
d.f.	42
e.s.e.	4.670

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42
s.e.d.	6.604

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	13.328

Stratum standard errors and coefficients of variation

Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	2.620	13.7
rep.*Units*	42	8.089	42.3

```
159                          "General                    Analysis                    of                  Variance"
160                                                                  BLOCK                                   rep
161                                                                  TREATMENTS                              genotype
162                                                  COVARIATE                           "No                 Covariate"
163                ANOVA     [PRINT=aovtable,information,means,%cv;    FACT=32;    CONTRASTS=7;    PCONTRASTS=7;    FPROB=yes;\
164                                                  PSE=diff,lsd,means;         LSDLEVEL=5]                 mass_t_ha
```

Analysis of variance

Variate: mass_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	1523.4	761.7	1.08	
rep.*Units* stratum					
genotype	21	18898.7	899.9	1.28	0.245
Residual	42	29613.7	705.1		
Total	65	50035.7			

Message: the following units have large residuals.

rep 3 *units* 8 -50.2 s.e. 21.2

rep 3 *units* 13 55.2 s.e. 21.2

Tables of means

Variate: mass_t_ha

Grand mean 82.5

genotype	BMR	HG	L001	p 178	p 179	p 304	p 40220
	75.9	86.0	103.1	77.0	107.4	97.9	74.2

genotype	p 40225	p 40249	p 506	P001	ss 003	ss 007	ss 008
	60.4	76.1	57.1	75.9	95.9	118.4	89.7

genotype	ss 016	ss 017	ss 019	ss 120	ss 27	ss 56	ss 63
	61.0	112.9	69.7	81.2	76.0	69.9	63.6

genotype	sswd
	86.4

Standard errors of means

Table	genotype
rep.	3
d.f.	42
e.s.e.	15.33

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42

s.e.d.	21.68

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	43.75

Stratum standard errors and coefficients of variation

Variate: mass_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	5.88	7.1
rep.*Units*	42	26.55	32.2

Anova Rustenburg 2011-2012

Genstat 64-bit Release 19.1 (PC/Windows 8) 24 February 2020 11:49:16
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Genstat Nineteenth Edition
Genstat Procedure Library Release PL27.1

```
 1  SET [WORKINGDIRECTORY='C:/Users/belindaj/Documents'; DIAGNOSTIC=messages]
 2  "Data taken from file: 'F:/2020/anova/2012 Rustenburg cult 2012.xls'"
 3  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
 4  READ [PRINT=*; SETNVALUES=yes] _stitle_
 8  PRINT [IPRINT=*] _stitle_; JUST=left
Data imported from Excel file: F:\2020\anova\2012 Rustenburg cult 2012.xls
on: 24-Feb-2020 11:49:38
taken from sheet "Sheet1", cells A2:F67
 9  DELETE [REDEFINE=yes] rep,entry,genotype,mass_t_ha,Brix_%,juice_t_ha
10  UNITS [NVALUES=*]
11  FACTOR [MODIFY=no; NVALUES=66; LEVELS=3; LABELS=*; REFERENCE=1] rep
12  READ rep; FREPRESENTATION=ordinal
    Identifier   Values   Missing   Levels
           rep       66         0        3
15  VARIATE [NVALUES=66] entry
16  READ entry
    Identifier   Minimum   Mean   Maximum   Values   Missing
         entry     1.000  11.50     22.00       66         0
20  FACTOR [MODIFY=no; NVALUES=66; LEVELS=22; LABELS=*; REFERENCE=1] genotype
21  READ genotype; FREPRESENTATION=ordinal
```

```
            Identifier       Values      Missing       Levels
            genotype             66            0           22
25                                                VARIATE                                [NVALUES=66]                                mass_t_ha
26                                                                                  READ                                mass_t_ha
            Identifier      Minimum         Mean      Maximum       Values      Missing
            mass_t_ha         3.936        22.47        55.87           66            0
33                                                VARIATE                                [NVALUES=66]                                Brix_%
34                                                                                  READ                                Brix_%
            Identifier      Minimum         Mean      Maximum       Values      Missing
            Brix_%            11.10        17.09        24.43           66            0

47                                                VARIATE                                [NVALUES=66]                                juice_t_ha
48                                                                                  READ                                juice_t_ha
            Identifier      Minimum         Mean      Maximum       Values      Missing
            juice_t_ha         0.192        2.038        12.38           66            0  Skew

56
57                                %PostMessage          1129;          0;          10000001          "Sheet          Update          Completed"
58                                              "General          Analysis          of          Variance"
59                                                                                  BLOCK                                rep
60                                                                              TREATMENTS                                genotype
61                                                                COVARIATE                                "No          Covariate"
62                                ANOVA          [PRINT=aovtable,information,means,%cv;          FACT=32;          CONTRASTS=7;          PCONTRASTS=7;          FPROB=yes;\
63                                                                PSE=diff,lsd,means;          LSDLEVEL=5]                                Brix_%
```

Analysis of variance

Variate: Brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	58.466	29.233	5.95	
rep.*Units* stratum					
genotype	21	317.250	15.107	3.08	<.001
Residual	42	206.315	4.912		
Total	65	582.031			

Message: the following units have large residuals.

rep 1 *units* 2	4.83	s.e.	1.77
rep 3 *units* 2	-5.55	s.e.	1.77

Tables of means

Variate: Brix_%

Grand mean 17.09

genotype	1	2	3	4	5	6	7
	17.18	16.59	17.63	18.04	18.80	21.32	19.26
genotype	8	9	10	11	12	13	14
	16.64	14.18	16.87	15.43	17.10	19.10	22.21
genotype	15	16	17	18	19	20	21
	14.52	16.48	14.71	17.46	16.57	13.54	13.97
genotype	22						
	18.38						

Standard errors of means

Table	genotype
rep.	3
d.f.	42
e.s.e.	1.280

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42
s.e.d.	1.810

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	3.652

Stratum standard errors and coefficients of variation

Variate: Brix_%

Stratum	d.f.	s.e.	cv%
rep	2	1.153	6.7
rep.*Units*	42	2.216	13.0

```
64                                  "General                    Analysis                    of                  Variance"
65                                                      BLOCK                                                   rep
66                                                  TREATMENTS                                                  genotype
67                                      COVARIATE                           "No                             Covariate"
68          ANOVA       [PRINT=aovtable,information,means,%cv;      FACT=32;    CONTRASTS=7;    PCONTRASTS=7;   FPROB=yes;\
69                          PSE=diff,lsd,means;                     LSDLEVEL=5]                                 mass_t_ha
```

Analysis of variance

Variate: mass_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	1558.3	779.2	7.65	
rep.*Units* stratum					
genotype	21	3884.4	185.0	1.82	0.049
Residual	42	4277.7	101.8		
Total	65	9720.4			

Message: the following units have large residuals.

rep 2 *units* 5	22.4	s.e.	8.1
rep 2 *units* 12	18.6	s.e.	8.1

Tables of means

Variate: mass_t_ha

Grand mean 22.5

genotype	1	2	3	4	5	6	7
	21.3	41.8	16.4	15.7	24.2	22.9	24.3

genotype	8	9	10	11	12	13	14
	14.8	20.6	17.1	11.1	29.1	28.9	18.8

genotype	15	16	17	18	19	20	21

	30.1	13.1	22.2	36.4	15.6	21.1	31.8
genotype	22						
	16.9						

Standard errors of means

Table	genotype
rep.	3
d.f.	42
e.s.e.	5.83

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42
s.e.d.	8.24

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	16.63

Stratum standard errors and coefficients of variation

Variate: mass_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	5.95	26.5
rep.*Units*	42	10.09	44.9

```
70 CALCULATE juice_t_ha_trans=LOG10(juice_t_ha)
71 FSPREADSHEET [SHEET=10000001; METHOD=replace; NOUNITS=yes] juice_t_ha_trans
72 "General Analysis of Variance"
73 BLOCK rep
74 TREATMENTS genotype
75 COVARIATE "No Covariate"
76 ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
77 PSE=diff,lsd,means; LSDLEVEL=5] juice_t_ha
```

Analysis of variance

Variate: juice_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	60.980	30.490	8.60	
rep.*Units* stratum					
genotype	21	135.097	6.433	1.81	0.050
Residual	42	148.967	3.547		
Total	65	345.043			

Message: the following units have large residuals.

rep 1 *units* 9	3.78	s.e. 1.50
rep 2 *units* 2	4.68	s.e. 1.50
rep 2 *units* 6	4.71	s.e. 1.50
rep 2 *units* 12	3.59	s.e. 1.50

Tables of means

Variate: juice_t_ha

Grand mean 2.04

genotype	1	2	3	4	5	6	7
	1.18	6.43	0.96	1.34	1.98	4.48	2.56
genotype	8	9	10	11	12	13	14
	0.86	2.88	1.70	1.25	3.20	1.70	1.92
genotype	15	16	17	18	19	20	21
	2.98	0.48	0.74	3.90	1.63	0.58	0.93
genotype	22						
	1.15						

Standard errors of means

Table	genotype
rep.	3
d.f.	42
e.s.e.	1.087

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42
s.e.d.	1.538

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	3.103

Stratum standard errors and coefficients of variation

Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	1.177	57.8
rep.*Units*	42	1.883	92.4

```
 78 "General Analysis of Variance"
 79 BLOCK rep
 80 TREATMENTS genotype
 81 COVARIATE "No Covariate"
 82 ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
 83 PSE=diff,lsd,means; LSDLEVEL=5] juice_t_ha_trans
```

Analysis of variance

Variate: juice_t_ha_trans

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	2.23441	1.11721	11.92	
rep.*Units* stratum					
genotype	21	4.46467	0.21260	2.27	0.012
Residual	42	3.93521	0.09370		
Total	65	10.63429			

Message: the following units have large residuals.

rep 2 *units* 9	-0.631	s.e.	0.244
rep 3 *units* 6	-0.626	s.e.	0.244

Tables of means

Variate: juice_t_ha_trans

Grand mean 0.116

genotype	1	2	3	4	5	6	7
	0.048	0.722	-0.115	0.026	0.213	0.303	0.365
genotype	8	9	10	11	12	13	14
	-0.102	0.284	-0.017	0.057	0.216	0.202	0.242
genotype	15	16	17	18	19	20	21
	0.468	-0.376	-0.217	0.477	0.109	-0.266	-0.130
genotype	22						
	0.047						

Standard errors of means

Table	genotype
rep.	3
d.f.	42
e.s.e.	0.1767

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42
s.e.d.	0.2499

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	0.5044

Stratum standard errors and coefficients of variation

Variate: juice_t_ha_trans

Stratum	d.f.	s.e.	cv%
rep	2	0.2253	194.0
rep.*Units*	42	0.3061	263.6

```
 84                                                                  CALCULATE                                  juice_t_ha_trans_lin=1.0*juice_t_ha
 85                    FSPREADSHEET          [SHEET=10000001;          METHOD=replace;          NOUNITS=yes]  juice_t_ha_trans_lin
 86                                  "General          Analysis          of          Variance"
 87                                                                  BLOCK                                  rep
 88                                                                  TREATMENTS                             genotype
 89                                                      COVARIATE                  "No                     Covariate"
 90          ANOVA          [PRINT=aovtable,information,means,%cv;          FACT=32;          CONTRASTS=7;          PCONTRASTS=7;          FPROB=yes;\
 91   PSE=diff,lsd,means; LSDLEVEL=5] juice_t_ha_trans_linAnalysis of variance
```

Variate: juice_t_ha_trans_lin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	60.980	30.490	8.60	
rep.*Units* stratum					
genotype	21	135.097	6.433	1.81	0.050
Residual	42	148.967	3.547		
Total	65	345.043			

Message: the following units have large residuals.

rep 1 *units* 9	3.78	s.e. 1.50
rep 2 *units* 2	4.68	s.e. 1.50
rep 2 *units* 6	4.71	s.e. 1.50
rep 2 *units* 12	3.59	s.e. 1.50

Tables of means

Variate: juice_t_ha_trans_lin

Grand mean 2.04

genotype	1	2	3	4	5	6	7
	1.18	6.43	0.96	1.34	1.98	4.48	2.56

genotype	8	9	10	11	12	13	14
	0.86	2.88	1.70	1.25	3.20	1.70	1.92

genotype	15	16	17	18	19	20	21
	2.98	0.48	0.74	3.90	1.63	0.58	0.93

genotype	22
	1.15

Standard errors of means

Table	genotype
rep.	3
d.f.	42
e.s.e.	1.087

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42
s.e.d.	1.538

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	3.103

Stratum standard errors and coefficients of variation

Variate: juice_t_ha_trans_lin

Stratum	d.f.	s.e.	cv%
rep	2	1.177	57.8
rep.*Units*	42	1.883	92.4

```
 92                                                    CALCULATE                                   juice_t_ha_trans_pow=juice_t_ha**1.0
 93                FSPREADSHEET      [SHEET=10000001;      METHOD=replace;      NOUNITS=yes]      juice_t_ha_trans_pow
 94                                "General      Analysis      of      Variance"
 95                                                    BLOCK                                       rep
 96                                                    TREATMENTS                                  genotype
 97                                          COVARIATE                     "No                   Covariate"
 98          ANOVA      [PRINT=aovtable,information,means,%cv;      FACT=32;      CONTRASTS=7;      PCONTRASTS=7;      FPROB=yes;\
 99  PSE=diff,lsd,means; LSDLEVEL=5] juice_t_ha_trans_powAnalysis of variance
```

Variate: juice_t_ha_trans_pow

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	60.980	30.490	8.60	
rep.*Units* stratum					
genotype	21	135.097	6.433	1.81	0.050
Residual	42	148.967	3.547		
Total	65	345.043			

Message: the following units have large residuals.

rep 1 *units* 9	3.78	s.e.	1.50
rep 2 *units* 2	4.68	s.e.	1.50
rep 2 *units* 6	4.71	s.e.	1.50
rep 2 *units* 12	3.59	s.e.	1.50

Tables of means

Variate: juice_t_ha_trans_pow

Grand mean 2.04

genotype	1	2	3	4	5	6	7
	1.18	6.43	0.96	1.34	1.98	4.48	2.56

genotype	8	9	10	11	12	13	14
	0.86	2.88	1.70	1.25	3.20	1.70	1.92

genotype	15	16	17	18	19	20	21
	2.98	0.48	0.74	3.90	1.63	0.58	0.93

genotype	22
	1.15

Standard errors of means

Table	genotype
rep.	3
d.f.	42
e.s.e.	1.087

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42
s.e.d.	1.538

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	3.103

Stratum standard errors and coefficients of variation

Variate: juice_t_ha_trans_pow

Stratum	d.f.	s.e.	cv%
rep	2	1.177	57.8
rep.*Units*	42	1.883	92.4

```
100 CALCULATE juice_t_ha_trans_sqr=SQRT(juice_t_ha)
101 FSPREADSHEET [SHEET=10000001; METHOD=replace; NOUNITS=yes] juice_t_ha_trans_sqr
102 "General Analysis of Variance"
103 BLOCK rep
104 TREATMENTS genotype
105 COVARIATE "No Covariate"
106 ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
107 PSE=diff,lsd,means; LSDLEVEL=5] juice_t_ha_trans_sqrAnalysis of variance
Variate: juice_t_ha_trans_sqr
Source of variation                 d.f.      s.s.      m.s.      v.r.  F pr.
rep stratum                            2    5.5558    2.7779     11.38
rep.*Units* stratum
genotype                              21   11.2380    0.5351      2.19  0.015
Residual                              42   10.2557    0.2442
Total                                 65   27.0495
Message: the following units have large residuals.
rep 1 *units* 9                                1.039      s.e.  0.394
rep 2 *units* 6                                1.058      s.e.  0.394
rep 2 *units* 9                               -1.035      s.e.  0.394
rep 2 *units* 12                               0.940      s.e.  0.394
rep 3 *units* 6                               -0.933      s.e.  0.394
Tables of means
Variate: juice_t_ha_trans_sqr
Grand mean  1.276

genotype        1      2      3      4      5      6      7
            1.073  2.410  0.931  1.094  1.341  1.804  1.560

genotype        8      9     10     11     12     13     14
            0.909  1.537  1.140  1.093  1.526  1.282  1.354

genotype       15     16     17     18     19     20     21
            1.719  0.672  0.818  1.867  1.210  0.747  0.915

genotype       22
            1.064

Standard errors of means
Table                   genotype
rep.                           3
d.f.                          42
e.s.e.                    0.2853
Standard errors of differences of means
Table                   genotype
rep.                           3
d.f.                          42
s.e.d.                    0.4035
Least significant differences of means (5% level)
Table                   genotype
rep.                           3
```

d.f.	42
l.s.d.	0.8142

Stratum standard errors and coefficients of variation

Variate: juice_t_ha_trans_sqr

Stratum	d.f.	s.e.	cv%
rep	2	0.3553	27.8
rep.*Units*	42	0.4941	38.7

2012-2013

Anova Bethlehem 2012-2013

```
176  "Data taken from file: 'H:/Vikus/Copy of 2013 BH cult coll  2013.xls'"
177  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
178  READ [PRINT=*; SETNVALUES=yes] _stitle_
182  PRINT [IPRINT=*]   stitle  ; JUST=left
Data imported from Excel file: H:\Vikus\Copy of 2013 BH cult coll 2013.xls
 on: 10-Oct-2017 8:20:18
 taken from sheet "stats data", cells A2:F67
183  DELETE [REDEFINE=yes] rep,Entry,cultivar,mass_t_ha,brix_%,juice_t_ha
184  UNITS [NVALUES=*]
185  FACTOR [MODIFY=no; NVALUES=66; LEVELS=3; LABELS=*; REFERENCE=1] rep
186  READ rep; FREPRESENTATION=ordinal
                          Identifier        Values       Missing        Levels
                          rep                   66             0             3
189  VARIATE [NVALUES=66] Entry
190  READ Entry
                          Identifier        Minimum      Mean         Maximum
                          Entry                   1        11.5            22          66           0

194  FACTOR [MODIFY=no; NVALUES=66; LEVELS=22; LABELS=!t('BMR','e3','HG','p 197',\
195  'p 225','p 249','p 868','p 888','p 893','SK','ss 001','ss 003','ss 007',\
196  'ss 008','ss 016','ss 017','ss 081','ss 120','ss 220','ss 56','ss 895',\
197  'supa'); REFERENCE=1] cultivar
198  READ cultivar; FREPRESENTATION=ordinal

                          Identifier        Values       Missing        Levels
                          cultivar              66             0            22

202  VARIATE [NVALUES=66] mass_t_ha
203  READ mass  t  ha

                          Identifier        Minimum      Mean         Maximum
```

mass_t_ha	10.53	32.44	76.01	66	0

```
220  VARIATE [NVALUES=66] brix_%
221  READ brix  %
```

Identifier	Minimum	Mean	Maximum		
brix_%	4.8	13.64	19.07	66	0

```
233  VARIATE [NVALUES=66] juice_t_ha
234  READ juice  t  ha
```

Identifier	Minimum	Mean	Maximum		
juice_t_ha	5.303	10	20.67	66	0

```
                250
251  %PostMessage 1129; 0; 100002 "Sheet Update Completed"
252  "One-way design in randomized blocks"
253  DELETE [REDEFINE=yes]   ibalance
256   SAVE=_a2save
Analysis of variance

Variate: mass_t_ha
```

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	54.8	27.4	0.27	
rep.*Units* stratum					
cultivar	21	5230.6	249.1	2.45	0.007
Residual	42	4268.6	101.6		
Total	65	9554			

```
Information summary

All terms orthogonal, none aliased.
Message: the following units have large residuals.
```

rep 1 *units* 22	-26.9	s.e.	8.0
rep 2 *units* 10	-21.8	s.e.	8.0
rep 3 *units* 10	25.6	s.e.	8.0
rep 3 *units* 22	25.5	s.e.	8.0

```
Tables of means

Variate: mass  t  ha

Grand mean  32.4
```

cultivar	BMR	e3	HG	p 197	p 225	p 249	p 868
	32.9	24.8	46.6	25.3	29.8	25.1	27.5

cultivar	p 888	p 893	SK	ss	ss 003	ss 007	ss 008
	12.3	25.1	24.6	28.8	51.5	35	35.8
cultivar	ss 016	ss 017	ss 081	ss	ss 220	ss 56	ss 895
	38	26	38.8	46.2	37.5	41.3	27.5
cultivar	supa						
	33.2						

Standard errors of differences of means

Table	cultivar
rep.	3
d.f.	42
s.e.d.	8.23

Least significant differences of means (5% level)

Table	cultivar
rep.	3
d.f.	42
l.s.d.	16.61

Stratum standard errors and coefficients of variation

Variate: mass t ha

Stratum	d.f.	s.e.	cv%
rep	2	1.12	3.4
rep.*Units*	42	10.08	31.1

```
 257  IF   ibalance.eq.0 .OR.   ibalance.eq.1
 258    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 260    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 262   FACTORIAL=9; SAVE=  a2save['save']] cultivar
```

Tukey's 95% confidence intervals
cultivar

Comparison		Lower	Upper	Significant
ss 003 vs HG	4.87	-26.69	36.43	no
ss 003 vs ss 120	5.25	-26.31	36.81	no
ss 003 vs ss 56	10.16	-21.4	41.72	no
ss 003 vs ss 081	12.73	-18.83	44.29	no
ss 003 vs ss 016	13.5	-18.06	45.06	no
ss 003 vs ss 220	14	-17.56	45.56	no
ss 003 vs ss 008	15.73	-15.83	47.29	no
ss 003 vs ss 007	16.45	-15.11	48	no
ss 003 vs supa	18.32	-13.24	49.87	no
ss 003 vs BMR	18.55	-13.01	50.11	no
ss 003 vs p 225	21.65	-9.91	53.2	no
ss 003 vs ss 001	22.67	-8.89	54.23	no
ss 003 vs ss 895	23.96	-7.59	55.52	no
ss 003 vs p 868	24.01	-7.55	55.57	no
ss 003 vs ss 017	25.51	-6.04	57.07	no
ss 003 vs p 197	26.21	-5.35	57.76	no
ss 003 vs p 893	26.36	-5.2	57.92	no

ss 003 vs p 249	26.41	-5.15	57.97	no
ss 003 vs e3	26.69	-4.87	58.25	no
ss 003 vs SK	26.87	-4.69	58.43	no
ss 003 vs p 888	39.21	7.65	70.77	yes
HG vs ss 120	0.38	-31.17	31.94	no
HG vs ss 56	5.29	-26.27	36.85	no
HG vs ss 081	7.86	-23.69	39.42	no
HG vs ss 016	8.63	-22.93	40.19	no
HG vs ss 220	9.13	-22.42	40.69	no
HG vs ss 008	10.86	-20.7	42.42	no
HG vs ss 007	11.58	-19.98	43.14	no
HG vs supa	13.45	-18.11	45.01	no
HG vs BMR	13.68	-17.88	45.24	no
HG vs p 225	16.78	-14.78	48.34	no
HG vs ss 001	17.8	-13.75	49.36	no
HG vs ss 895	19.1	-12.46	50.66	no
HG vs p 868	19.14	-12.42	50.7	no
HG vs ss 017	20.65	-10.91	52.21	no
HG vs p 197	21.34	-10.22	52.9	no
HG vs p 893	21.49	-10.07	53.05	no
HG vs p 249	21.55	-10.01	53.1	no
HG vs e3	21.83	-9.73	53.38	no
HG vs SK	22.01	-9.55	53.56	no
HG vs p 888	34.34	2.78	65.9	yes
ss 120 vs ss 56	4.91	-26.65	36.46	no
ss 120 vs ss 081	7.48	-24.08	39.04	no
ss 120 vs ss 016	8.25	-23.31	39.81	no
ss 120 vs ss 220	8.75	-22.81	40.31	no
ss 120 vs ss 008	10.48	-21.08	42.04	no
ss 120 vs ss 007	11.19	-20.36	42.75	no
ss 120 vs supa	13.06	-18.49	44.62	no
ss 120 vs BMR	13.3	-18.26	44.85	no
ss 120 vs p 225	16.4	-15.16	47.95	no
ss 120 vs ss 001	17.42	-14.14	48.98	no
ss 120 vs ss 895	18.71	-12.84	50.27	no
ss 120 vs p 868	18.76	-12.8	50.32	no
ss 120 vs ss 017	20.26	-11.29	51.82	no
ss 120 vs p 197	20.95	-10.6	52.51	no
ss 120 vs p 893	21.11	-10.45	52.67	no
ss 120 vs p 249	21.16	-10.4	52.72	no
ss 120 vs e3	21.44	-10.12	53	no
ss 120 vs SK	21.62	-9.94	53.18	no
ss 120 vs p 888	33.96	2.4	65.51	yes
ss 56 vs ss 081	2.57	-28.98	34.13	no
ss 56 vs ss 016	3.34	-28.22	34.9	no
ss 56 vs ss 220	3.84	-27.71	35.4	no
ss 56 vs ss 008	5.57	-25.99	37.13	no
ss 56 vs ss 007	6.29	-25.27	37.85	no
ss 56 vs supa	8.16	-23.4	39.72	no
ss 56 vs BMR	8.39	-23.17	39.95	no
ss 56 vs p 225	11.49	-20.07	43.05	no
ss 56 vs ss 001	12.51	-19.04	44.07	no
ss 56 vs ss 895	13.81	-17.75	45.37	no
ss 56 vs p 868	13.85	-17.71	45.41	no

ss 56 vs ss 017	15.36	-16.2	46.92	no
ss 56 vs p 197	16.05	-15.51	47.61	no
ss 56 vs p 893	16.2	-15.36	47.76	no
ss 56 vs p 249	16.26	-15.3	47.81	no
ss 56 vs e3	16.54	-15.02	48.09	no
ss 56 vs SK	16.72	-14.84	48.27	no
ss 56 vs p 888	29.05	-2.51	60.61	no
ss 081 vs ss 016	0.77	-30.79	32.33	no
ss 081 vs ss 220	1.27	-30.29	32.83	no
ss 081 vs ss 008	3	-28.56	34.56	no
ss 081 vs ss 007	3.71	-27.84	35.27	no
ss 081 vs supa	5.58	-25.97	37.14	no
ss 081 vs BMR	5.82	-25.74	37.37	no
ss 081 vs p 225	8.91	-22.64	40.47	no
ss 081 vs ss 001	9.94	-21.62	41.5	no
ss 081 vs ss 895	11.23	-20.33	42.79	no
ss 081 vs p 868	11.28	-20.28	42.84	no
ss 081 vs ss 017	12.78	-18.78	44.34	no
ss 081 vs p 197	13.47	-18.08	45.03	no
ss 081 vs p 893	13.63	-17.93	45.19	no
ss 081 vs p 249	13.68	-17.88	45.24	no
ss 081 vs e3	13.96	-17.6	45.52	no
ss 081 vs SK	14.14	-17.42	45.7	no
ss 081 vs p 888	26.48	-5.08	58.03	no
ss 016 vs ss 220	0.5	-31.06	32.06	no
ss 016 vs ss 008	2.23	-29.33	33.79	no
ss 016 vs ss 007	2.95	-28.61	34.5	no
ss 016 vs supa	4.82	-26.74	36.37	no
ss 016 vs BMR	5.05	-26.51	36.6	no
ss 016 vs p 225	8.15	-23.41	39.7	no
ss 016 vs ss 001	9.17	-22.39	40.73	no
ss 016 vs ss 895	10.46	-21.09	42.02	no
ss 016 vs p 868	10.51	-21.05	42.07	no
ss 016 vs ss 017	12.01	-19.54	43.57	no
ss 016 vs p 197	12.71	-18.85	44.26	no
ss 016 vs p 893	12.86	-18.7	44.42	no
ss 016 vs p 249	12.91	-18.65	44.47	no
ss 016 vs e3	13.19	-18.37	44.75	no
ss 016 vs SK	13.37	-18.19	44.93	no
ss 016 vs p 888	25.71	-5.85	57.27	no
ss 220 vs ss 008	1.73	-29.83	33.29	no
ss 220 vs ss 007	2.45	-29.11	34	no
ss 220 vs supa	4.32	-27.24	35.87	no
ss 220 vs BMR	4.55	-27.01	36.1	no
ss 220 vs p 225	7.65	-23.91	39.2	no
ss 220 vs ss 001	8.67	-22.89	40.23	no
ss 220 vs ss 895	9.96	-21.59	41.52	no
ss 220 vs p 868	10.01	-21.55	41.57	no
ss 220 vs ss 017	11.51	-20.04	43.07	no
ss 220 vs p 197	12.21	-19.35	43.76	no
ss 220 vs p 893	12.36	-19.2	43.92	no
ss 220 vs p 249	12.41	-19.15	43.97	no
ss 220 vs e3	12.69	-18.87	44.25	no
ss 220 vs SK	12.87	-18.69	44.43	no

ss 220 vs p 888	25.21	-6.35	56.76	no
ss 008 vs ss 007	0.72	-30.84	32.28	no
ss 008 vs supa	2.59	-28.97	34.15	no
ss 008 vs BMR	2.82	-28.74	34.38	no
ss 008 vs p 225	5.92	-25.64	37.48	no
ss 008 vs ss 001	6.94	-24.62	38.5	no
ss 008 vs ss 895	8.24	-23.32	39.79	no
ss 008 vs p 868	8.28	-23.28	39.84	no
ss 008 vs ss 017	9.79	-21.77	41.34	no
ss 008 vs p 197	10.48	-21.08	42.04	no
ss 008 vs p 893	10.63	-20.93	42.19	no
ss 008 vs p 249	10.68	-20.87	42.24	no
ss 008 vs e3	10.96	-20.59	42.52	no
ss 008 vs SK	11.14	-20.41	42.7	no
ss 008 vs p 888	23.48	-8.08	55.04	no
ss 007 vs supa	1.87	-29.69	33.43	no
ss 007 vs BMR	2.1	-29.46	33.66	no
ss 007 vs p 225	5.2	-26.36	36.76	no
ss 007 vs ss 001	6.22	-25.33	37.78	no
ss 007 vs ss 895	7.52	-24.04	39.08	no
ss 007 vs p 868	7.56	-24	39.12	no
ss 007 vs ss 017	9.07	-22.49	40.63	no
ss 007 vs p 197	9.76	-21.8	41.32	no
ss 007 vs p 893	9.91	-21.64	41.47	no
ss 007 vs p 249	9.97	-21.59	41.52	no
ss 007 vs e3	10.25	-21.31	41.81	no
ss 007 vs SK	10.43	-21.13	41.98	no
ss 007 vs p 888	22.76	-8.8	54.32	no
supa vs BMR	0.23	-31.33	31.79	no
supa vs p 225	3.33	-28.23	34.89	no
supa vs ss 001	4.35	-27.2	35.91	no
supa vs ss 895	5.65	-25.91	37.21	no
supa vs p 868	5.69	-25.87	37.25	no
supa vs ss 017	7.2	-24.36	38.76	no
supa vs p 197	7.89	-23.67	39.45	no
supa vs p 893	8.04	-23.51	39.6	no
supa vs p 249	8.1	-23.46	39.65	no
supa vs e3	8.38	-23.18	39.94	no
supa vs SK	8.56	-23	40.11	no
supa vs p 888	20.89	-10.67	52.45	no
BMR vs p 225	3.1	-28.46	34.66	no
BMR vs ss 001	4.12	-27.43	35.68	no
BMR vs ss 895	5.42	-26.14	36.98	no
BMR vs p 868	5.46	-26.1	37.02	no
BMR vs ss 017	6.97	-24.59	38.53	no
BMR vs p 197	7.66	-23.9	39.22	no
BMR vs p 893	7.81	-23.74	39.37	no
BMR vs p 249	7.87	-23.69	39.42	no
BMR vs e3	8.15	-23.41	39.7	no
BMR vs SK	8.33	-23.23	39.88	no
BMR vs p 888	20.66	-10.9	52.22	no
p 225 vs ss 001	1.02	-30.53	32.58	no
p 225 vs ss 895	2.32	-29.24	33.88	no
p 225 vs p 868	2.36	-29.2	33.92	no

p 225 vs ss 017	3.87	-27.69	35.43	no
p 225 vs p 197	4.56	-27	36.12	no
p 225 vs p 893	4.71	-26.84	36.27	no
p 225 vs p 249	4.77	-26.79	36.32	no
p 225 vs e3	5.05	-26.51	36.6	no
p 225 vs SK	5.23	-26.33	36.78	no
p 225 vs p 888	17.56	-14	49.12	no
ss 001 vs ss 895	1.29	-30.26	32.85	no
ss 001 vs p 868	1.34	-30.22	32.9	no
ss 001 vs ss 017	2.84	-28.71	34.4	no
ss 001 vs p 197	3.54	-28.02	35.09	no
ss 001 vs p 893	3.69	-27.87	35.25	no
ss 001 vs p 249	3.74	-27.82	35.3	no
ss 001 vs e3	4.02	-27.54	35.58	no
ss 001 vs SK	4.2	-27.36	35.76	no
ss 001 vs p 888	16.54	-15.02	48.09	no
ss 895 vs p 868	0.04	-31.51	31.6	no
ss 895 vs ss 017	1.55	-30.01	33.11	no
ss 895 vs p 197	2.24	-29.32	33.8	no
ss 895 vs p 893	2.4	-29.16	33.95	no
ss 895 vs p 249	2.45	-29.11	34.01	no
ss 895 vs e3	2.73	-28.83	34.29	no
ss 895 vs SK	2.91	-28.65	34.47	no
ss 895 vs p 888	15.24	-16.32	46.8	no
p 868 vs ss 017	1.51	-30.05	33.06	no
p 868 vs p 197	2.2	-29.36	33.76	no
p 868 vs p 893	2.35	-29.21	33.91	no
p 868 vs p 249	2.4	-29.15	33.96	no
p 868 vs e3	2.68	-28.87	34.24	no
p 868 vs SK	2.86	-28.69	34.42	no
p 868 vs p 888	15.2	-16.36	46.76	no
ss 017 vs p 197	0.69	-30.87	32.25	no
ss 017 vs p 893	0.85	-30.71	32.4	no
ss 017 vs p 249	0.9	-30.66	32.46	no
ss 017 vs e3	1.18	-30.38	32.74	no
ss 017 vs SK	1.36	-30.2	32.92	no
ss 017 vs p 888	13.69	-17.87	45.25	no
p 197 vs p 893	0.15	-31.4	31.71	no
p 197 vs p 249	0.21	-31.35	31.76	no
p 197 vs e3	0.49	-31.07	32.04	no
p 197 vs SK	0.67	-30.89	32.22	no
p 197 vs p 888	13	-18.56	44.56	no
p 893 vs p 249	0.05	-31.51	31.61	no
p 893 vs e3	0.33	-31.23	31.89	no
p 893 vs SK	0.51	-31.05	32.07	no
p 893 vs p 888	12.85	-18.71	44.41	no
p 249 vs e3	0.28	-31.28	31.84	no
p 249 vs SK	0.46	-31.1	32.02	no
p 249 vs p 888	12.79	-18.76	44.35	no
e3 vs SK	0.18	-31.38	31.74	no
e3 vs p 888	12.51	-19.04	44.07	no
SK vs p 888	12.33	-19.22	43.89	no

	Mean	
ss 003	51.49	a
HG	46.62	a
ss 120	46.24	a
ss 56	41.33	ab
ss 081	38.76	ab
ss 016	37.99	ab
ss 220	37.49	ab
ss 008	35.76	ab
ss 007	35.04	ab
supa	33.17	ab
BMR	32.94	ab
p 225	29.84	ab
ss 001	28.82	ab
ss 895	27.53	ab
p 868	27.48	ab
ss 017	25.98	ab
p 197	25.28	ab
p 893	25.13	ab
p 249	25.08	ab
e3	24.8	ab
SK	24.62	ab
p 888	12.28	b

263 ENDIF
264 SET [IN=*]

Analysis of variance

Variate: brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	6.227	3.114	0.95	
rep.*Units* stratum					
cultivar	21	301.694	14.366	4.38	<.001
Residual	42	137.811	3.281		
Total	65	445.732			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 2 *units* 4	-3.38	s.e. 1.45
rep 2 *units* 5	3.39	s.e. 1.45
rep 3 *units* 4	3.87	s.e. 1.45
rep 3 *units* 5	-5.92	s.e. 1.45

Tables of means

Variate: brix_%

Grand mean 13.64

cultivar	BMR	e3	HG	p 197	p 225	p 249	p 868
	12.44	11.73	14.63	10.77	13.23	9.5	14.33
cultivar	p 888	p 893	SK	ss	ss 003	ss 007	ss 008
	12.58	11.92	11.02	16.23	16.31	18.38	15.68
cultivar	ss 016	ss 017	ss 081	ss	ss 220	ss 56	ss 895
	13.74	15.36	13.87	16.82	11.99	12.98	12.76
cultivar	supa						
	13.77						

Standard errors of differences of means

Table	cultivar
rep.	3
d.f.	42
s.e.d.	1.479

Least significant differences of means (5% level)

Table	cultivar
rep.	3
d.f.	42
l.s.d.	2.985

Stratum standard errors and coefficients of variation

Variate: brix %

Stratum	d.f.	s.e.	cv%
rep	2	0.376	2.8
rep.*Units*	42	1.811	13.3

```
 492  IF   ibalance.eq.0 .OR.   ibalance.eq.1
 493    DELETE [REDEFINE=yes]   mean,   rep,   var,   rdf
 495    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 497   FACTORIAL=9; SAVE=  a2save['save']] cultivar
```

Tukey's 95% confidence intervals
cultivar

Comparison		Lower	Upper	Significant
ss 007 vs ss 120	1.556	-4.115	7.226	no
ss 007 vs ss 003	2.067	-3.604	7.737	no
ss 007 vs ss 001	2.144	-3.526	7.815	no
ss 007 vs ss 008	2.7	-2.97	8.37	no
ss 007 vs ss 017	3.022	-2.648	8.693	no
ss 007 vs HG	3.744	-1.926	9.415	no
ss 007 vs p 868	4.044	-1.626	9.715	no
ss 007 vs ss 081	4.511	-1.159	10.181	no
ss 007 vs supa	4.611	-1.059	10.281	no
ss 007 vs ss 016	4.633	-1.037	10.304	no
ss 007 vs p 225	5.144	-0.526	10.815	no

ss 007 vs ss 56	5.4	-0.27	11.07	no
ss 007 vs ss 895	5.622	-0.048	11.293	no
ss 007 vs p 888	5.8	0.13	11.47	yes
ss 007 vs BMR	5.933	0.263	11.604	yes
ss 007 vs ss 220	6.384	0.714	12.055	yes
ss 007 vs p 893	6.456	0.785	12.126	yes
ss 007 vs e3	6.644	0.974	12.315	yes
ss 007 vs SK	7.356	1.685	13.026	yes
ss 007 vs p 197	7.611	1.941	13.281	yes
ss 007 vs p 249	8.878	3.207	14.548	yes
ss 120 vs ss 003	0.511	-5.159	6.181	no
ss 120 vs ss 001	0.589	-5.081	6.259	no
ss 120 vs ss 008	1.144	-4.526	6.815	no
ss 120 vs ss 017	1.467	-4.204	7.137	no
ss 120 vs HG	2.189	-3.481	7.859	no
ss 120 vs p 868	2.489	-3.181	8.159	no
ss 120 vs ss 081	2.956	-2.715	8.626	no
ss 120 vs supa	3.056	-2.615	8.726	no
ss 120 vs ss 016	3.078	-2.593	8.748	no
ss 120 vs p 225	3.589	-2.081	9.259	no
ss 120 vs ss 56	3.844	-1.826	9.515	no
ss 120 vs ss 895	4.067	-1.604	9.737	no
ss 120 vs p 888	4.244	-1.426	9.915	no
ss 120 vs BMR	4.378	-1.293	10.048	no
ss 120 vs ss 220	4.829	-0.841	10.499	no
ss 120 vs p 893	4.9	-0.77	10.57	no
ss 120 vs e3	5.089	-0.581	10.759	no
ss 120 vs SK	5.8	0.13	11.47	yes
ss 120 vs p 197	6.056	0.385	11.726	yes
ss 120 vs p 249	7.322	1.652	12.993	yes
ss 003 vs ss 001	0.078	-5.593	5.748	no
ss 003 vs ss 008	0.633	-5.037	6.304	no
ss 003 vs ss 017	0.956	-4.715	6.626	no
ss 003 vs HG	1.678	-3.993	7.348	no
ss 003 vs p 868	1.978	-3.693	7.648	no
ss 003 vs ss 081	2.444	-3.226	8.115	no
ss 003 vs supa	2.544	-3.126	8.215	no
ss 003 vs ss 016	2.567	-3.104	8.237	no
ss 003 vs p 225	3.078	-2.593	8.748	no
ss 003 vs ss 56	3.333	-2.337	9.004	no
ss 003 vs ss 895	3.556	-2.115	9.226	no
ss 003 vs p 888	3.733	-1.937	9.404	no
ss 003 vs BMR	3.867	-1.804	9.537	no
ss 003 vs ss 220	4.318	-1.353	9.988	no
ss 003 vs p 893	4.389	-1.281	10.059	no
ss 003 vs e3	4.578	-1.093	10.248	no
ss 003 vs SK	5.289	-0.381	10.959	no
ss 003 vs p 197	5.544	-0.126	11.215	no
ss 003 vs p 249	6.811	1.141	12.481	yes
ss 001 vs ss 008	0.556	-5.115	6.226	no
ss 001 vs ss 017	0.878	-4.793	6.548	no
ss 001 vs HG	1.6	-4.07	7.27	no
ss 001 vs p 868	1.9	-3.77	7.57	no
ss 001 vs ss 081	2.367	-3.304	8.037	no

ss 001 vs supa	2.467	-3.204	8.137	no
ss 001 vs ss 016	2.489	-3.181	8.159	no
ss 001 vs p 225	3	-2.67	8.67	no
ss 001 vs ss 56	3.256	-2.415	8.926	no
ss 001 vs ss 895	3.478	-2.193	9.148	no
ss 001 vs p 888	3.656	-2.015	9.326	no
ss 001 vs BMR	3.789	-1.881	9.459	no
ss 001 vs ss 220	4.24	-1.43	9.91	no
ss 001 vs p 893	4.311	-1.359	9.981	no
ss 001 vs e3	4.5	-1.17	10.17	no
ss 001 vs SK	5.211	-0.459	10.881	no
ss 001 vs p 197	5.467	-0.204	11.137	no
ss 001 vs p 249	6.733	1.063	12.404	yes
ss 008 vs ss 017	0.322	-5.348	5.993	no
ss 008 vs HG	1.044	-4.626	6.715	no
ss 008 vs p 868	1.344	-4.326	7.015	no
ss 008 vs ss 081	1.811	-3.859	7.481	no
ss 008 vs supa	1.911	-3.759	7.581	no
ss 008 vs ss 016	1.933	-3.737	7.604	no
ss 008 vs p 225	2.444	-3.226	8.115	no
ss 008 vs ss 56	2.7	-2.97	8.37	no
ss 008 vs ss 895	2.922	-2.748	8.593	no
ss 008 vs p 888	3.1	-2.57	8.77	no
ss 008 vs BMR	3.233	-2.437	8.904	no
ss 008 vs ss 220	3.684	-1.986	9.355	no
ss 008 vs p 893	3.756	-1.915	9.426	no
ss 008 vs e3	3.944	-1.726	9.615	no
ss 008 vs SK	4.656	-1.015	10.326	no
ss 008 vs p 197	4.911	-0.759	10.581	no
ss 008 vs p 249	6.178	0.507	11.848	yes
ss 017 vs HG	0.722	-4.948	6.393	no
ss 017 vs p 868	1.022	-4.648	6.693	no
ss 017 vs ss 081	1.489	-4.181	7.159	no
ss 017 vs supa	1.589	-4.081	7.259	no
ss 017 vs ss 016	1.611	-4.059	7.281	no
ss 017 vs p 225	2.122	-3.548	7.793	no
ss 017 vs ss 56	2.378	-3.293	8.048	no
ss 017 vs ss 895	2.6	-3.07	8.27	no
ss 017 vs p 888	2.778	-2.893	8.448	no
ss 017 vs BMR	2.911	-2.759	8.581	no
ss 017 vs ss 220	3.362	-2.308	9.033	no
ss 017 vs p 893	3.433	-2.237	9.104	no
ss 017 vs e3	3.622	-2.048	9.293	no
ss 017 vs SK	4.333	-1.337	10.004	no
ss 017 vs p 197	4.589	-1.081	10.259	no
ss 017 vs p 249	5.856	0.185	11.526	yes
HG vs p 868	0.3	-5.37	5.97	no
HG vs ss 081	0.767	-4.904	6.437	no
HG vs supa	0.867	-4.804	6.537	no
HG vs ss 016	0.889	-4.781	6.559	no
HG vs p 225	1.4	-4.27	7.07	no
HG vs ss 56	1.656	-4.015	7.326	no
HG vs ss 895	1.878	-3.793	7.548	no
HG vs p 888	2.056	-3.615	7.726	no

HG vs BMR	2.189	-3.481	7.859	no
HG vs ss 220	2.64	-3.03	8.31	no
HG vs p 893	2.711	-2.959	8.381	no
HG vs e3	2.9	-2.77	8.57	no
HG vs SK	3.611	-2.059	9.281	no
HG vs p 197	3.867	-1.804	9.537	no
HG vs p 249	5.133	-0.537	10.804	no
p 868 vs ss 081	0.467	-5.204	6.137	no
p 868 vs supa	0.567	-5.104	6.237	no
p 868 vs ss 016	0.589	-5.081	6.259	no
p 868 vs p 225	1.1	-4.57	6.77	no
p 868 vs ss 56	1.356	-4.315	7.026	no
p 868 vs ss 895	1.578	-4.093	7.248	no
p 868 vs p 888	1.756	-3.915	7.426	no
p 868 vs BMR	1.889	-3.781	7.559	no
p 868 vs ss 220	2.34	-3.33	8.01	no
p 868 vs p 893	2.411	-3.259	8.081	no
p 868 vs e3	2.6	-3.07	8.27	no
p 868 vs SK	3.311	-2.359	8.981	no
p 868 vs p 197	3.567	-2.104	9.237	no
p 868 vs p 249	4.833	-0.837	10.504	no
ss 081 vs supa	0.1	-5.57	5.77	no
ss 081 vs ss 016	0.122	-5.548	5.793	no
ss 081 vs p 225	0.633	-5.037	6.304	no
ss 081 vs ss 56	0.889	-4.781	6.559	no
ss 081 vs ss 895	1.111	-4.559	6.781	no
ss 081 vs p 888	1.289	-4.381	6.959	no
ss 081 vs BMR	1.422	-4.248	7.093	no
ss 081 vs ss 220	1.873	-3.797	7.544	no
ss 081 vs p 893	1.944	-3.726	7.615	no
ss 081 vs e3	2.133	-3.537	7.804	no
ss 081 vs SK	2.844	-2.826	8.515	no
ss 081 vs p 197	3.1	-2.57	8.77	no
ss 081 vs p 249	4.367	-1.304	10.037	no
supa vs ss 016	0.022	-5.648	5.693	no
supa vs p 225	0.533	-5.137	6.204	no
supa vs ss 56	0.789	-4.881	6.459	no
supa vs ss 895	1.011	-4.659	6.681	no
supa vs p 888	1.189	-4.481	6.859	no
supa vs BMR	1.322	-4.348	6.993	no
supa vs ss 220	1.773	-3.897	7.444	no
supa vs p 893	1.844	-3.826	7.515	no
supa vs e3	2.033	-3.637	7.704	no
supa vs SK	2.744	-2.926	8.415	no
supa vs p 197	3	-2.67	8.67	no
supa vs p 249	4.267	-1.404	9.937	no
ss 016 vs p 225	0.511	-5.159	6.181	no
ss 016 vs ss 56	0.767	-4.904	6.437	no
ss 016 vs ss 895	0.989	-4.681	6.659	no
ss 016 vs p 888	1.167	-4.504	6.837	no
ss 016 vs BMR	1.3	-4.37	6.97	no
ss 016 vs ss 220	1.751	-3.919	7.421	no
ss 016 vs p 893	1.822	-3.848	7.493	no
ss 016 vs e3	2.011	-3.659	7.681	no

ss 016 vs SK	2.722	-2.948	8.393	no
ss 016 vs p 197	2.978	-2.693	8.648	no
ss 016 vs p 249	4.244	-1.426	9.915	no
p 225 vs ss 56	0.256	-5.415	5.926	no
p 225 vs ss 895	0.478	-5.193	6.148	no
p 225 vs p 888	0.656	-5.015	6.326	no
p 225 vs BMR	0.789	-4.881	6.459	no
p 225 vs ss 220	1.24	-4.43	6.91	no
p 225 vs p 893	1.311	-4.359	6.981	no
p 225 vs e3	1.5	-4.17	7.17	no
p 225 vs SK	2.211	-3.459	7.881	no
p 225 vs p 197	2.467	-3.204	8.137	no
p 225 vs p 249	3.733	-1.937	9.404	no
ss 56 vs ss 895	0.222	-5.448	5.893	no
ss 56 vs p 888	0.4	-5.27	6.07	no
ss 56 vs BMR	0.533	-5.137	6.204	no
ss 56 vs ss 220	0.984	-4.686	6.655	no
ss 56 vs p 893	1.056	-4.615	6.726	no
ss 56 vs e3	1.244	-4.426	6.915	no
ss 56 vs SK	1.956	-3.715	7.626	no
ss 56 vs p 197	2.211	-3.459	7.881	no
ss 56 vs p 249	3.478	-2.193	9.148	no
ss 895 vs p 888	0.178	-5.493	5.848	no
ss 895 vs BMR	0.311	-5.359	5.981	no
ss 895 vs ss 220	0.762	-4.908	6.433	no
ss 895 vs p 893	0.833	-4.837	6.504	no
ss 895 vs e3	1.022	-4.648	6.693	no
ss 895 vs SK	1.733	-3.937	7.404	no
ss 895 vs p 197	1.989	-3.681	7.659	no
ss 895 vs p 249	3.256	-2.415	8.926	no
p 888 vs BMR	0.133	-5.537	5.804	no
p 888 vs ss 220	0.584	-5.086	6.255	no
p 888 vs p 893	0.656	-5.015	6.326	no
p 888 vs e3	0.844	-4.826	6.515	no
p 888 vs SK	1.556	-4.115	7.226	no
p 888 vs p 197	1.811	-3.859	7.481	no
p 888 vs p 249	3.078	-2.593	8.748	no
BMR vs ss 220	0.451	-5.219	6.121	no
BMR vs p 893	0.522	-5.148	6.193	no
BMR vs e3	0.711	-4.959	6.381	no
BMR vs SK	1.422	-4.248	7.093	no
BMR vs p 197	1.678	-3.993	7.348	no
BMR vs p 249	2.944	-2.726	8.615	no
ss 220 vs p 893	0.071	-5.599	5.741	no
ss 220 vs e3	0.26	-5.41	5.93	no
ss 220 vs SK	0.971	-4.699	6.641	no
ss 220 vs p 197	1.227	-4.444	6.897	no
ss 220 vs p 249	2.493	-3.177	8.164	no
p 893 vs e3	0.189	-5.481	5.859	no
p 893 vs SK	0.9	-4.77	6.57	no
p 893 vs p 197	1.156	-4.515	6.826	no
p 893 vs p 249	2.422	-3.248	8.093	no
e3 vs SK	0.711	-4.959	6.381	no
e3 vs p 197	0.967	-4.704	6.637	no

e3 vs p 249	2.233	-3.437	7.904	no
SK vs p 197	0.256	-5.415	5.926	no
SK vs p 249	1.522	-4.148	7.193	no
p 197 vs p 249	1.267	-4.404	6.937	no

	Mean	
ss 007	18.38	a
ss 120	16.82	ab
ss 003	16.31	abc
ss 001	16.23	abc
ss 008	15.68	abc
ss 017	15.36	abc
HG	14.63	abcd
p 868	14.33	abcd
ss 081	13.87	abcd
supa	13.77	abcd
ss 016	13.74	abcd
p 225	13.23	abcd
ss 56	12.98	abcd
ss 895	12.76	abcd
p 888	12.58	bcd
BMR	12.44	bcd
ss 220	11.99	bcd
p 893	11.92	bcd
e3	11.73	bcd
SK	11.02	cd
p 197	10.77	cd
p 249	9.5	d

```
 498  ENDIF
 499  SET [IN=*]
 505  "One-way design in randomized blocks"
 506  DELETE [REDEFINE=yes]   ibalance
 509   SAVE=_a2save
```

Analysis of variance

Variate: juice t ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	11.23	5.615	1	
rep.*Units* stratum					
cultivar	21	378.563	18.027	3.2	<.001
Residual	42	236.571	5.633		
Total	65	626.364			

Information summary

All terms orthogonal, none aliased.
Message: the following units have large residuals.

rep 1 *units* 22	-6.16	s.e. 1.89
rep 3 *units* 22	6.32	s.e. 1.89

Tables of means

Variate: juice_t_ha

Grand mean 10.00

cultivar	BMR	e3	HG	p 197	p 225	p 249	p 868
	8.74	6.74	14.86	7.17	8.38	8.22	10.99
cultivar	p 888	p 893	SK	ss	ss 003	ss 007	ss 008
	6.81	7.3	9.57	10.17	14.73	12.3	10.73
cultivar	ss 016	ss 017	ss 081	ss	ss 220	ss 56	ss 895
	9.97	8.43	10.2	14.68	10.03	10.3	8.27
cultivar	supa						
	11.5						

Standard errors of differences of means

Table	cultivar
rep.	3
d.f.	42
s.e.d.	1.938

Least significant differences of means (5% level)

Table	cultivar
rep.	3
d.f.	42
l.s.d.	3.911

Stratum standard errors and coefficients of variation

Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	0.505	5.1
rep.*Units*	42	2.373	23.7

```
 510  IF   ibalance.eq.0 .OR.   ibalance.eq.1
 511    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 513    AKEEP [SAVE=  a2save['save']] #  resid; DF=  rdf
 515   FACTORIAL=9; SAVE=_a2save['save']] cultivar
```

Tukey's 95% confidence intervals
cultivar

Comparison		Lower	Upper	Significant
HG vs ss 003	0.128	-7.301	7.56	no
HG vs ss 120	0.179	-7.25	7.61	no
HG vs ss 007	2.562	-4.868	9.99	no
HG vs supa	3.356	-4.074	10.79	no
HG vs p 868	3.865	-3.564	11.29	no
HG vs ss 008	4.124	-3.305	11.55	no
HG vs ss 56	4.56	-2.869	11.99	no
HG vs ss 081	4.662	-2.767	12.09	no
HG vs ss 001	4.688	-2.741	12.12	no
HG vs ss 220	4.829	-2.601	12.26	no

HG vs ss 016	4.893	-2.536	12.32	no
HG vs SK	5.29	-2.139	12.72	no
HG vs BMR	6.123	-1.307	13.55	no
HG vs ss 017	6.43	-0.999	13.86	no
HG vs p 225	6.481	-0.948	13.91	no
HG vs ss 895	6.584	-0.846	14.01	no
HG vs p 249	6.639	-0.79	14.07	no
HG vs p 893	7.557	0.128	14.99	yes
HG vs p 197	7.685	0.256	15.11	yes
HG vs p 888	8.044	0.614	15.47	yes
HG vs e3	8.121	0.691	15.55	yes
ss 003 vs ss 120	0.051	-7.378	7.48	no
ss 003 vs ss 007	2.434	-4.996	9.86	no
ss 003 vs supa	3.228	-4.202	10.66	no
ss 003 vs p 868	3.737	-3.692	11.17	no
ss 003 vs ss 008	3.996	-3.433	11.43	no
ss 003 vs ss 56	4.432	-2.998	11.86	no
ss 003 vs ss 081	4.534	-2.895	11.96	no
ss 003 vs ss 001	4.56	-2.869	11.99	no
ss 003 vs ss 220	4.7	-2.729	12.13	no
ss 003 vs ss 016	4.765	-2.665	12.19	no
ss 003 vs SK	5.162	-2.267	12.59	no
ss 003 vs BMR	5.994	-1.435	13.42	no
ss 003 vs ss 017	6.302	-1.128	13.73	no
ss 003 vs p 225	6.353	-1.076	13.78	no
ss 003 vs ss 895	6.456	-0.974	13.88	no
ss 003 vs p 249	6.511	-0.918	13.94	no
ss 003 vs p 893	7.429	0	14.86	no
ss 003 vs p 197	7.557	0.128	14.99	yes
ss 003 vs p 888	7.916	0.486	15.35	yes
ss 003 vs e3	7.993	0.563	15.42	yes
ss 120 vs ss 007	2.382	-5.047	9.81	no
ss 120 vs supa	3.177	-4.253	10.61	no
ss 120 vs p 868	3.686	-3.743	11.12	no
ss 120 vs ss 008	3.945	-3.484	11.37	no
ss 120 vs ss 56	4.381	-3.049	11.81	no
ss 120 vs ss 081	4.483	-2.946	11.91	no
ss 120 vs ss 001	4.509	-2.921	11.94	no
ss 120 vs ss 220	4.649	-2.78	12.08	no
ss 120 vs ss 016	4.714	-2.716	12.14	no
ss 120 vs SK	5.111	-2.319	12.54	no
ss 120 vs BMR	5.943	-1.486	13.37	no
ss 120 vs ss 017	6.251	-1.179	13.68	no
ss 120 vs p 225	6.302	-1.128	13.73	no
ss 120 vs ss 895	6.404	-1.025	13.83	no
ss 120 vs p 249	6.46	-0.969	13.89	no
ss 120 vs p 893	7.378	-0.052	14.81	no
ss 120 vs p 197	7.506	0.076	14.94	yes
ss 120 vs p 888	7.865	0.435	15.29	yes
ss 120 vs e3	7.941	0.512	15.37	yes
ss 007 vs supa	0.794	-6.635	8.22	no
ss 007 vs p 868	1.304	-6.126	8.73	no
ss 007 vs ss 008	1.563	-5.867	8.99	no
ss 007 vs ss 56	1.998	-5.431	9.43	no

ss 007 vs ss 081	2.101	-5.329	9.53	no
ss 007 vs ss 001	2.126	-5.303	9.56	no
ss 007 vs ss 220	2.267	-5.163	9.7	no
ss 007 vs ss 016	2.331	-5.098	9.76	no
ss 007 vs SK	2.728	-4.701	10.16	no
ss 007 vs BMR	3.561	-3.869	10.99	no
ss 007 vs ss 017	3.868	-3.561	11.3	no
ss 007 vs p 225	3.919	-3.51	11.35	no
ss 007 vs ss 895	4.022	-3.407	11.45	no
ss 007 vs p 249	4.078	-3.352	11.51	no
ss 007 vs p 893	4.995	-2.434	12.42	no
ss 007 vs p 197	5.123	-2.306	12.55	no
ss 007 vs p 888	5.482	-1.947	12.91	no
ss 007 vs e3	5.559	-1.87	12.99	no
supa vs p 868	0.51	-6.92	7.94	no
supa vs ss 008	0.769	-6.661	8.2	no
supa vs ss 56	1.204	-6.225	8.63	no
supa vs ss 081	1.306	-6.123	8.74	no
supa vs ss 001	1.332	-6.097	8.76	no
supa vs ss 220	1.473	-5.957	8.9	no
supa vs ss 016	1.537	-5.892	8.97	no
supa vs SK	1.934	-5.495	9.36	no
supa vs BMR	2.767	-4.663	10.2	no
supa vs ss 017	3.074	-4.355	10.5	no
supa vs p 225	3.125	-4.304	10.55	no
supa vs ss 895	3.228	-4.202	10.66	no
supa vs p 249	3.283	-4.146	10.71	no
supa vs p 893	4.201	-3.228	11.63	no
supa vs p 197	4.329	-3.1	11.76	no
supa vs p 888	4.688	-2.741	12.12	no
supa vs e3	4.765	-2.665	12.19	no
p 868 vs ss 008	0.259	-7.17	7.69	no
p 868 vs ss 56	0.694	-6.735	8.12	no
p 868 vs ss 081	0.797	-6.632	8.23	no
p 868 vs ss 001	0.823	-6.607	8.25	no
p 868 vs ss 220	0.963	-6.466	8.39	no
p 868 vs ss 016	1.027	-6.402	8.46	no
p 868 vs SK	1.425	-6.005	8.85	no
p 868 vs BMR	2.257	-5.172	9.69	no
p 868 vs ss 017	2.565	-4.865	9.99	no
p 868 vs p 225	2.616	-4.814	10.05	no
p 868 vs ss 895	2.718	-4.711	10.15	no
p 868 vs p 249	2.774	-4.656	10.2	no
p 868 vs p 893	3.692	-3.738	11.12	no
p 868 vs p 197	3.82	-3.61	11.25	no
p 868 vs p 888	4.178	-3.251	11.61	no
p 868 vs e3	4.255	-3.174	11.68	no
ss 008 vs ss 56	0.435	-6.994	7.86	no
ss 008 vs ss 081	0.538	-6.891	7.97	no
ss 008 vs ss 001	0.564	-6.866	7.99	no
ss 008 vs ss 220	0.704	-6.725	8.13	no
ss 008 vs ss 016	0.769	-6.661	8.2	no
ss 008 vs SK	1.166	-6.264	8.59	no
ss 008 vs BMR	1.998	-5.431	9.43	no

ss 008 vs ss 017	2.306	-5.124	9.73	no
ss 008 vs p 225	2.357	-5.073	9.79	no
ss 008 vs ss 895	2.459	-4.97	9.89	no
ss 008 vs p 249	2.515	-4.914	9.94	no
ss 008 vs p 893	3.433	-3.997	10.86	no
ss 008 vs p 197	3.561	-3.869	10.99	no
ss 008 vs p 888	3.919	-3.51	11.35	no
ss 008 vs e3	3.996	-3.433	11.43	no
ss 56 vs ss 081	0.102	-7.327	7.53	no
ss 56 vs ss 001	0.128	-7.301	7.56	no
ss 56 vs ss 220	0.269	-7.161	7.7	no
ss 56 vs ss 016	0.333	-7.096	7.76	no
ss 56 vs SK	0.73	-6.699	8.16	no
ss 56 vs BMR	1.563	-5.867	8.99	no
ss 56 vs ss 017	1.87	-5.559	9.3	no
ss 56 vs p 225	1.921	-5.508	9.35	no
ss 56 vs ss 895	2.024	-5.406	9.45	no
ss 56 vs p 249	2.079	-5.35	9.51	no
ss 56 vs p 893	2.997	-4.432	10.43	no
ss 56 vs p 197	3.125	-4.304	10.55	no
ss 56 vs p 888	3.484	-3.945	10.91	no
ss 56 vs e3	3.561	-3.869	10.99	no
ss 081 vs ss 001	0.026	-7.404	7.45	no
ss 081 vs ss 220	0.166	-7.263	7.6	no
ss 081 vs ss 016	0.231	-7.199	7.66	no
ss 081 vs SK	0.628	-6.802	8.06	no
ss 081 vs BMR	1.46	-5.969	8.89	no
ss 081 vs ss 017	1.768	-5.662	9.2	no
ss 081 vs p 225	1.819	-5.611	9.25	no
ss 081 vs ss 895	1.921	-5.508	9.35	no
ss 081 vs p 249	1.977	-5.452	9.41	no
ss 081 vs p 893	2.895	-4.535	10.32	no
ss 081 vs p 197	3.023	-4.407	10.45	no
ss 081 vs p 888	3.381	-4.048	10.81	no
ss 081 vs e3	3.458	-3.971	10.89	no
ss 001 vs ss 220	0.141	-7.289	7.57	no
ss 001 vs ss 016	0.205	-7.224	7.63	no
ss 001 vs SK	0.602	-6.827	8.03	no
ss 001 vs BMR	1.435	-5.995	8.86	no
ss 001 vs ss 017	1.742	-5.687	9.17	no
ss 001 vs p 225	1.793	-5.636	9.22	no
ss 001 vs ss 895	1.896	-5.534	9.33	no
ss 001 vs p 249	1.951	-5.478	9.38	no
ss 001 vs p 893	2.869	-4.56	10.3	no
ss 001 vs p 197	2.997	-4.432	10.43	no
ss 001 vs p 888	3.356	-4.074	10.79	no
ss 001 vs e3	3.433	-3.997	10.86	no
ss 220 vs ss 016	0.064	-7.365	7.49	no
ss 220 vs SK	0.461	-6.968	7.89	no
ss 220 vs BMR	1.294	-6.135	8.72	no
ss 220 vs ss 017	1.601	-5.828	9.03	no
ss 220 vs p 225	1.653	-5.777	9.08	no
ss 220 vs ss 895	1.755	-5.674	9.18	no
ss 220 vs p 249	1.811	-5.619	9.24	no

ss 220 vs p 893	2.729	-4.701	10.16	no
ss 220 vs p 197	2.857	-4.573	10.29	no
ss 220 vs p 888	3.215	-4.214	10.64	no
ss 220 vs e3	3.292	-4.137	10.72	no
ss 016 vs SK	0.397	-7.032	7.83	no
ss 016 vs BMR	1.23	-6.2	8.66	no
ss 016 vs ss 017	1.537	-5.892	8.97	no
ss 016 vs p 225	1.588	-5.841	9.02	no
ss 016 vs ss 895	1.691	-5.739	9.12	no
ss 016 vs p 249	1.746	-5.683	9.18	no
ss 016 vs p 893	2.664	-4.765	10.09	no
ss 016 vs p 197	2.792	-4.637	10.22	no
ss 016 vs p 888	3.151	-4.278	10.58	no
ss 016 vs e3	3.228	-4.202	10.66	no
SK vs BMR	0.833	-6.597	8.26	no
SK vs ss 017	1.14	-6.289	8.57	no
SK vs p 225	1.191	-6.238	8.62	no
SK vs ss 895	1.294	-6.136	8.72	no
SK vs p 249	1.349	-6.08	8.78	no
SK vs p 893	2.267	-5.162	9.7	no
SK vs p 197	2.395	-5.034	9.82	no
SK vs p 888	2.754	-4.676	10.18	no
SK vs e3	2.831	-4.599	10.26	no
BMR vs ss 017	0.307	-7.122	7.74	no
BMR vs p 225	0.359	-7.071	7.79	no
BMR vs ss 895	0.461	-6.968	7.89	no
BMR vs p 249	0.517	-6.913	7.95	no
BMR vs p 893	1.435	-5.995	8.86	no
BMR vs p 197	1.563	-5.867	8.99	no
BMR vs p 888	1.921	-5.508	9.35	no
BMR vs e3	1.998	-5.431	9.43	no
ss 017 vs p 225	0.051	-7.378	7.48	no
ss 017 vs ss 895	0.154	-7.276	7.58	no
ss 017 vs p 249	0.209	-7.22	7.64	no
ss 017 vs p 893	1.127	-6.302	8.56	no
ss 017 vs p 197	1.255	-6.174	8.68	no
ss 017 vs p 888	1.614	-5.815	9.04	no
ss 017 vs e3	1.691	-5.739	9.12	no
p 225 vs ss 895	0.102	-7.327	7.53	no
p 225 vs p 249	0.158	-7.271	7.59	no
p 225 vs p 893	1.076	-6.353	8.51	no
p 225 vs p 197	1.204	-6.225	8.63	no
p 225 vs p 888	1.563	-5.867	8.99	no
p 225 vs e3	1.64	-5.79	9.07	no
ss 895 vs p 249	0.056	-7.374	7.48	no
ss 895 vs p 893	0.973	-6.456	8.4	no
ss 895 vs p 197	1.102	-6.328	8.53	no
ss 895 vs p 888	1.46	-5.969	8.89	no
ss 895 vs e3	1.537	-5.892	8.97	no
p 249 vs p 893	0.918	-6.512	8.35	no
p 249 vs p 197	1.046	-6.383	8.48	no
p 249 vs p 888	1.405	-6.025	8.83	no
p 249 vs e3	1.481	-5.948	8.91	no
p 893 vs p 197	0.128	-7.301	7.56	no

p 893 vs p 888	0.487	-6.943	7.92	no
p 893 vs e3	0.564	-6.866	7.99	no
p 197 vs p 888	0.359	-7.071	7.79	no
p 197 vs e3	0.435	-6.994	7.86	no
p 888 vs e3	0.077	-7.353	7.51	no

	Mean	
HG	14.86	a
ss 003	14.73	ab
ss 120	14.68	ab
ss 007	12.3	abc
supa	11.5	abc
p 868	10.99	abc
ss 008	10.73	abc
ss 56	10.3	abc
ss 081	10.2	abc
ss 001	10.17	abc
ss 220	10.03	abc
ss 016	9.97	abc
SK	9.57	abc
BMR	8.74	abc
ss 017	8.43	abc
p 225	8.38	abc
ss 895	8.27	abc
p 249	8.22	abc
p 893	7.3	bc
p 197	7.17	c
p 888	6.81	c
e3	6.74	c

```
516  ENDIF
517  SET [IN=*]
```

Anova Potchefstroom 2012-2013

```
Genstat 64-bit Release 18.1 ( PC/Windows 8) 10 October 2017 08:43:38
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                    ____________________________________________

                    Genstat Eighteenth Edition
                    Genstat Procedure Library Release PL26.1
                    ____________________________________________

  1  SET [WORKINGDIRECTORY='C:/Users/maalis/Documents']
  2  "Data taken from file: 'H:/Vikus/Copy of 2013 P cult coll 2013.xls'"
  3  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
  4  READ [PRINT=*; SETNVALUES=yes] _stitle_
  8  PRINT [IPRINT=*] _stitle_; JUST=left

Data imported from Excel file: H:\Vikus\Copy of 2013 P cult coll 2013.xls
 on: 10-Oct-2017 8:44:02
 taken from sheet "stats data", cells A2:F67

  9  DELETE [REDEFINE=yes] rep,Entry,genotype,mass_t_ha,brix_%,juice_t_ha
 10  UNITS [NVALUES=*]
 11  FACTOR [MODIFY=no; NVALUES=66; LEVELS=3; LABELS=*; REFERENCE=1] rep
 12  READ rep; FREPRESENTATION=ordinal


                     Identifier        Values       Missing      Levels
                    rep                    66             0           3

 15  VARIATE [NVALUES=66] Entry
 16  READ Entry


                     Identifier       Minimum        Mean       Maximum      Values     Missing
                    Entry                  1        11.5            22          66           0

 20  FACTOR [MODIFY=no; NVALUES=66; LEVELS=22; LABELS=!t('BMR','e3','HG','p 197',\
 21  'p 220','p 225','p 249','p 868','p 888','p 893','p 895','SK','ss 001',\
 22  'ss 003','ss 007','ss 008','ss 016','ss 017','ss 081','ss 120','ss 56',\
 23  'supa'); REFERENCE=1] genotype
 24  READ genotype; FREPRESENTATION=ordinal


                     Identifier        Values       Missing      Levels
                    genotype               66             0          22

 28  VARIATE [NVALUES=66] mass_t_ha
```

```
 29  READ mass_t_ha

                    Identifier      Minimum      Mean      Maximum    Values    Missing
                    mass_t_ha         18.06      41.9        74.39        66          0

 46  VARIATE [NVALUES=66] brix_%
 47  READ brix_%

                    Identifier      Minimum      Mean      Maximum    Values    Missing
                    brix_%            11.03     16.18           21        66          0

 62  VARIATE [NVALUES=66] juice_t_ha
 63  READ juice_t_ha

                    Identifier      Minimum      Mean      Maximum    Values    Missing
                    juice_t_ha         5.38     9.045         17.06        66          0

             80
 81  %PostMessage 1129; 0; 100001 "Sheet Update Completed"
 82  "One-way design in randomized blocks"
 83  DELETE [REDEFINE=yes] _ibalance
 84  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype; BLOCKS=rep; FPROB=yes;\
 85   PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=_ibalance] mass_t_ha;\
 86   SAVE=_a2save
Analysis of variance

Variate: mass_t_ha

Source of variation      d.f.          s.s.          m.s.          v.r.          F pr.

rep stratum                 2         175.2          87.6          0.42

rep.*Units* stratum
genotype                   21        5274.9         251.2           1.2         0.303
Residual                   42        8821.1           210

Total                      65       14271.2
Information summary

All terms orthogonal, none aliased.


Message: the following units have large residuals.

rep 1 *units* 7                  28.3     s.e.   11.6
```

Tables of means

Variate: mass_t_ha

Grand mean 41.9

genotype	BMR	e3	HG	p 197	p 220	p 225	p 249
	57.5	30.3	38.4	53.5	56.1	62.5	36.1
genotype	p 868	p 888	p 893	p 895	SK	ss 001	ss 003
	40.6	31.8	35.9	40.7	34	28.9	39.3
genotype	ss 007	ss 008	ss 016	ss 017	ss 081	ss 120	ss 56
	46.3	40.4	42	36.5	35.9	51	38.8
genotype	supa						
	45.3						

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42
s.e.d.	11.83

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	23.88

Stratum standard errors and coefficients of variation

Variate: mass_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	2	4.8
rep.*Units*	42	14.49	34.6

```
 87  IF _ibalance.eq.0 .OR. _ibalance.eq.1
 88    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 89    AKEEP [SAVE=_a2save['save']] genotype; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
 90    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 91    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=_mean; REPLICATION=_rep; VARIANCE=_var;\
 92   DF=_rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

	Mean	Lower	Upper
genotype			

BMR	57.54	24.074	91
e3	30.28	-3.183	63.74
HG	38.4	4.937	71.86
p 197	53.54	20.077	87
p 220	56.1	22.639	89.56
p 225	62.51	29.043	95.97
p 249	36.09	2.632	69.56
p 868	40.58	7.115	74.04
p 888	31.82	-1.646	65.28
p 893	35.89	2.427	69.35
p 895	40.71	7.243	74.17
SK	33.97	0.506	67.43
ss 001	28.87	-4.592	62.33
ss 003	39.35	5.885	72.81
ss 007	46.34	12.879	79.8
ss 008	40.37	6.91	73.84
ss 016	42.04	8.575	75.5
ss 017	36.45	2.991	69.92
ss 081	35.92	2.453	69.38
ss 120	50.95	17.49	84.42
ss 56	38.76	5.296	72.22
supa	45.29	11.829	78.75

```
 93   AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 94   SAVE=_a2save['save']] genotype
```

Tukey's 95% confidence intervals
genotype

	Mean	
p 225	62.51	a
BMR	57.54	a
p 220	56.1	a
p 197	53.54	a
ss 120	50.95	a
ss 007	46.34	a
supa	45.29	a
ss 016	42.04	a
p 895	40.71	a
p 868	40.58	a
ss 008	40.37	a
ss 003	39.35	a
ss 56	38.76	a
HG	38.4	a
ss 017	36.45	a
p 249	36.09	a
ss 081	35.92	a
p 893	35.89	a

SK	33.97	a
p 888	31.82	a
e3	30.28	a
ss 001	28.87	a

```
 95  ENDIF
 96  SET [IN=*]
102  "One-way design in randomized blocks"
103  DELETE [REDEFINE=yes] _ibalance
104  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype; BLOCKS=rep; FPROB=yes;\
105   PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=_ibalance] juice_t_ha;\
106   SAVE=_a2save
```

Analysis of variance

Variate: juice_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	8.659	4.33	0.55	
rep.*Units* stratum					
genotype	21	178.854	8.517	1.08	0.408
Residual	42	332.699	7.921		
Total	65	520.212			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 1 *units* 7 5.99 s.e. 2.25

Tables of means

Variate: juice_t_ha

Grand mean 9.04

genotype	BMR	e3	HG	p 197	p 220	p 225	p 249
	12.76	7.2	8.97	11.17	11.6	12.12	8.17
genotype	p 868	p 888	p 893	p 895	SK	ss 001	ss 003
	9.3	7.38	7.79	7.76	7.2	7.76	9.17
genotype	ss 007	ss 008	ss 016	ss 017	ss 081	ss 120	ss 56
	10.35	8.48	8.17	7.69	7.07	10.03	9.09
genotype	supa						
	9.76						

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42
s.e.d.	2.298

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	4.638

Stratum standard errors and coefficients of variation

Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	0.444	4.9
rep.*Units*	42	2.814	31.1

```
 107  IF _ibalance.eq.0 .OR. _ibalance.eq.1
 108    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 109    AKEEP [SAVE=_a2save['save']] genotype; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
 110    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 111    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=_mean; REPLICATION=_rep;
VARIANCE=_var;\
 112   DF=_rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

	Mean	Lower	Upper
genotype			
BMR	12.757	6.259	19.26
e3	7.198	0.7	13.7
HG	8.966	2.467	15.46
p 197	11.169	4.67	17.67
p 220	11.605	5.106	18.1
p 225	12.117	5.618	18.62
p 249	8.172	1.673	14.67
p 868	9.299	2.8	15.8
p 888	7.378	0.879	13.88
p 893	7.788	1.289	14.29
p 895	7.762	1.263	14.26
SK	7.198	0.7	13.7
ss 001	7.762	1.263	14.26
ss 003	9.171	2.672	15.67
ss 007	10.349	3.851	16.85
ss 008	8.479	1.981	14.98

ss 016	8.172	1.673	14.67
ss 017	7.685	1.186	14.18
ss 081	7.07	0.572	13.57
ss 120	10.029	3.53	16.53
ss 56	9.094	2.595	15.59
supa	9.76	3.261	16.26

```
 113   AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 114   SAVE=_a2save['save']] genotype
```

Tukey's 95% confidence intervals
genotype

	Mean	
BMR	12.757	a
p 225	12.117	a
p 220	11.605	a
p 197	11.169	a
ss 007	10.349	a
ss 120	10.029	a
supa	9.76	a
p 868	9.299	a
ss 003	9.171	a
ss 56	9.094	a
HG	8.966	a
ss 008	8.479	a
ss 016	8.172	a
p 249	8.172	a
p 893	7.788	a
ss 001	7.762	a
p 895	7.762	a
ss 017	7.685	a
p 888	7.378	a
e3	7.198	a
SK	7.198	a
ss 081	7.07	a

```
 115  ENDIF
 116  SET [IN=*]
 122  "One-way design in randomized blocks"
 123  DELETE [REDEFINE=yes] _ibalance
 124  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype; BLOCKS=rep; FPROB=yes;\
 125   PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=_ibalance] brix_%; SAVE=_a2save
```

Analysis of variance

Variate: brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	4.192	2.096	0.51	

rep.*Units* stratum					
genotype	21	207.794	9.895	2.41	0.008
Residual	42	172.612	4.11		
Total	65	384.598			

Information summary

All terms orthogonal, none aliased.

Tables of means

Variate: brix_%

Grand mean 16.18

genotype	BMR	e3	HG	p 197	p 220	p 225	p 249
	17.96	16.26	14.62	14.98	14.61	13.31	15.67
genotype	p 868	p 888	p 893	p 895	SK	ss 001	ss 003
	17.99	14.42	13.21	13.73	17.5	17.28	17.27
				ss			
genotype	ss 007	ss 008	ss 016	017	ss 081	ss 120	ss 56
	19.44	18.84	16.64	15	16.67	15.07	18.03
genotype	supa						
	17.49						

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42
s.e.d.	1.655

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42
l.s.d.	3.34

Stratum standard errors and coefficients of variation

Variate: brix_%

Stratum	d.f.	s.e.	cv%
rep	2	0.309	1.9
rep.*Units*	42	2.027	12.5

```
126  IF _ibalance.eq.0 .OR. _ibalance.eq.1
127    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
```

```
 128    AKEEP [SAVE=_a2save['save']] genotype; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
 129    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 130    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=_mean; REPLICATION=_rep;
VARIANCE=_var;\
 131   DF=_rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

	Mean	Lower	Upper
genotype			
BMR	17.96	13.27	22.64
e3	16.26	11.57	20.94
HG	14.62	9.94	19.3
p 197	14.98	10.3	19.66
p 220	14.61	9.93	19.29
p 225	13.31	8.63	17.99
p 249	15.67	10.99	20.35
p 868	17.99	13.31	22.67
p 888	14.42	9.74	19.1
p 893	13.21	8.53	17.89
p 895	13.73	9.05	18.41
SK	17.5	12.82	22.18
ss 001	17.28	12.6	21.96
ss 003	17.27	12.59	21.95
ss 007	19.44	14.76	24.13
ss 008	18.84	14.16	23.53
ss 016	16.64	11.96	21.33
ss 017	15	10.32	19.68
ss 081	16.67	11.99	21.35
ss 120	15.07	10.39	19.75
ss 56	18.03	13.35	22.71
supa	17.49	12.81	22.17

```
 132    AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 133   SAVE=_a2save['save']] genotype
```

Tukey's 95% confidence intervals

genotype

	Mean	
ss 007	19.44	a
ss 008	18.84	a
ss 56	18.03	a
p 868	17.99	a

BMR	17.96	a
SK	17.5	a
supa	17.49	a
ss 001	17.28	a
ss 003	17.27	a
ss 081	16.67	a
ss 016	16.64	a
e3	16.26	a
p 249	15.67	a
ss 120	15.07	a
ss 017	15	a
p 197	14.98	a
HG	14.62	a
p 220	14.61	a
p 888	14.42	a
p 895	13.73	a
p 225	13.31	a
p 893	13.21	a

```
134  ENDIF
135  SET [IN=*]
```

Anova Rustenburg 2012-2013

```
Genstat 64-bit Release 18.1 ( PC/Windows 8) 04 October 2017 17:40:45
Copyright 2015, VSN International Ltd.
Registered to: ARC-Grain Crops Institute
```

Genstat Eighteenth Edition
Genstat Procedure Library Release PL26.1

```
  1                                    SET                                    [WORKINGDIRECTORY='C:/Users/mavunganidzez/Documents']
  2                          "Data          taken          from          file:          '\
 -3  C:/Users/mavunganidzez/Documents/Wikus/2013          RB          cult          collection          2013.xls'"
  4                  DELETE  [REDEFINE=yes]          _stitle_:          TEXT          _stitle_
  5                    READ  [PRINT=*;          SETNVALUES=yes]          _stitle_
  9                   PRINT  [IPRINT=*]          _stitle_;          JUST=left
Data imported from Excel file: C:\Users\mavunganidzez\Documents\Wikus\2013 RB cult collection 2013.xls
 on: 4-Oct-2017 17:41:19
 taken from sheet "stats data", cells A2:F64
```

```
10 DELETE [REDEFINE=yes] rep,Entry,genotype,mass_t_ha,brix_%,juice_t_ha
11 UNITS [NVALUES=*]
12 FACTOR [MODIFY=no; NVALUES=63; LEVELS=3; LABELS=*; REFERENCE=1] rep
13 READ rep; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
rep	63	0	3

```
16 VARIATE [NVALUES=63] Entry
17 READ Entry
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Entry	1.000	11.00	21.00	63	0

```
21 FACTOR [MODIFY=no; NVALUES=63; LEVELS=21; LABELS=!t('BMR','e3','HG','p 197',\
22 'p 220','p 225','p 249','p 868','p 888','p 893','p 895','SK','ss 001',\
23 'ss 003','ss 007','ss 008','ss 016','ss 017','ss 120','ss 56','supa')\
24 ; REFERENCE=1] genotype
25 READ genotype; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
genotype	63	0	21

```
29 VARIATE [NVALUES=63] mass_t_ha
30 READ mass_t_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
mass_t_ha	13.22	74.89	129.1	63	0

```
47 VARIATE [NVALUES=63] brix_%
48 READ brix_%
```

Identifier	Minimum	Mean	Maximum	Values	Missing
brix_%	6.833	15.34	23.47	63	0

```
62 VARIATE [NVALUES=63] juice_t_ha
63 READ juice_t_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
juice_t_ha	7.147	16.95	32.20	63	0

```
80
81 %PostMessage 1129; 0; 100001 "Sheet Update Completed"
82 "General Analysis of Variance"
83 BLOCK rep
84 TREATMENTS genotype
85 COVARIATE "No Covariate"
86 ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
87 PSE=diff,lsd,means; LSDLEVEL=5] brix_%
```

Analysis of variance

Variate: brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.962	0.481	0.07	
rep.*Units* stratum					
genotype	20	558.000	27.900	4.10	<.001
Residual	40	271.919	6.798		
Total	62	830.881			

Message: the following units have large residuals.

rep 1 *units* 18 -5.85 s.e. 2.08

rep 3 *units* 13 -4.66 s.e. 2.08

Tables of means
Variate: brix_%
Grand mean 15.34

genotype	BMR	e3	HG	p 197	p 220	p 225	p 249
	16.50	16.19	17.61	12.23	13.43	10.94	10.51

genotype	p 868	p 888	p 893	p 895	SK	ss 001	ss 003
	16.99	13.34	10.80	11.50	18.46	15.14	16.87

genotype	ss 007	ss 008	ss 016	ss 017	ss 120	ss 56	supa
	20.02	14.64	18.36	13.73	18.46	20.26	16.21

Standard errors of means

Table	genotype
rep.	3
d.f.	40
e.s.e.	1.505

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	40
s.e.d.	2.129

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	40
l.s.d.	4.303

Stratum standard errors and coefficients of variation
Variate: brix_%

Stratum	d.f.	s.e.	cv%
rep	2	0.151	1.0
rep.*Units*	40	2.607	17.0

```
88                          "General                    Analysis                    of                  Variance"
89                                                BLOCK                                                 rep
90                                           TREATMENTS                                                 genotype
91                                  COVARIATE                                "No                        Covariate"
92          ANOVA  [PRINT=aovtable,information,means,%cv;  FACT=32;  CONTRASTS=7;  PCONTRASTS=7;  FPROB=yes;\
93                                 PSE=diff,lsd,means;  LSDLEVEL=5]                                        juice_t_ha
```

Analysis of variance
Variate: juice_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	9.85	4.92	0.23	
rep.*Units* stratum					
genotype	20	1296.13	64.81	3.03	0.001
Residual	40	856.19	21.40		
Total	62	2162.17			

Message: the following units have large residuals.

rep 1 *units* 2 -8.42 s.e. 3.69

Tables of means
Variate: juice_t_ha
Grand mean 16.95

genotype	BMR	e3	HG	p 197	p 220	p 225	p 249
	21.54	13.83	21.72	12.22	14.45	9.99	14.22

genotype	p 868	p 888	p 893	p 895	SK	ss 001	ss 003
	16.11	11.02	13.55	13.91	20.06	22.30	25.05

genotype	ss 007	ss 008	ss 016	ss 017	ss 120	ss 56	supa
	19.76	22.06	12.16	21.08	21.29	10.85	18.70

Standard errors of means

Table	genotype
rep.	3
d.f.	40
e.s.e.	2.671

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	40
s.e.d.	3.778

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	40
l.s.d.	7.635

Stratum standard errors and coefficients of variation
Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	0.484	2.9
rep.*Units*	40	4.627	27.3

```
 94                                "General                    Analysis                    of                  Variance"
 95                                                      BLOCK                                                   rep
 96                                                 TREATMENTS                                                   genotype
 97                                        COVARIATE                          "No                              Covariate"
 98          ANOVA     [PRINT=aovtable,information,means,%cv;     FACT=32;     CONTRASTS=7;     PCONTRASTS=7;     FPROB=yes;\
 99                                        PSE=diff,lsd,means;          LSDLEVEL=5]                                mass_t_ha
```

Analysis of variance
Variate: mass_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	849.6	424.8	1.18	
rep.*Units* stratum					
genotype	20	16917.7	845.9	2.35	0.010
Residual	40	14374.8	359.4		
Total	62	32142.1			

Message: the following units have large residuals.

rep 1 *units* 20 -37.0 s.e. 15.1

Tables of means

Variate: mass_t_ha

Grand mean 74.9

genotype	BMR	e3	HG	p 197	p 220	p 225	p 249
	98.9	55.4	97.1	83.0	84.3	71.4	73.1

genotype	p 868	p 888	p 893	p 895	SK	ss 001	ss 003
	72.1	51.3	69.7	85.4	82.7	77.6	103.4

genotype	ss 007	ss 008	ss 016	ss 017	ss 120	ss 56	supa
	66.6	80.7	39.6	77.2	87.8	44.9	70.7

Standard errors of means

Table	genotype
rep.	3
d.f.	40
e.s.e.	10.94

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	40
s.e.d.	15.48

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	40
l.s.d.	31.28

Stratum standard errors and coefficients of variation

Variate: mass_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	4.50	6.0
rep.*Units*	40	18.96	25.3

2013 - 2014

Anova Bethlehem 2013-2014

```
Genstat 64-bit Release 18.1 ( PC/Windows 8) 04 October 2017 17:35:55
Copyright 2015, VSN International Ltd.
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                                  ___________________________________

                                  Genstat Eighteenth Edition
                                  Genstat Procedure Library Release PL26.1

                                  ___________________________________

 1                                                    SET                                                [WORKINGDIRECTORY='C:/Users/mavunganidzez/Documents']
 2                                                          "Data                taken                from                file:                '\
-3                                  C:/Users/mavunganidzez/Documents/Wikus/2014                BH                cult                coll                2014.xls'"
 4                                             DELETE                [REDEFINE=yes]                _stitle_:                TEXT                _stitle_
 5                                                  READ                [PRINT=*;                SETNVALUES=yes]                _stitle_
 9                                                  PRINT                [IPRINT=*]                _stitle_;                JUST=left
Data imported from Excel file: C:\Users\mavunganidzez\Documents\Wikus\2014 BH cult coll 2014.xls
 on: 4-Oct-2017 17:36:38
 taken from sheet "stats data", cells A2:F49
 10                                                  DELETE                [REDEFINE=yes]                rep,entry,genotype,mass_t_ha,brix_%,juice_t_ha
 11                                                                                UNITS                [NVALUES=*]
 12                                       FACTOR                [MODIFY=no;                NVALUES=48;                LEVELS=3;                LABELS=*;                REFERENCE=1]                rep
 13                                                  READ                rep;                FREPRESENTATION=ordinal
     Identifier   Values    Missing    Levels
            rep       48          0         3
 16                                                                VARIATE                [NVALUES=48]                entry
 17                                                                READ                entry
     Identifier    Minimum       Mean    Maximum     Values    Missing
          entry      1.000      8.500      16.00         48          0
 20                                       FACTOR                [MODIFY=no;                NVALUES=48;                LEVELS=16;                LABELS=!t('HG','p                868','p                888',\
 21                                  'p                893','p                895','SK','ss                001','ss                003','ss                007','ss                008','ss                016','ss                017',\
 22                                            'ss                081','ss                120','ss                27','supa');                REFERENCE=1]                genotype
 23                                                  READ                genotype;                FREPRESENTATION=ordinal
     Identifier      Values     Missing      Levels
       genotype          48           0          16

 26                                                                VARIATE                [NVALUES=48]                mass_t_ha
 27                                                                READ                mass_t_ha
     Identifier    Minimum       Mean    Maximum     Values    Missing
      mass_t_ha     0.7685      16.15      51.49         48          0
 40                                                                VARIATE                [NVALUES=48]                brix_%
 41                                                                READ                brix_%
     Identifier    Minimum       Mean    Maximum     Values    Missing
          brix_%     0.0000      13.80      21.17         48          0
```

```
51                                                  VARIATE                  [NVALUES=48]                  juice_t_ha
52                                                                  READ                                      juice_t_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing	
juice_t_ha	0.0000	2.586	14.22	48	0	Skew

```
64
65                          %PostMessage          1129;          0;          100001          "Sheet          Update          Completed"
66                                    "General                    Analysis                    of                    Variance"
67                                                                BLOCK                                         rep
68                                                            TREATMENTS                                        genotype
69                                                  COVARIATE                          "No                    Covariate"
70                          ANOVA          [PRINT=aovtable,information,means,%cv;          FACT=32;          CONTRASTS=7;          PCONTRASTS=7;          FPROB=yes;\
71                                                  PSE=diff,lsd,means;                    LSDLEVEL=5]                    brix_%
```

Analysis of variance

Variate: brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	21.23	10.62	0.49	
rep.*Units* stratum					
genotype	15	437.66	29.18	1.34	0.239
Residual	30	652.12	21.74		
Total	47	1111.01			

Message: the following units have large residuals.

rep 1 *units* 3	8.10	s.e.	3.69
rep 1 *units* 5	-10.02	s.e.	3.69
rep 1 *units* 10	-11.51	s.e.	3.69
rep 3 *units* 5	9.52	s.e.	3.69

Tables of means

Variate: brix_%

Grand mean 13.80

genotype	HG	p 868	p 888	p 893	p 895	SK	ss 001
	13.07	14.47	3.63	15.87	10.86	16.33	14.18

genotype	ss 003	ss 007	ss 008	ss 016	ss 017	ss 081	ss 120
	15.63	16.72	12.34	14.61	15.04	15.86	13.86

genotype	ss 27	supa
	14.97	13.32

Standard errors of means

Table	genotype
rep.	3
d.f.	30
e.s.e.	2.692

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	30
s.e.d.	3.807

Least significant differences of means (5% level)

Table	genotype
rep.	3

d.f.	30
l.s.d.	7.774

Stratum standard errors and coefficients of variation

Variate: brix_%

Stratum	d.f.	s.e.	cv%
rep	2	0.815	5.9
rep.*Units*	30	4.662	33.8

```
 72 "General Analysis of Variance"
 73 BLOCK rep
 74 TREATMENTS genotype
 75 COVARIATE "No Covariate"
 76 ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
 77 PSE=diff,lsd,means; LSDLEVEL=5] juice_t_ha
```

Analysis of variance

Variate: juice_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	41.793	20.896	4.38	
rep.*Units* stratum					
genotype	15	81.823	5.455	1.14	0.365
Residual	30	143.269	4.776		
Total	47	266.884			

Message: the following units have large residuals.

rep 3 *units* 15 6.68 s.e. 1.73

Tables of means

Variate: juice_t_ha

Grand mean 2.59

genotype	HG	p 868	p 888	p 893	p 895	SK	ss 001
	4.10	3.71	0.77	2.69	1.67	3.46	1.28

genotype	ss 003	ss 007	ss 008	ss 016	ss 017	ss 081	ss 120
	2.82	2.05	2.31	1.67	2.82	1.92	2.56

genotype	ss 27	supa
	6.28	1.28

Standard errors of means

Table	genotype
rep.	3
d.f.	30
e.s.e.	1.262

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	30
s.e.d.	1.784

Least significant differences of means (5% level)

Table	genotype
rep.	3

d.f. 30
l.s.d. 3.644

Stratum standard errors and coefficients of variation
Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	1.143	44.2
rep.*Units*	30	2.185	84.5

```
 78 "General Analysis of Variance"
 79 BLOCK rep
 80 TREATMENTS genotype
 81 COVARIATE "No Covariate"
 82 ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
 83 PSE=diff,lsd,means; LSDLEVEL=5] mass_t_ha
```

Analysis of variance
Variate: mass_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	635.07	317.54	4.95	
rep.*Units* stratum					
genotype	15	1063.26	70.88	1.11	0.393
Residual	30	1923.79	64.13		
Total	47	3622.13			

Message: the following units have large residuals.

rep 3 *units* 1 14.2 s.e. 6.3
rep 3 *units* 15 20.5 s.e. 6.3

Tables of means
Variate: mass_t_ha
Grand mean 16.1

genotype	HG	p 868	p 888	p 893	p 895	SK	ss 001
	21.3	14.3	8.7	19.5	11.5	20.6	10.9

genotype	ss 003	ss 007	ss 008	ss 016	ss 017	ss 081	ss 120
	16.1	16.8	15.8	11.0	17.5	12.4	22.4

genotype	ss 27	supa
	26.1	13.3

Standard errors of means

Table	genotype
rep.	3
d.f.	30
e.s.e.	4.62

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	30
s.e.d.	6.54

Least significant differences of means (5% level)

Table	genotype
rep.	3

d.f.	30
l.s.d.	13.35

Stratum standard errors and coefficients of variation

Variate: mass_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	4.45	27.6
rep.*Units*	30	8.01	49.6

Anova Potchefstroom 2013-2014

```
321  "Data taken from file: 'H:/Vikus/Copy of 2014 Potch cult data.xls'"
 322  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
 323  READ [PRINT=*; SETNVALUES=yes] _stitle_
 327  PRINT [IPRINT=*] _stitle_; JUST=left

Data imported from Excel file: H:\Vikus\Copy of 2014 Potch cult data.xls
 on: 10-Oct-2017 8:50:44
 taken from sheet "stats data", cells A2:F52

 328  DELETE [REDEFINE=yes] rep,entry,genotype,mass_t_ha,brix_%,juice_t_ha
 329  UNITS [NVALUES=*]
 330  FACTOR [MODIFY=no; NVALUES=51; LEVELS=3; LABELS=*; REFERENCE=1] rep
 331  READ rep; FREPRESENTATION=ordinal

                                Identifier          Values         Missing      Levels
                                rep                     51               0           3

 334  VARIATE [NVALUES=51] entry
 335  READ entry

                                Identifier         Minimum        Mean         Maximum        Values      Missing
                                entry                     1           9              17            51            0

 338  FACTOR [MODIFY=no; NVALUES=51; LEVELS=17; LABELS=!t('BMR','HG','p 868',\
 339  'p 888','p 893','p 895','SK','ss 001','ss 003','ss 007','ss 008','ss 016',\
 340  'ss 017','ss 081','ss 120','ss 56','supa'); REFERENCE=1] genotype
 341  READ genotype; FREPRESENTATION=ordinal

                                Identifier          Values         Missing      Levels
                                genotype                51               0          17

 344  VARIATE [NVALUES=51] mass_t_ha
 345  READ mass_t_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
mass_t_ha	20.7	78	145.3	51	0

```
356  VARIATE [NVALUES=51] brix_%
357  READ brix_%
```

Identifier	Minimum	Mean	Maximum	Values	Missing
brix_%	10.77	15.48	20.1	51	0

```
368  VARIATE [NVALUES=51] juice_t_ha
369  READ juice_t_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
juice_t_ha	0.7655	15.17	36.03	51	0

```
                    376
377  %PostMessage 1129; 0; 100003 "Sheet Update Completed"
378  "One-way design in randomized blocks"
379  DELETE [REDEFINE=yes] _ibalance
380  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype; BLOCKS=rep; FPROB=yes;\
381   PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=_ibalance] mass_t_ha;\
382   SAVE=_a2save
```

Analysis of variance

Variate: mass_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	3325.4	1662.7	2.05	
rep.*Units* stratum					
genotype	16	18244.4	1140.3	1.4	0.201
Residual	32	25988.3	812.1		
Total	50	47558.1			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 3 *units* 12 51.4 s.e. 22.6

Tables of means

Variate: mass_t_ha

Grand mean 78.0

genotype	BMR	HG	p 868	p 888	p 893	p 895	SK
	106.7	122.2	80.2	55.7	80.2	100.3	65.1

genotype	ss 001	ss 003	ss 007	ss 008	ss 016	ss 017	ss 081
	68.2	82.8	79.9	79.4	63.2	79.1	58.3

genotype	ss 120	ss 56	supa
	95.5	55.6	53.5

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	32
s.e.d.	23.27

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	32
l.s.d.	47.4

Stratum standard errors and coefficients of variation

Variate: mass_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	9.89	12.7
rep.*Units*	32	28.5	36.5

```
383  IF _ibalance.eq.0 .OR. _ibalance.eq.1
384    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
385    AKEEP [SAVE=_a2save['save']] genotype; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
386    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
387    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=_mean; REPLICATION=_rep; VARIANCE=_var;\
388   DF=_rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

genotype	Mean	Lower	Upper
BMR	106.69	42.19	171.2
HG	122.16	57.65	186.7
p 868	80.19	15.69	144.7
p 888	55.75	-8.75	120.3
p 893	80.24	15.74	144.7
p 895	100.31	35.8	164.8
SK	65.06	0.56	129.6

ss 001	68.23	3.73	132.7
ss 003	82.79	18.28	147.3
ss 007	79.9	15.4	144.4
ss 008	79.41	14.91	143.9
ss 016	63.25	-1.25	127.8
ss 017	79.06	14.55	143.6
ss 081	58.29	-6.21	122.8
ss 120	95.51	31.01	160
ss 56	55.58	-8.92	120.1
supa	53.54	-10.96	118

```
 389   AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 390   SAVE=_a2save['save']] genotype
```

Tukey's 95% confidence intervals
genotype

	Mean	
HG	122.16	a
BMR	106.69	a
p 895	100.31	a
ss 120	95.51	a
ss 003	82.79	a
p 893	80.24	a
p 868	80.19	a
ss 007	79.9	a
ss 008	79.41	a
ss 017	79.06	a
ss 001	68.23	a
SK	65.06	a
ss 016	63.25	a
ss 081	58.29	a
p 888	55.75	a
ss 56	55.58	a
supa	53.54	a

```
 391  ENDIF
 392  SET [IN=*]
 398  "One-way design in randomized blocks"
 399  DELETE [REDEFINE=yes] _ibalance
 400  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype; BLOCKS=rep; FPROB=yes;\
 401   PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=_ibalance] juice_t_ha;\
 402   SAVE=_a2save
```

Analysis of variance

Variate: juice_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	76.33	38.17	0.72	

rep.*Units* stratum					
genotype	16	1550.82	96.93	1.83	0.071
Residual	32	1692.73	52.9		
Total	50	3319.88			

Information summary

All terms orthogonal, none aliased.
Message: the following units have large residuals.

rep 1 *units* 8	-12.9	s.e. 5.8
rep 2 *units* 4	-13.3	s.e. 5.8
rep 3 *units* 15	-14.5	s.e. 5.8

Tables of means

Variate: juice_t_ha

Grand mean 15.2

genotype	BMR	HG	p 868	p 888	p 893	p 895	SK
	21.1	26.9	17.4	11	12.4	21.8	11.6

genotype	ss 001	ss 003	ss 007	ss 008	ss 016	ss 017	ss 081
	13	15.4	15.4	12.6	10.1	12.1	15.3

genotype	ss 120	ss 56	supa
	25.7	8.7	7.5

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	32
s.e.d.	5.94

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	32
l.s.d.	12.1

Stratum standard errors and coefficients of variation

Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%

rep	2	1.5	9.9
rep.*Units*	32	7.27	47.9

```
 403  IF _ibalance.eq.0 .OR. _ibalance.eq.1
 404    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 405    AKEEP [SAVE=_a2save['save']] genotype; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
 406    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 407    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=_mean; REPLICATION=_rep; VARIANCE=_var;\
 408   DF=_rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

genotype			
BMR	21.08	4.618	37.54
HG	26.86	10.393	43.32
p 868	17.38	0.914	33.84
p 888	10.96	-5.501	27.42
p 893	12.39	-4.07	28.85
p 895	21.85	5.386	38.31
SK	11.6	-4.862	28.06
ss 001	13.04	-3.418	29.51
ss 003	15.39	-1.067	31.86
ss 007	15.38	-1.08	31.84
ss 008	12.65	-3.814	29.11
ss 016	10.08	-6.382	26.54
ss 017	12.14	-4.325	28.6
ss 081	15.28	-1.182	31.74
ss 120	25.68	9.218	42.14
ss 56	8.69	-7.775	25.15
supa	7.47	-8.989	23.94

```
 409    AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 410   SAVE=_a2save['save']] genotype
```

Tukey's 95% confidence intervals

genotype

	Mean	
HG	26.86	a
ss 120	25.68	a
p 895	21.85	a
BMR	21.08	a
p 868	17.38	a
ss 003	15.39	a
ss 007	15.38	a
ss 081	15.28	a
ss 001	13.04	a
ss 008	12.65	a
p 893	12.39	a

ss 017	12.14	a
SK	11.6	a
p 888	10.96	a
ss 016	10.08	a
ss 56	8.69	a
supa	7.47	a

```
 411  ENDIF
 412  SET [IN=*]
 418  "One-way design in randomized blocks"
 419  DELETE [REDEFINE=yes] _ibalance
 420  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype; BLOCKS=rep; FPROB=yes;\
 421   PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=_ibalance] brix_%; SAVE=_a2save
```

Analysis of variance

Variate: brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.499	0.249	0.04	
rep.*Units* stratum					
genotype	16	70.862	4.429	0.75	0.721
Residual	32	187.938	5.873		
Total	50	259.299			

Information summary

All terms orthogonal, none aliased.
Message: the following units have large residuals.

rep 1 *units* 2	4.18	s.e.	1.92
rep 1 *units* 8	-4.24	s.e.	1.92
rep 1 *units* 9	-4.23	s.e.	1.92

Tables of means

Variate: brix_%

Grand mean 15.48

genotype	BMR	HG	p 868	p 888	p 893	p 895	SK
	12.63	14.14	16.26	14.56	15	14.03	15.82

genotype	ss 001	ss 003	ss 007	ss 008	ss 016	ss 017	ss 081
	17.26	14.99	16.44	17.03	16.5	15.11	15.02

genotype	ss 120	ss 56	supa
	15.5	16.39	16.42

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	32
s.e.d.	1.979

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	32
l.s.d.	4.031

Stratum standard errors and coefficients of variation

Variate: brix_%

Stratum	d.f.	s.e.	cv%
rep	2	0.121	0.8
rep.*Units*	32	2.423	15.7

```
 422  IF _ibalance.eq.0 .OR. _ibalance.eq.1
 423    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 424    AKEEP [SAVE=_a2save['save']] genotype; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
 425    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 426    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=_mean; REPLICATION=_rep; VARIANCE=_var;\
 427   DF=_rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

	Mean	Lower	Upper
genotype			
BMR	12.63	7.148	18.12
HG	14.14	8.659	19.63
p 868	16.26	10.77	21.74
p 888	14.56	9.07	20.04
p 893	15	9.515	20.49
p 895	14.03	8.548	19.52
SK	15.82	10.337	21.31
ss 001	17.26	11.77	22.74
ss 003	14.99	9.504	20.47
ss 007	16.44	10.959	21.93
ss 008	17.03	11.548	22.52
ss 016	16.5	11.015	21.99
ss 017	15.11	9.626	20.6
ss 081	15.02	9.537	20.51
ss 120	15.5	10.015	20.99
ss 56	16.39	10.904	21.87
supa	16.42	10.937	21.91

```
 428   AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 429   SAVE=_a2save['save']] genotype
```

Tukey's 95% confidence intervals
genotype

	Mean	
ss 001	17.26	a
ss 008	17.03	a
ss 016	16.5	a
ss 007	16.44	a
supa	16.42	a
ss 56	16.39	a
p 868	16.26	a
SK	15.82	a
ss 120	15.5	a
ss 017	15.11	a
ss 081	15.02	a
p 893	15	a
ss 003	14.99	a
p 888	14.56	a
HG	14.14	a
p 895	14.03	a
BMR	12.63	a

```
 430  ENDIF
 431  SET [IN=*]
```

Anova Rustenburg 2013-2014

```
Genstat 64-bit Release 19.1 ( PC/Windows 8) 24 February 2020 12:03:50
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                                                  ___________________________________

                                                  Genstat Nineteenth Edition
                                                  Genstat Procedure Library Release PL27.1

                                                  ___________________________________

 1                                        SET       [WORKINGDIRECTORY='C:/Users/belindaj/Documents';                    DIAGNOSTIC=messages]
 2                          "Data  taken  from  file:  'F:/2020/anova/2014  Rb  2014.xls'"
 3                                 DELETE  [REDEFINE=yes]  _stitle_:  TEXT  _stitle_
 4                                   READ  [PRINT=*;  SETNVALUES=yes]  _stitle_
 8                                  PRINT  [IPRINT=*]  _stitle_;  JUST=left
Data imported from Excel file: F:\2020\anova\2014 Rb 2014.xls
 on: 24-Feb-2020 12:04:34
 taken from sheet "stats", cells A2:H52
 9                                 DELETE  [REDEFINE=yes]  rep,entry,genotype,mass_t_ha,height_m,diameter_cm,\
10                                                                                                  brix_%,juice_t_ha
11                                                                  UNITS  [NVALUES=*]
12                       FACTOR  [MODIFY=no;  NVALUES=51;  LEVELS=3;  LABELS=*;  REFERENCE=1]  rep
13                                READ  rep;  FREPRESENTATION=ordinal
    Identifier  Values  Missing  Levels
           rep      51        0       3
16                                  VARIATE  [NVALUES=51]  entry
17                                    READ  entry
    Identifier  Minimum  Mean  Maximum  Values  Missing
         entry    1.000  10.65    21.00      51        0
20                       FACTOR  [MODIFY=no;  NVALUES=51;  LEVELS=17;  LABELS=*;  REFERENCE=1]  genotype
21                                READ  genotype;  FREPRESENTATION=ordinal
    Identifier  Values  Missing  Levels
      genotype      51        0      17
24                                  VARIATE  [NVALUES=51]  mass_t_ha
25                                    READ  mass_t_ha
    Identifier  Minimum  Mean  Maximum  Values  Missing
     mass_t_ha   17.25  46.89    70.92      51        0
36                                  VARIATE  [NVALUES=51]  height_m
37                                    READ  height_m
    Identifier  Minimum  Mean  Maximum  Values  Missing
      height_m   1.573  2.290    3.237      51        0
48                                  VARIATE  [NVALUES=51]  diameter_cm
49                                    READ  diameter_cm
    Identifier  Minimum  Mean  Maximum  Values  Missing  Skew
   diameter_cm  0.6000  1.006    2.300      51        0

58                                  VARIATE  [NVALUES=51]  brix_%
59                                    READ  brix_%
```

Identifier	Minimum	Mean	Maximum	Values	Missing
brix_%	11.57	18.86	23.13	51	0

```
70 VARIATE [NVALUES=51] juice_t_ha
71 READ juice_t_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
juice_t_ha	15.33	92.72	161.0	51	0

```
81
82 %PostMessage 1129; 0; 10000001 "Sheet Update Completed"
83 "General Analysis of Variance"
84 BLOCK rep
85 TREATMENTS genotype
86 COVARIATE "No Covariate"
87 ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
88 PSE=diff,lsd,means; LSDLEVEL=5] brix_%Analysis of variance
```

Variate: brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	9.403	4.701	0.61	
rep.*Units* stratum					
genotype	16	98.024	6.127	0.79	0.685
Residual	32	248.070	7.752		
Total	50	355.497			

Message: the following units have large residuals.

rep 1 *units* 14 -5.17 s.e. 2.21
rep 2 *units* 6 -5.26 s.e. 2.21

Tables of means

Variate: brix_%

Grand mean 18.86

genotype	1	2	3	4	5	6	7
	18.70	19.47	18.06	19.32	19.61	17.27	17.88

genotype	8	9	10	11	12	13	14
	19.78	15.29	19.04	18.83	19.87	17.79	17.96

genotype	15	16	17
	19.46	21.40	20.84

Standard errors of means

Table	genotype
rep.	3
d.f.	32
e.s.e.	1.608

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	32
s.e.d.	2.273

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	32

l.s.d. 4.631

Stratum standard errors and coefficients of variation

Variate: brix_%

Stratum	d.f.	s.e.	cv%
rep	2	0.526	2.8
rep.*Units*	32	2.784	14.8

```
 89                                    "General                    Analysis                    of                    Variance"
 90                                                                BLOCK                                             rep
 91                                                                TREATMENTS                                        genotype
 92                                                  COVARIATE                         "No                           Covariate"
 93                    ANOVA          [PRINT=aovtable,information,means,%cv;          FACT=32;      CONTRASTS=7;    PCONTRASTS=7;   FPROB=yes;\
 94
111                    ANOVA          [PRINT=aovtable,information,means,%cv;          FACT=32;      CONTRASTS=7;    PCONTRASTS=7;   FPROB=yes;\
112   PSE=diff,lsd,means; LSDLEVEL=5] juice_t_haAnalysis of variance
```

Variate: juice_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	1664.	832.	0.61	
rep.*Units* stratum					
genotype	16	26061.	1629.	1.19	0.329
Residual	32	43949.	1373.		
Total	50	71674.			

Message: the following units have large residuals.

rep 1 *units* 15 73.3 s.e. 29.4

rep 2 *units* 1 83.1 s.e. 29.4

Tables of means

Variate: juice_t_ha

Grand mean 92.7

genotype	1	2	3	4	5	6	7
	54.9	115.0	54.9	104.1	107.3	88.2	98.4

genotype	8	9	10	11	12	13	14
	129.1	93.3	100.9	104.8	84.9	58.8	79.2

genotype	15	16	17
	81.8	85.2	135.6

Standard errors of means

Table	genotype
rep.	3
d.f.	32
e.s.e.	21.40

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	32
s.e.d.	30.26

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	32
l.s.d.	61.64

Stratum standard errors and coefficients of variation
Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	7.00	7.5
rep.*Units*	32	37.06	40.0

```
113 "General Analysis of Variance"
114 BLOCK rep
115 TREATMENTS genotype
116 COVARIATE "No Covariate"
117 ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
118 PSE=diff,lsd,means; LSDLEVEL=5] mass_t_haAnalysis of variance
```

Variate: mass_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	510.4	255.2	1.43	
rep.*Units* stratum					
genotype	16	2752.6	172.0	0.96	0.515
Residual	32	5719.0	178.7		
Total	50	8982.1			

Message: the following units have large residuals.

rep 1 *units* 15	27.3	s.e.	10.6
rep 2 *units* 9	-26.6	s.e.	10.6

Tables of means
Variate: mass_t_ha
Grand mean 46.9

genotype	1	2	3	4	5	6	7
	37.6	51.7	30.0	52.6	48.8	48.8	43.7

genotype	8	9	10	11	12	13	14
	58.8	47.7	49.6	56.1	46.1	41.8	47.0

genotype	15	16	17
	39.7	39.8	57.4

Standard errors of means

Table	genotype
rep.	3
d.f.	32
e.s.e.	7.72

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	32
s.e.d.	10.92

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	32
l.s.d.	22.23

Stratum standard errors and coefficients of variation

Variate: mass_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	3.87	8.3
rep.*Units*	32	13.37	28.5

Rustenburg 2914 Juice yield - Transformation square root:

Analysis of variance

Variate: juice_t_ha_trans_sqr

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	5.5558	2.7779	11.38	
rep.*Units* stratum					
genotype	21	11.2380	0.5351	2.19	0.015
Residual	42	10.2557	0.2442		
Total	65	27.0495			

Message: the following units have large residuals.

rep 1 *units* 9	1.039	s.e.	0.394
rep 2 *units* 6	1.058	s.e.	0.394
rep 2 *units* 9	-1.035	s.e.	0.394
rep 2 *units* 12	0.940	s.e.	0.394
rep 3 *units* 6	-0.933	s.e.	0.394

Tables of means

Variate: juice_t_ha_trans_sqr

Grand mean 1.276

genotype	1	2	3	4	5	6	7
	1.073	2.410	0.931	1.094	1.341	1.804	1.560

genotype	8	9	10	11	12	13	14
	0.909	1.537	1.140	1.093	1.526	1.282	1.354

genotype	15	16	17	18	19	20	21
	1.719	0.672	0.818	1.867	1.210	0.747	0.915

genotype	22
	1.064

Standard errors of means

Table	genotype
rep.	3
d.f.	42
e.s.e.	0.2853

Standard errors of differences of means

Table	genotype
rep.	3
d.f.	42
s.e.d.	0.4035

Least significant differences of means (5% level)

Table	genotype
rep.	3
d.f.	42

l.s.d. 0.8142

Stratum standard errors and coefficients of variation

Variate: juice_t_ha_trans_sqr

Stratum	d.f.	s.e.	cv%
rep	2	0.3553	27.8
rep.*Units*	42	0.4941	38.7

Appendix J 2

Nitrogen application levels

2011-2012

Vaalharts 2011-2012

```
Genstat 64-bit Release 18.1 ( PC/Windows 8) 10 October 2017 08:02:10
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                    ------------------------------------------

                    Genstat Eighteenth Edition
                    Genstat Procedure Library Release PL26.1

                    ------------------------------------------

  1  SET [WORKINGDIRECTORY='C:/Users/maalis/Documents']
  2  "Data taken from file: 'H:/Vikus/Copy of 2012 VH nitro coll 2012.xls'"
  3  DELETE [REDEFINE=yes]   stitle  : TEXT   stitle
  4  READ [PRINT=*; SETNVALUES=yes] _stitle_
  8  PRINT [IPRINT=*] _stitle_; JUST=left

Data imported from Excel file: H:\Vikus\Copy of 2012 VH nitro coll 2012.xls
 on: 10-Oct-2017 8:03:58
 taken from sheet "stats data", cells A2:G31

  9  DELETE [REDEFINE=yes] rep,genotype,treatment_N_kg_ha,treat_level,\
 10  biomass  t  ha,brix  %,juice  t  ha
 11  UNITS [NVALUES=*]
 12  FACTOR [MODIFY=no; NVALUES=30; LEVELS=2; LABELS=*; REFERENCE=1] rep
 13  READ rep; FREPRESENTATION=ordinal

                     Identifier          Values       Missing       Levels
                    rep                      30             0            2

 15  FACTOR [MODIFY=no; NVALUES=30; LEVELS=3; LABELS=!t('PX 174','ss 120',\
 16  'ss 27'); REFERENCE=1] genotype
 17  READ genotype; FREPRESENTATION=ordinal

                     Identifier          Values       Missing       Levels
                    genotype                 30             0            3

 19  VARIATE [NVALUES=30] treatment  N  kg  ha
 20  READ treatment_N_kg_ha

                     Identifier          Minimum      Mean
```

treatment_N_kg_ha	0	60	120	30	0

```
 23  FACTOR [MODIFY=no; NVALUES=30; LEVELS=5; LABELS=*; REFERENCE=1] treat_level
 24  READ treat  level; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
treat_level	30	0	5

```
 26  VARIATE [NVALUES=30] biomass_t_ha
 27  READ biomass  t  ha
```

Identifier	Minimum	Mean			
biomass_t_ha	16.03	30.26	46.66	30	0

```
 31  VARIATE [NVALUES=30] brix_%
 32  READ brix_%
```

Identifier	Minimum	Mean			
brix_%	17	23.18	28.25	30	0

```
 36  VARIATE [NVALUES=30] juice_t_ha
 37  READ juice_t_ha
```

Identifier	Minimum	Mean			
juice_t_ha	1.44	3.472	6.624	30	0

```
                41
 42  %PostMessage 1129; 0; 100001 "Sheet Update Completed"
 43  "Two-way design in randomized blocks"
 44  DELETE [REDEFINE=yes] _ibalance
 45  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,treat_level; BLOCKS=rep;\
 46   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=  ibalance]\
 47   biomass_t_ha; SAVE=_a2save
```

Analysis of variance

Variate: biomass t ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	9.41	9.41	0.18	
rep.*Units* stratum					
genotype	2	369.65	184.83	3.49	0.059
treat level	4	277.98	69.5	1.31	0.313
genotype.treat level	8	182.86	22.86	0.43	0.883
Residual	14	741.39	52.96		
Total	29	1581.29			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 1 *units* 14	-13.3	s.e. 5.0
rep 2 *units* 14	13.3	s.e. 5.0

Tables of means

Variate: biomass t ha

Grand mean 30.3

genotype	PX 174	ss 120	ss 27
	34.3	25.8	30.7

treat level	1	2	3	4	5
	24.9	32.2	31.5	33.6	29.1

genotype	treat level	1	2	3	4	5
PX 174		25.3	40.9	35.1	39.7	30.5
ss 120		21.4	27.6	28	28.2	23.6
ss 27		28.1	28.2	31.4	32.8	33

Standard errors of differences of means

Table	genotype	treat_level	genotype treat level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	3.25	4.2	7.28

Least significant differences of means (5% level)

Table	genotype	treat level	genotype treat_level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	6.98	9.01	15.61

Stratum standard errors and coefficients of variation

Variate: biomass t ha

Stratum	d.f.	s.e.	cv%
rep	1	0.79	2.6
rep.*Units*	14	7.28	24

```
 48  IF   ibalance.eq.0 .OR.   ibalance.eq.1
```

```
 49   DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 50   AKEEP [SAVE=  a2save['save']] genotype; MEAN=  mean; REP=  rep; VARIANCE=  var; RTERM=  resid
 51   AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 52   AMCOMPARISON [PRINT=letter; METHOD=duncan; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 53  SAVE=_a2save['save']] genotype
```

Duncan's multiple range test

genotype

	Mean	
PX 174	34.32	a
ss 27	30.71	ab
ss 120	25.76	b

```
 54  ENDIF
 55  SET [IN=*]
 61  "Two-way design in randomized blocks"
 62  DELETE [REDEFINE=yes] _ibalance
 63  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,treat_level; BLOCKS=rep;\
 64   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=  ibalance]\
 65   brix_%; SAVE=_a2save
```

Analysis of variance

Variate: brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	0.02	0.02	0.01	
rep.*Units* stratum					
genotype	2	37.758	18.879	6.65	0.009
treat level	4	53.535	13.384	4.71	0.013
genotype.treat level	8	14.251	1.781	0.63	0.743
Residual	14	39.751	2.839		
Total	29	145.315			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 1 *units* 3	-2.57	s.e.	1.15
rep 2 *units* 3	2.57	s.e.	1.15

Tables of means

Variate: brix_%

Grand mean 23.18

genotype	PX 174	ss 120	ss 27			
	22.03	22.8	24.7			
treat level	1	2	3	4	5	
	20.7	23	23.55	24.22	24.42	
genotype	treat_level	1	2	3	4	5
PX 174		19.1	22.07	22.55	23.62	22.8
ss 120		20.62	22.81	24.35	22.7	23.52
ss 27		22.37	24.12	23.75	26.32	26.92

Standard errors of differences of means

Table	genotype	treat_level	genotype treat level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	0.754	0.973	1.685

Least significant differences of means (5% level)

Table	genotype	treat level	genotype treat_level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	1.616	2.087	3.614

Stratum standard errors and coefficients of variation

Variate: brix_%

Stratum	d.f.	s.e.	cv%
rep	1	0.037	0.2
rep.*Units*	14	1.685	7.3

```
 66  IF   ibalance.eq.0 .OR.   ibalance.eq.1
 67    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 68    AKEEP [SAVE=_a2save['save']] genotype; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
 69    AKEEP [SAVE=  a2save['save']] #  resid; DF=  rdf
 70    AMCOMPARISON [PRINT=letter; METHOD=duncan; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 71   SAVE=  a2save['save']] genotype
```

Duncan's multiple range test

genotype

	Mean	
ss 27	24.7	a
ss 120	22.8	b
PX 174	22.03	b

```
 72  ENDIF
 73  SET [IN=*]
 79  "Two-way design in randomized blocks"
 80  DELETE [REDEFINE=yes] _ibalance
 81  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,treat_level; BLOCKS=rep;\
 82   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=  ibalance]\
 83   juice_t_ha; SAVE=_a2save
```

Analysis of variance

Variate: juice_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	0.069	0.069	0.04	
rep.*Units* stratum					
genotype	2	12.262	6.131	3.98	0.043
treat level	4	15.031	3.758	2.44	0.096
genotype.treat_level	8	5.559	0.695	0.45	0.871
Residual	14	21.584	1.542		
Total	29	54.505			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 1 *units* 14	-2.5	s.e.	0.85
rep 2 *units* 14	2.5	s.e.	0.85

Tables of means

Variate: juice_t_ha

Grand mean 3.47

genotype	PX 174	ss 120	ss 27		
	4.27	2.71	3.44		
treat_level	1	2	3	4	5
	2.54	4.08	3.66	4.32	2.75

genotype	treat level	1	2	3	4	5
PX 174		2.64	5.57	4.37	5.66	3.12
ss 120		1.92	3.31	3.17	3.22	1.92
ss 27		3.07	3.36	3.46	4.08	3.22

Standard errors of differences of means

Table	genotype	treat level	genotype treat_level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	0.555	0.717	1.242

Least significant differences of means (5% level)

Table	genotype	treat level	genotype treat_level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	1.191	1.538	2.663

Stratum standard errors and coefficients of variation

Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
rep	1	0.068	2
rep.*Units*	14	1.242	35.8

```
 84  IF   ibalance.eq.0 .OR.   ibalance.eq.1
 85    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 86    AKEEP [SAVE=  a2save['save']] genotype; MEAN=  mean; REP=  rep; VARIANCE=  var; RTERM=  resid
 87    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 88    AMCOMPARISON [PRINT=letter; METHOD=duncan; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 89   SAVE=_a2save['save']] genotype
```

Duncan's multiple range test

genotype

	Mean	
PX 174	4.272	a
ss 27	3.437	ab
ss 120	2.707	b

```
 90  ENDIF
```

```
 91  SET [IN=*]
```

Wilgeboom 2011-2012

```
Genstat 64-bit Release 18.1 ( PC/Windows 8) 10 October 2017 08:14:59
Copyright 2015, VSN International Ltd.
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                    Genstat Eighteenth Edition
                    Genstat Procedure Library Release PL26.1


  1  SET [WORKINGDIRECTORY='C:/Users/maalis/Documents']
  2  "Data taken from file: 'H:/Vikus/2012 WB nitro data analaysis.xls'"
  3  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
  4  READ [PRINT=*; SETNVALUES=yes] _stitle_
  8  PRINT [IPRINT=*]   stitle  ; JUST=left

Data imported from Excel file: H:\Vikus\2012 WB nitro data analaysis.xls
 on: 10-Oct-2017 8:15:22
 taken from sheet "stats data", cells A2:H31

  9  DELETE [REDEFINE=yes] Rep,entry,genotype,N  kg  ha,treat  level,mass  t  ha,\
 10  ave_brix_%,juice_t_ha
 11  UNITS [NVALUES=*]
 12  FACTOR [MODIFY=no; NVALUES=30; LEVELS=2; LABELS=*; REFERENCE=1]
 13  READ Rep; FREPRESENTATION=ordinal

                     Identifier        Values       Missing       Levels
                     Rep                     30             0            2

 15  VARIATE [NVALUES=30] entry
 16  READ entry

                     Identifier        Minimum      Mean                    Values       Missing
                     entry                   31         45.5           60           30             0

 19  FACTOR [MODIFY=no; NVALUES=30; LEVELS=3; LABELS=!t('BMR','ss 120','ss
 20  ; REFERENCE=1] genotype
 21  READ genotype; FREPRESENTATION=ordinal

                     Identifier        Values       Missing       Levels
```

genotype	30	0	3

```
 23  VARIATE [NVALUES=30] N_kg_ha
 24  READ N  kg  ha
```

Identifier	Minimum	Mean	Values	Missing
N_kg_ha	0	60	120	30	0

```
 27  FACTOR [MODIFY=no; NVALUES=30; LEVELS=5; LABELS=*; REFERENCE=1] treat_level
 28  READ treat  level; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
treat_level	30	0	5

```
 30  VARIATE [NVALUES=30] mass_t_ha
 31  READ mass_t_ha
```

Identifier	Minimum	Mean	Values	Missing
mass_t_ha	20.54	45.83	76.36	30	0

```
 35  VARIATE [NVALUES=30] ave_brix_%
 36  READ ave_brix_%
```

Identifier	Minimum	Mean	Values	Missing
ave_brix_%	8.033	12.97	17.53	30	0

```
 43  VARIATE [NVALUES=30] juice_t_ha
 44  READ juice_t_ha
```

Identifier	Minimum	Mean	Values	Missing
juice_t_ha	1.286	5.63	10.38	30	0

```
                48
 49  %PostMessage 1129; 0; 100001 "Sheet Update Completed"
 50  "Two-way design in randomized blocks"
 51  DELETE [REDEFINE=yes]   ibalance
 52  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,treat_level; BLOCKS=Rep;\
 53   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present;
 54   mass_t_ha; SAVE=_a2save
Analysis of variance

Variate: mass  t  ha
```

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	262.7	262.7	1.59	
Rep.*Units* stratum					
genotype	2	107.5	53.7	0.32	0.728
treat level	4	2022.1	505.5	3.06	0.053
genotype.treat level	8	1480	185	1.12	0.408
Residual	14	2315.3	165.4		
Total	29	6187.5			

Information summary

All terms orthogonal, none aliased.

Tables of means

Variate: mass t ha

Grand mean 45.8

genotype	BMR	ss 120	ss 27
	43.8	48.4	45.3

treat level	1	2	3	4	5
	42.7	51.5	58.4	34.9	41.6

genotype	treat level	1	2	3	4	5
BMR		38.8	44.7	67.4	39.7	28.6
ss 120		53.8	58.4	56.1	25.8	47.7
ss 27		35.6	51.5	51.6	39.1	48.6

Standard errors of differences of means

Table	genotype	treat_level	genotype treat level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	5.75	7.42	12.86

Least significant differences of means (5% level)

Table	genotype	treat level	genotype treat_level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	12.34	15.92	27.58

Stratum standard errors and coefficients of variation

Variate: mass t ha

Stratum	d.f.	s.e.	cv%
Rep	1	4.18	9.1
Rep.*Units*	14	12.86	28.1

55 IF ibalance.eq.0 .OR. ibalance.eq.1

```
 56   DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 57   AKEEP [SAVE=  a2save['save']] genotype; MEAN=  mean; REP=  rep; VARIANCE=  var; RTERM=  resid
 58   AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 59   AMCOMPARISON [PRINT=comparison,letter; METHOD=tukey; DIRECTION=descending; PROB=0.05;\
 60  FACTORIAL=9; SAVE=_a2save['save']] genotype
```

Tukey's 95% confidence intervals

genotype

		Lower	Upper	
ss 120 vs	3.083	-11.97	18.14	no
ss 120 vs	4.54	-10.51	19.59	no
ss 27 vs	1.457	-13.6	16.51	no

	Mean	
ss 120	48.37	a
ss 27	45.29	a
BMR	43.83	a

```
 61  ENDIF
 62  SET [IN=*]
 68  "Two-way design in randomized blocks"
 69  DELETE [REDEFINE=yes] _ibalance
 70  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,treat  level; BLOCKS=Rep;\
 71   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present;
 72   juice_t_ha; SAVE=_a2save
```

Analysis of variance

Variate: juice_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	1.224	1.224	0.26	
Rep.*Units* stratum					
genotype	2	45.212	22.606	4.79	0.026
treat level	4	18.802	4.701	1	0.442
genotype.treat_level	8	59.061	7.383	1.56	0.222
Residual	14	66.135	4.724		
Total	29	190.434			

Information summary

All terms orthogonal, none aliased.

Tables of means

Variate: juice_t_ha

Grand mean 5.63

genotype	BMR	ss 120	ss 27
	4.75	4.77	7.37

treat_level	1	2	3	4	5
	5.73	5.64	6.99	4.59	5.19

genotype	treat_level	1	2	3	4	5
BMR		5.06	2.57	9.21	3.47	3.44
ss 120		4.59	7.43	4.23	3.69	3.92
ss 27		7.55	6.91	7.53	6.62	8.22

Standard errors of differences of means

Table	genotype	treat_level	genotype treat_level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	0.972	1.255	2.173

Least significant differences of means (5% level)

Table	genotype	treat_level	genotype treat_level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	2.085	2.691	4.662

Stratum standard errors and coefficients of variation

Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
Rep	1	0.286	5.1
Rep.*Units*	14	2.173	38.6

```
 73  IF _ibalance.eq.0 .OR. _ibalance.eq.1
 74    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 75    AKEEP [SAVE=  a2save['save']] genotype; MEAN=  mean; REP=  rep; VARIANCE=  var; RTERM=  resid
 76    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 77    AMCOMPARISON [PRINT=comparison,letter; METHOD=tukey; DIRECTION=descending; PROB=0.05;\
 78   FACTORIAL=9; SAVE=  a2save['save']] genotype
```

Tukey's 95% confidence intervals

genotype

		Lower	Upper	
ss 27 vs ss	2.594	0.0498	5.138	yes
ss 27 vs	2.614	0.0705	5.158	yes
ss 120 vs	0.021	-2.5232	2.565	no

	Mean	
ss 27	7.366	a
ss 120	4.772	b
BMR	4.752	b

```
 79  ENDIF
 80  SET [IN=*]
 86  "Two-way design in randomized blocks"
 87  DELETE [REDEFINE=yes]   ibalance
 88  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,treat_level; BLOCKS=Rep;\
 89   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present;
 90   ave  brix  %; SAVE=  a2save
```

Analysis of variance

Variate: ave brix %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	1.083	1.083	0.34	
Rep.*Units* stratum					
genotype	2	62.166	31.083	9.76	0.002
treat_level	4	18.638	4.659	1.46	0.266
genotype.treat level	8	39.034	4.879	1.53	0.232
Residual	14	44.595	3.185		
Total	29	165.516			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Rep 1 *units* 1	-2.59	s.e.
Rep 2 *units* 1	2.59	s.e.

Tables of means

Variate: ave brix %

Grand mean 12.97

genotype	BMR	ss 120	ss 27
	11.79	12.12	15

treat_level	1	2	3	4	5
	12.22	12.41	14.36	12.55	13.32

genotype	treat level	1	2	3	4	5
BMR		10.82	12.68	13.2	11.33	10.92
ss 120		11.52	11.93	14.95	10.03	12.18
ss 27		14.32	12.6	14.92	16.28	16.87

Standard errors of differences of means

Table	genotype	treat_level	genotype treat_level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	0.798	1.03	1.785

Least significant differences of means (5% level)

Table	genotype	treat_level	genotype treat level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	1.712	2.21	3.828

Stratum standard errors and coefficients of variation

Variate: ave brix %

Stratum	d.f.	s.e.	cv%
Rep	1	0.269	2.1
Rep.*Units*	14	1.785	13.8

```
 91 IF   ibalance.eq.0 .OR.   ibalance.eq.1
 92   DELETE [REDEFINE=yes]   mean,   rep,   var,   rdf
 93   AKEEP [SAVE=_a2save['save']] genotype; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
 94   AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 95   AMCOMPARISON [PRINT=comparison,letter; METHOD=tukey; DIRECTION=descending; PROB=0.05;\
 96  FACTORIAL=9; SAVE=_a2save['save']] genotype
```

Tukey's 95% confidence intervals

genotype

		Lower	Upper	
ss 27 vs ss	2.873	0.784	4.962	yes
ss 27 vs	3.207	1.118	5.296	yes
ss 120 vs	0.333	-1.756	2.422	no

	Mean	
ss 27	15	a
ss 120	12.12	b
BMR	11.79	b

```
 97  ENDIF
 98  SET [IN=*]
104  "Two-way design in randomized blocks"
105  DELETE [REDEFINE=yes] _ibalance
106  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,treat_level; BLOCKS=Rep;\
107   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present;
108   ave_brix_%; SAVE=_a2save
```

Analysis of variance

Variate: ave_brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	1.083	1.083	0.34	
Rep.*Units* stratum					
genotype	2	62.166	31.083	9.76	0.002
treat level	4	18.638	4.659	1.46	0.266
genotype.treat_level	8	39.034	4.879	1.53	0.232
Residual	14	44.595	3.185		
Total	29	165.516			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Rep 1 *units* 1	-2.59	s.e.
Rep 2 *units* 1	2.59	s.e.

Tables of means

Variate: ave_brix_%

Grand mean 12.97

genotype	BMR	ss 120	ss 27			
	11.79	12.12	15			
treat_level	1	2	3	4	5	
	12.22	12.41	14.36	12.55	13.32	
genotype	treat level	1	2	3	4	5
BMR		10.82	12.68	13.2	11.33	10.92
ss 120		11.52	11.93	14.95	10.03	12.18
ss 27		14.32	12.6	14.92	16.28	16.87

Standard errors of differences of means

Table	genotype	treat_level	genotype treat level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	0.798	1.03	1.785

Least significant differences of means (5% level)

Table	genotype	treat level	genotype treat_level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	1.712	2.21	3.828

Stratum standard errors and coefficients of variation

Variate: ave brix %

Stratum	d.f.	s.e.	cv%
Rep	1	0.269	2.1
Rep.*Units*	14	1.785	13.8

```
 109  IF   ibalance.eq.0 .OR.   ibalance.eq.1
 110    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 111    AKEEP [SAVE=  a2save['save']] treat  level; MEAN=  mean; REP=  rep; VARIANCE=  var; RTERM=  resid
 112    AKEEP [SAVE=  a2save['save']] #  resid; DF=  rdf
 113    AMCOMPARISON [PRINT=comparison,letter; METHOD=tukey; DIRECTION=descending; PROB=0.05;\
 114   FACTORIAL=9; SAVE=_a2save['save']] treat_level
```

Tukey's 95% confidence intervals

treat_level

Comparison		Lower	Upper	
3 vs 5	1.033	-2.177	4.244	no
3 vs 4	1.806	-1.405	5.016	no
3 vs 2	1.95	-1.261	5.161	no
3 vs 1	2.139	-1.072	5.35	no
5 vs 4	0.772	-2.439	3.983	no
5 vs 2	0.917	-2.294	4.127	no
5 vs 1	1.106	-2.105	4.316	no
4 vs 2	0.144	-3.066	3.355	no
4 vs 1	0.333	-2.877	3.544	no
2 vs 1	0.189	-3.022	3.4	no

	Mean	
3	14.36	a
5	13.32	a
4	12.55	a
2	12.41	a
1	12.22	a

```
 115  ENDIF
 116  SET [IN=*]
 122  "Two-way design in randomized blocks"
 123  DELETE [REDEFINE=yes] _ibalance
 124  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,treat  level; BLOCKS=Rep;\
 125   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present;
 126   juice_t_ha; SAVE=_a2save
```

Analysis of variance

Variate: juice_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	1.224	1.224	0.26	
Rep.*Units* stratum					
genotype	2	45.212	22.606	4.79	0.026
treat_level	4	18.802	4.701	1	0.442
genotype.treat_level	8	59.061	7.383	1.56	0.222
Residual	14	66.135	4.724		
Total	29	190.434			

Information summary

All terms orthogonal, none aliased.

Tables of means

Variate: juice_t_ha

Grand mean 5.63

genotype	BMR	ss 120	ss 27			
	4.75	4.77	7.37			
treat_level	1	2	3	4	5	
	5.73	5.64	6.99	4.59	5.19	
genotype	treat_level	1	2	3	4	5
BMR		5.06	2.57	9.21	3.47	3.44
ss 120		4.59	7.43	4.23	3.69	3.92
ss 27		7.55	6.91	7.53	6.62	8.22

Standard errors of differences of means

Table	genotype	treat level	genotype treat_level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	0.972	1.255	2.173

Least significant differences of means (5% level)

Table	genotype	treat_level	genotype treat_level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	2.085	2.691	4.662

Stratum standard errors and coefficients of variation

Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
Rep	1	0.286	5.1
Rep.*Units*	14	2.173	38.6

```
 127  IF _ibalance.eq.0 .OR. _ibalance.eq.1
 128    DELETE [REDEFINE=yes]  mean,  rep,  var,  rdf
 129    AKEEP [SAVE= a2save['save']] genotype; MEAN= mean; REP= rep; VARIANCE= var; RTERM= resid
 130    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 131    AMCOMPARISON [PRINT=comparison,letter; METHOD=tukey; DIRECTION=descending; PROB=0.05;\
 132   FACTORIAL=9; SAVE= a2save['save']] genotype
```

Tukey's 95% confidence intervals

genotype

		Lower	Upper	
ss 27 vs ss	2.594	0.0498	5.138	yes
ss 27 vs	2.614	0.0705	5.158	yes
ss 120 vs	0.021	-2.5232	2.565	no

	Mean	
ss 27	7.366	a
ss 120	4.772	b
BMR	4.752	b

```
 133  ENDIF
 134  SET [IN=*]
 140  "Two-way design in randomized blocks"
 141  DELETE [REDEFINE=yes]   ibalance
 142  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,treat_level; BLOCKS=Rep;\
 143   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present;
 144   juice  t  ha; SAVE=  a2save
```

Analysis of variance

Variate: juice t ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	1.224	1.224	0.26	
Rep.*Units* stratum					
genotype	2	45.212	22.606	4.79	0.026
treat_level	4	18.802	4.701	1	0.442
genotype.treat level	8	59.061	7.383	1.56	0.222
Residual	14	66.135	4.724		
Total	29	190.434			

Information summary

All terms orthogonal, none aliased.

Tables of means

Variate: juice t ha

Grand mean 5.63

genotype	BMR	ss 120	ss 27		
	4.75	4.77	7.37		
treat_level	1	2	3	4	5
	5.73	5.64	6.99	4.59	5.19

genotype	treat_level	1	2	3	4	5
BMR		5.06	2.57	9.21	3.47	3.44
ss 120		4.59	7.43	4.23	3.69	3.92
ss 27		7.55	6.91	7.53	6.62	8.22

Standard errors of differences of means

Table	genotype	treat_level	genotype treat level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	0.972	1.255	2.173

Least significant differences of means (5% level)

Table	genotype	treat_level	genotype treat level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	2.085	2.691	4.662

Stratum standard errors and coefficients of variation

Variate: juice t ha

Stratum	d.f.	s.e.	cv%
Rep	1	0.286	5.1
Rep.*Units*	14	2.173	38.6

```
145 IF  ibalance.eq.0 .OR.  ibalance.eq.1
146   DELETE [REDEFINE=yes]  mean,  rep,  var,  rdf
147   AKEEP [SAVE=_a2save['save']] treat_level; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
148   AKEEP [SAVE=_a2save['save']] #  resid; DF=  rdf
149   AMCOMPARISON [PRINT=comparison,letter; METHOD=tukey; DIRECTION=descending; PROB=0.05;\
150  FACTORIAL=9; SAVE=_a2save['save']] treat_level
```

Tukey's 95% confidence intervals

treat level

Comparison		Lower	Upper	
3 vs 1	1.259	-2.651	5.169	no
3 vs 2	1.356	-2.554	5.266	no
3 vs 5	1.798	-2.112	5.708	no
3 vs 4	2.4	-1.51	6.31	no
1 vs 2	0.097	-3.813	4.007	no

1 vs 5	0.539	-3.371	4.45	no
1 vs 4	1.141	-2.769	5.051	no
2 vs 5	0.443	-3.467	4.353	no
2 vs 4	1.044	-2.866	4.954	no
5 vs 4	0.602	-3.308	4.512	no

	Mean	
3	6.993	a
1	5.734	a
2	5.637	a
5	5.194	a
4	4.593	a

```
 151  ENDIF
 152  SET [IN=*]
 158  "Two-way design in randomized blocks"
 159  DELETE [REDEFINE=yes] _ibalance
 160  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,treat_level; BLOCKS=Rep;\
 161   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present;
 162   ave_brix_%; SAVE=_a2save
```

Analysis of variance

Variate: ave_brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	1.083	1.083	0.34	
Rep.*Units* stratum					
genotype	2	62.166	31.083	9.76	0.002
treat_level	4	18.638	4.659	1.46	0.266
genotype.treat_level	8	39.034	4.879	1.53	0.232
Residual	14	44.595	3.185		
Total	29	165.516			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Rep 1 *units* 1	-2.59	s.e.
Rep 2 *units* 1	2.59	s.e.

Tables of means

Variate: ave_brix_%

Grand mean 12.97

genotype	BMR	ss 120	ss 27
	11.79	12.12	15

treat_level	1	2	3	4	5
	12.22	12.41	14.36	12.55	13.32

genotype	treat level	1	2	3	4	5
BMR		10.82	12.68	13.2	11.33	10.92
ss 120		11.52	11.93	14.95	10.03	12.18
ss 27		14.32	12.6	14.92	16.28	16.87

Standard errors of differences of means

Table	genotype	treat_level	genotype treat level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	0.798	1.03	1.785

Least significant differences of means (5% level)

Table	genotype	treat level	genotype treat_level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	1.712	2.21	3.828

Stratum standard errors and coefficients of variation

Variate: ave brix %

Stratum	d.f.	s.e.	cv%
Rep	1	0.269	2.1
Rep.*Units*	14	1.785	13.8

```
 163  IF   ibalance.eq.0 .OR.   ibalance.eq.1
 164    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 165    AKEEP [SAVE=  a2save['save']] treat  level; MEAN=  mean; REP=  rep; VARIANCE=  var; RTERM=  resid
 166    AKEEP [SAVE=  a2save['save']] #  resid; DF=  rdf
 167    AMCOMPARISON [PRINT=comparison,letter; METHOD=tukey; DIRECTION=descending; PROB=0.05;\
 168   FACTORIAL=9; SAVE=_a2save['save']] treat_level
```

Tukey's 95% confidence intervals

treat_level

Comparison		Lower	Upper	
3 vs 5	1.033	-2.177	4.244	no
3 vs 4	1.806	-1.405	5.016	no
3 vs 2	1.95	-1.261	5.161	no
3 vs 1	2.139	-1.072	5.35	no
5 vs 4	0.772	-2.439	3.983	no
5 vs 2	0.917	-2.294	4.127	no
5 vs 1	1.106	-2.105	4.316	no
4 vs 2	0.144	-3.066	3.355	no
4 vs 1	0.333	-2.877	3.544	no
2 vs 1	0.189	-3.022	3.4	no

	Mean	
3	14.36	a
5	13.32	a
4	12.55	a
2	12.41	a
1	12.22	a

```
169  ENDIF
170  SET [IN=*]
```

2012-2013

Vaalharts

```
141  "Data taken from file: 'H:/Vikus/Copy of 2013 VH nitro coll.xls'"
 142  DELETE [REDEFINE=yes]   stitle  : TEXT   stitle
 143  READ [PRINT=*; SETNVALUES=yes] _stitle_
 147  PRINT [IPRINT=*] _stitle_; JUST=left

Data imported from Excel file: H:\Vikus\Copy of 2013 VH nitro coll.xls
 on: 10-Oct-2017 8:46:43
 taken from sheet "stats data", cells A2:I31

 148  DELETE [REDEFINE=yes] Block,Entry,rep,genotype,N_appl_kg_ha,n_level,\
 149  mass  t  ha,brix  %,juice  t  ha
Warning 1, code VA 19, statement 1 on line 149

Command: DELETE [REDEFINE=yes] Block,Entry,rep,genotype,N_appl_kg_ha,n_level,mas
Inconsistent structure(s).


***** Block  Entry  rep  genotype  N_appl_kg_ha  n_level  mass_t_ha  brix_%  juice_t_ha
```

***** Having been redefined, the following structure(s) were found to be inconsistent:
***** mean
*****
and they have been destroyed.

```
150  UNITS [NVALUES=*]
151  VARIATE [NVALUES=30] Block
152  READ Block
```

Identifier		Mean			Missing
Block	1	15.5	30	30	0

```
155  VARIATE [NVALUES=30] Entry
156  READ Entry
```

Identifier		Mean			Missing
Entry	1	8	15	30	0

```
158  FACTOR [MODIFY=no; NVALUES=30; LEVELS=2; LABELS=*; REFERENCE=1] rep
159  READ rep; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
rep	30	0	2

```
161  FACTOR [MODIFY=no; NVALUES=30; LEVELS=3; LABELS=!t('p 868','ss 120','ss 63')\
162  ; REFERENCE=1] genotype
163  READ genotype; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
genotype	30	0	3

```
165  VARIATE [NVALUES=30] N_appl_kg_ha
166  READ N_appl_kg_ha
```

Identifier		Mean			Missing
N_appl_kg_ha	0	60	120	30	0

```
169  FACTOR [MODIFY=no; NVALUES=30; LEVELS=5; LABELS=*; REFERENCE=1] n_level
170  READ n_level; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
n_level	30	0	5

```
172  VARIATE [NVALUES=30] mass_t_ha
173  READ mass_t_ha
```

Identifier		Mean			Missing
mass_t_ha	17.75	57.12	113.2	30	0

```
182  VARIATE [NVALUES=30] brix_%
183  READ brix_%
```

Identifier		Mean			Missing
brix_%	4.967	12.17	20.87	30	0

```
190  VARIATE [NVALUES=30] juice  t  ha
191  READ juice_t_ha

                 Identifier                 Mean                          Missing
                juice_t_ha        8.684     16.77     31.43       30            0

              199
200  %PostMessage 1129; 0; 100002 "Sheet Update Completed"
201  "Two-way design in randomized blocks"
202  DELETE [REDEFINE=yes] _ibalance
203  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,n_level; BLOCKS=rep;\
204   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=_ibalance]\
205   mass  t  ha; SAVE=  a2save
Analysis of variance

Variate: mass  t  ha

Source of variation   d.f.        s.s.        m.s.        v.r.        F pr.

rep stratum                1       343.3       343.3        1.19

rep.*Units* stratum
genotype                   2      5580.6      2790.3        9.68       0.002
n_level                    4      2372.5       593.1        2.06       0.141
genotype.n  level          8       821.7       102.7        0.36       0.927
Residual                  14      4033.5       288.1

Total                     29     13151.7


Information summary

All terms orthogonal, none aliased.


Message: the following units have large residuals.

rep 1 *units* 5          25.6     s.e.
rep 2 *units* 4         -25.6     s.e.


Tables of means

Variate: mass  t  ha

Grand mean  57.1

                genotype        p 868       ss 120       ss 63
                                 54.9         74.8         41.7

                n_level             1            2            3            4            5
                                 46.8         65.5         47.2         58.1           68
```

genotype	n_level	1	2	3	4	5
p 868		43.3	72.4	43.7	56.8	58.1
ss 120		58.7	76.9	69.5	73.8	95.3
ss 63		38.3	47.2	28.4	43.6	50.7

Standard errors of differences of means

Table	genotype	n_level	genotype n level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	7.59	9.8	16.97

Least significant differences of means (5% level)

Table	genotype	n_level	genotype n level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	16.28	21.02	36.4

Stratum standard errors and coefficients of variation

Variate: mass t ha

Stratum	d.f.	s.e.	cv%
rep	1	4.78	8.4
rep.*Units*	14	16.97	29.7

```
206 IF   ibalance.eq.0 .OR.   ibalance.eq.1
207    DELETE [REDEFINE=yes]   mean,   rep,   var,   rdf
208    AKEEP [SAVE=_a2save['save']] genotype; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
209    AKEEP [SAVE=_a2save['save']] #  resid; DF=  rdf
210    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=_mean; REPLICATION=_rep; VARIANCE=_var;\
211   DF=_rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

	Mean	Lower	Upper
genotype			
p 868	54.87	40.42	69.32
ss 120	74.83	60.39	89.28
ss 63	41.65	27.21	56.1

```
 212   AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 213  SAVE=  a2save['save']] genotype
```

Tukey's 95% confidence intervals

genotype

	Mean	
ss 120	74.83	a
p 868	54.87	b
ss 63	41.65	b

```
 214  ENDIF
 215  SET [IN=*]
 221  "Two-way design in randomized blocks"
 222  DELETE [REDEFINE=yes] _ibalance
 223  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,n_level; BLOCKS=rep;\
 224   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=  ibalance]\
 225   juice_t_ha; SAVE=_a2save
```

Analysis of variance

Variate: juice_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	8.93	8.93	0.63	
rep.*Units* stratum					
genotype	2	592.68	296.34	20.83	<.001
n level	4	95.39	23.85	1.68	0.211
genotype.n_level	8	62.89	7.86	0.55	0.799
Residual	14	199.19	14.23		
Total	29	959.08			

Information summary

All terms orthogonal, none aliased.

Tables of means

Variate: juice_t_ha

Grand mean 16.77

genotype	p 868	ss 120	ss 63		
	14.04	23.04	13.23		
n_level	1	2	3	4	5
	14.61	18.82	15.24	16.29	18.89

genotype	n level	1	2	3	4	5
p 868		12.91	17.64	11.37	13.53	14.76
ss 120		18.87	24.25	20.52	23.4	28.17
ss 63		12.07	14.56	13.83	11.95	13.76

Standard errors of differences of means

Table	genotype	n level	genotype n_level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	1.687	2.178	3.772

Least significant differences of means (5% level)

Table	genotype	n level	genotype n_level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	3.618	4.671	8.09

Stratum standard errors and coefficients of variation

Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
rep	1	0.772	4.6
rep.*Units*	14	3.772	22.5

```
 226  IF   ibalance.eq.0 .OR.   ibalance.eq.1
 227    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 228    AKEEP [SAVE=  a2save['save']] genotype; MEAN=  mean; REP=  rep; VARIANCE=  var; RTERM=  resid
 229    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 230    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=_mean; REPLICATION=_rep; VARIANCE=_var;\
 231   DF=_rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

	Mean	Lower	Upper
genotype			
p 868	14.04	10.83	17.25
ss 120	23.04	19.83	26.25
ss 63	13.23	10.02	16.44

```
 232    AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 233   SAVE=_a2save['save']] genotype
```

Tukey's 95% confidence intervals

genotype

	Mean	
ss 120	23.04	a
p 868	14.04	b
ss 63	13.23	b

```
 234  ENDIF
 235  SET [IN=*]
 241  "Two-way design in randomized blocks"
 242  DELETE [REDEFINE=yes] _ibalance
 243  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,n  level; BLOCKS=rep;\
 244   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=_ibalance]\
 245   brix_%; SAVE=_a2save
```

Analysis of variance

Variate: brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	0.972	0.972	0.1	
rep.*Units* stratum					
genotype	2	193.931	96.965	10.06	0.002
n_level	4	91.702	22.926	2.38	0.102
genotype.n_level	8	82.93	10.366	1.08	0.432
Residual	14	134.926	9.638		
Total	29	504.461			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 1 *units* 9	5.07	s.e.
rep 2 *units* 8	-5.07	s.e.

Tables of means

Variate: brix_%

Grand mean 12.17

genotype	p 868	ss 120	ss 63
	14.19	13.75	8.59

n_level	1	2	3	4	5
	12.45	10.17	13.47	10.22	14.56

genotype	n_level	1	2	3	4	5
p 868		13.35	12.97	12.88	12.98	18.75
ss 120		14.13	10.05	14.9	12.22	17.43
ss 63		9.87	7.5	12.62	5.45	7.5

Standard errors of differences of means

Table	genotype	n_level	genotype n_level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	1.388	1.792	3.104

Least significant differences of means (5% level)

Table	genotype	n_level	genotype n_level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	2.978	3.844	6.658

Stratum standard errors and coefficients of variation

Variate: brix_%

Stratum	d.f.	s.e.	cv%
rep	1	0.255	2.1
rep.*Units*	14	3.104	25.5

```
246  IF _ibalance.eq.0 .OR. _ibalance.eq.1
247    DELETE [REDEFINE=yes] mean, rep, var, rdf
248    AKEEP [SAVE= a2save['save']] genotype; MEAN= mean; REP= rep; VARIANCE= var; RTERM= resid
249    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
250    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=_mean; REPLICATION=_rep; VARIANCE=_var;\
251   DF= rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

	Mean	Lower	Upper
genotype			
p 868	14.19	11.544	16.83
ss 120	13.75	11.104	16.39
ss 63	8.59	5.944	11.23

```
 252   AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 253  SAVE=  a2save['save']] genotype
```

Tukey's 95% confidence intervals

genotype

	Mean	
p 868	14.19	a
ss 120	13.75	a
ss 63	8.59	b

```
 254  ENDIF
 255  SET [IN=*]
 261  "Two-way design in randomized blocks"
 262  DELETE [REDEFINE=yes]   ibalance
 263  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,n_level; BLOCKS=rep;\
 264   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=_ibalance]\
 265   brix  %; SAVE=  a2save
```

Analysis of variance

Variate: brix %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	0.972	0.972	0.1	
rep.*Units* stratum					
genotype	2	193.931	96.965	10.06	0.002
n_level	4	91.702	22.926	2.38	0.102
genotype.n_level	8	82.93	10.366	1.08	0.432
Residual	14	134.926	9.638		
Total	29	504.461			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 1 *units* 9 5.07 s.e.

rep 2 *units* 8 -5.07 s.e.

Tables of means

Variate: brix_%

Grand mean 12.17

genotype	p 868	ss 120	ss 63		
	14.19	13.75	8.59		

n_level	1	2	3	4	5
	12.45	10.17	13.47	10.22	14.56

genotype	n_level	1	2	3	4	5
p 868		13.35	12.97	12.88	12.98	18.75
ss 120		14.13	10.05	14.9	12.22	17.43
ss 63		9.87	7.5	12.62	5.45	7.5

Standard errors of differences of means

Table	genotype	n_level	genotype n_level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	1.388	1.792	3.104

Least significant differences of means (5% level)

Table	genotype	n_level	genotype n level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	2.978	3.844	6.658

Stratum standard errors and coefficients of variation

Variate: brix %

Stratum	d.f.	s.e.	cv%
rep	1	0.255	2.1
rep.*Units*	14	3.104	25.5

```
266 IF   ibalance.eq.0 .OR.   ibalance.eq.1
267    DELETE [REDEFINE=yes]   mean,   rep,   var,   rdf
268    AKEEP [SAVE=_a2save['save']] n_level; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
269    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
270    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=  mean; REPLICATION=  rep; VARIANCE=  var;\
```

271 DF=_rdf

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

n level	Mean	Lower	Upper
1	12.45	8.317	16.58
2	10.17	6.04	14.3
3	13.47	9.334	17.6
4	10.22	6.084	14.35
5	14.56	10.429	18.69

272 AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
273 SAVE=_a2save['save']] n_level

Tukey's 95% confidence intervals

n_level

	Mean	
5	14.56	a
3	13.47	a
1	12.45	a
4	10.22	a
2	10.17	a

274 ENDIF
275 SET [IN=*]
281 "Two-way design in randomized blocks"
282 DELETE [REDEFINE=yes] _ibalance
283 A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,n level; BLOCKS=rep;\
284 FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=_ibalance]\
285 mass_t_ha; SAVE=_a2save

Analysis of variance

Variate: mass_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	343.3	343.3	1.19	
rep.*Units* stratum					
genotype	2	5580.6	2790.3	9.68	0.002
n level	4	2372.5	593.1	2.06	0.141
genotype.n_level	8	821.7	102.7	0.36	0.927
Residual	14	4033.5	288.1		

Total	29	13151.7

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 1 *units* 5	25.6	s.e.
rep 2 *units* 4	-25.6	s.e.

Tables of means

Variate: mass t ha

Grand mean 57.1

genotype	p 868	ss 120	ss 63		
	54.9	74.8	41.7		

n_level	1	2	3	4	5
	46.8	65.5	47.2	58.1	68

genotype	n_level	1	2	3	4	5
p 868		43.3	72.4	43.7	56.8	58.1
ss 120		58.7	76.9	69.5	73.8	95.3
ss 63		38.3	47.2	28.4	43.6	50.7

Standard errors of differences of means

Table	genotype	n level	genotype n level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	7.59	9.8	16.97

Least significant differences of means (5% level)

Table	genotype	n level	genotype n_level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	16.28	21.02	36.4

Stratum standard errors and coefficients of variation

Variate: mass_t_ha

Stratum	d.f.	s.e.	cv%
rep	1	4.78	8.4
rep.*Units*	14	16.97	29.7

```
 286  IF _ibalance.eq.0 .OR. _ibalance.eq.1
 287    DELETE [REDEFINE=yes]   mean,   rep,   var,   rdf
 288    AKEEP [SAVE=_a2save['save']] n_level; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
 289    AKEEP [SAVE=  a2save['save']] #  resid; DF=  rdf
 290    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=_mean; REPLICATION=_rep; VARIANCE=_var;\
 291   DF=_rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

n_level	Mean	Lower	Upper
1	46.78	24.19	69.38
2	65.5	42.91	88.1
3	47.2	24.61	69.79
4	58.09	35.49	80.68
5	68.03	45.43	90.62

```
 292    AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 293   SAVE=_a2save['save']] n_level
```

Tukey's 95% confidence intervals

n level

	Mean	
5	68.03	a
2	65.5	a
4	58.09	a
3	47.2	a
1	46.78	a

```
 294  ENDIF
 295  SET [IN=*]
 301  "Two-way design in randomized blocks"
 302  DELETE [REDEFINE=yes]   ibalance
 303  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=genotype,n_level; BLOCKS=rep;\
 304   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=  ibalance]\
 305   juice  t  ha; SAVE=  a2save
```

Analysis of variance

Variate: juice t ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	8.93	8.93	0.63	
rep.*Units* stratum					
genotype	2	592.68	296.34	20.83	<.001
n level	4	95.39	23.85	1.68	0.211
genotype.n_level	8	62.89	7.86	0.55	0.799
Residual	14	199.19	14.23		
Total	29	959.08			

Information summary

All terms orthogonal, none aliased.

Tables of means

Variate: juice_t_ha

Grand mean 16.77

genotype	p 868	ss 120	ss 63
	14.04	23.04	13.23

n level	1	2	3	4	5
	14.61	18.82	15.24	16.29	18.89

genotype	n level	1	2	3	4	5
p 868		12.91	17.64	11.37	13.53	14.76
ss 120		18.87	24.25	20.52	23.4	28.17
ss 63		12.07	14.56	13.83	11.95	13.76

Standard errors of differences of means

Table	genotype	n_level	genotype n_level
rep.	10	6	2
d.f.	14	14	14
s.e.d.	1.687	2.178	3.772

Least significant differences of means (5% level)

Table	genotype	n level	genotype n level
rep.	10	6	2
d.f.	14	14	14
l.s.d.	3.618	4.671	8.09

Stratum standard errors and coefficients of variation

Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
rep	1	0.772	4.6
rep.*Units*	14	3.772	22.5

```
306  IF _ibalance.eq.0 .OR. _ibalance.eq.1
307    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
308    AKEEP [SAVE=  a2save['save']] n  level; MEAN=  mean; REP=  rep; VARIANCE=  var; RTERM=  resid
309    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
310    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=_mean; REPLICATION=_rep; VARIANCE=_var;\
311   DF=  rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

n level	Mean	Lower	Upper
1	14.61	9.59	19.64
2	18.82	13.79	23.84
3	15.24	10.22	20.26
4	16.29	11.27	21.31
5	18.89	13.87	23.91

```
312    AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
313   SAVE=  a2save['save']] n  level
```

Tukey's 95% confidence intervals

n_level

	Mean	
5	18.89	a
2	18.82	a
4	16.29	a
3	15.24	a
1	14.61	a

```
314  ENDIF
315  SET [IN=*]
```

2013-2014

Wilgeboom

```
437  "Data taken from file: 'H:/Vikus/Copy of 2014 WB nitro.xls'"
438  DELETE [REDEFINE=yes]   stitle  : TEXT   stitle
439  READ [PRINT=*; SETNVALUES=yes] _stitle_
443  PRINT [IPRINT=*] _stitle_; JUST=left

Data imported from Excel file: H:\Vikus\Copy of 2014 WB nitro.xls
 on: 10-Oct-2017 8:53:53
 taken from sheet "stats data", cells A2:G37

444  DELETE [REDEFINE=yes] rep,cult,N_appl_kg_ha,n_level,mass_t_ha,brix_%_ave,\
445  juice_t_ha
446  UNITS [NVALUES=*]
447  FACTOR [MODIFY=no; NVALUES=36; LEVELS=2; LABELS=*; REFERENCE=1] rep
448  READ rep; FREPRESENTATION=ordinal

                    Identifier          Values        Missing      Levels
                    rep                      36              0             2

450  FACTOR [MODIFY=no; NVALUES=36; LEVELS=3; LABELS=!t('p 888','ss 120','ss 27')\
451  ; REFERENCE=1] cult
452  READ cult; FREPRESENTATION=ordinal

                    Identifier          Values        Missing      Levels
                    cult                     36              0             3

454  VARIATE [NVALUES=36] N_appl_kg_ha
455  READ N_appl_kg_ha

                    Identifier          Minimum      Mean                    Values        Missing
                    N_appl_kg_ha               0       83.33          200            36              0

458  FACTOR [MODIFY=no; NVALUES=36; LEVELS=6; LABELS=*; REFERENCE=1] n_level
459  READ n_level; FREPRESENTATION=ordinal

                    Identifier          Values        Missing      Levels
                    n_level                  36              0             6

461  VARIATE [NVALUES=36] mass_t_ha
462  READ mass_t_ha

                    Identifier          Minimum      Mean                    Values        Missing
                    mass_t_ha               1.96       8.296        14.09          36              0

468  VARIATE [NVALUES=36] brix_%_ave
469  READ brix_%_ave

                    Identifier          Minimum      Mean                    Values        Missing
```

brix_%_ave	8.183	13.25	17.5	36	0

```
 475  VARIATE [NVALUES=36] juice_t_ha
 476  READ juice  t  ha
```

Identifier	Minimum	Mean	Values	Missing
juice_t_ha	0.575	1.652	4.006	36	0

```
          481
 482  %PostMessage 1129; 0; 100004 "Sheet Update Completed"
 483  "Two-way design in randomized blocks"
 484  DELETE [REDEFINE=yes] _ibalance
 485  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=cult,n_level; BLOCKS=rep;\
 486   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=  ibalance]\
 487   mass_t_ha; SAVE=_a2save
```

Analysis of variance

Variate: mass_t_ha

Source of	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	0.03572	0.03572	0.54	
rep.*Units* stratum					
cult	2	14.38819	7.19409	108.6	<.001
n level	5	41.73237	8.34647	126	<.001
cult.n_level	10	260.90065	26.09006	393.86	<.001
Residual	17	1.12611	0.06624		
Total	35	318.18304			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 1 *units* 5	0.446	s.e.
rep 1 *units* 16	-0.468	s.e.
rep 2 *units* 5	-0.446	s.e.
rep 2 *units* 16	0.468	s.e.

Tables of means

Variate: mass t ha

Grand mean 8.296

cult	p 888	ss 120	ss 27
	8.729	8.757	7.402

n_level	1	2	3	4	5	6	
	8.149	9.39	6.321	9.599	7.937	8.381	

cult	n_level	1	2	3	4	5	6
p 888		13.181	10.319	6.349	8.033	9.8	4.691
ss 120		1.989	11.45	6.568	13.97	6.951	11.616
ss 27		9.276	6.402	6.045	6.794	7.06	8.837

Standard errors of differences of means

Table	cult	n_level	cult n_level
rep.	12	6	2
d.f.	17	17	17
s.e.d.	0.1051	0.1486	0.2574

Least significant differences of means (5% level)

Table	cult	n_level	cult n_level
rep.	12	6	2
d.f.	17	17	17
l.s.d.	0.2217	0.3135	0.543

Stratum standard errors and coefficients of variation

Variate: mass t ha

Stratum	d.f.	s.e.	cv%
rep	1	0.0445	0.5
rep.*Units*	17	0.2574	3.1

```
 488 IF   ibalance.eq.0 .OR.   ibalance.eq.1
 489    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 490    AKEEP [SAVE=_a2save['save']] cult; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
 491    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 492    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=  mean; REPLICATION=  rep; VARIANCE=  var;\
 493   DF=_rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

	Mean	Lower	Upper
cult			
p 888	8.729	8.533	8.925
ss 120	8.757	8.562	8.953

ss 27	7.402	7.207	7.598

```
 494   AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 495  SAVE=_a2save['save']] cult
```

Tukey's 95% confidence intervals

cult

	Mean	
ss 120	8.757	a
p 888	8.729	a
ss 27	7.402	b

```
 496  ENDIF
 497  SET [IN=*]
 503  "Two-way design in randomized blocks"
 504  DELETE [REDEFINE=yes] _ibalance
 505  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=cult,n_level; BLOCKS=rep;\
 506   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=  ibalance]\
 507   brix_%_ave; SAVE=_a2save
```

Analysis of variance

Variate: brix_%_ave

Source of	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	16.4475	16.4475	42.61	
rep.*Units* stratum					
cult	2	66.9704	33.4852	86.74	<.001
n level	5	64.2436	12.8487	33.28	<.001
cult.n level	10	75.3784	7.5378	19.53	<.001
Residual	17	6.5627	0.386		
Total	35	229.6027			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 1 *units* 8	1.22	s.e.	0.43
rep 2 *units* 8	-1.22	s.e.	0.43

Tables of means

Variate: brix_%_ave

Grand mean 13.25

cult	p 888	ss 120	ss 27
	11.33	14.34	14.09

n level	1	2	3	4	5	6
	10.65	14.5	12.56	13.68	13.62	14.51

cult	n_level	1	2	3	4	5	6
p 888		12.53	11.52	10.22	11.16	11.45	11.08
ss 120		10.18	16.3	13.19	13.5	16.18	16.7
ss 27		9.24	15.67	14.27	16.39	13.21	15.73

Standard errors of differences of means

Table	cult	n_level	cult n level
rep.	12	6	2
d.f.	17	17	17
s.e.d.	0.254	0.359	0.621

Least significant differences of means (5% level)

Table	cult	n level	cult n_level
rep.	12	6	2
d.f.	17	17	17
l.s.d.	0.535	0.757	1.311

Stratum standard errors and coefficients of variation

Variate: brix_%_ave

Stratum	d.f.	s.e.	cv%
rep	1	0.956	7.2
rep.*Units*	17	0.621	4.7

```
 508 IF   ibalance.eq.0 .OR.   ibalance.eq.1
 509    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 510    AKEEP [SAVE=_a2save['save']] cult; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
 511    AKEEP [SAVE=  a2save['save']] #  resid; DF=  rdf
 512    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=_mean; REPLICATION=_rep; VARIANCE=_var;\
 513   DF=  rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

	Mean	Lower	Upper
cult			
p 888	11.33	10.86	11.8
ss 120	14.34	13.87	14.81
ss 27	14.09	13.61	14.56

```
 514   AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 515  SAVE=_a2save['save']] cult
```

Tukey's 95% confidence intervals

cult

	Mean	
ss 120	14.34	a
ss 27	14.09	a
p 888	11.33	b

```
 516  ENDIF
 517  SET [IN=*]
 523  "Two-way design in randomized blocks"
 524  DELETE [REDEFINE=yes] _ibalance
 525  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=cult,n  level; BLOCKS=rep;\
 526   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=_ibalance]\
 527   juice_t_ha; SAVE=_a2save
```

Analysis of variance

Variate: juice_t_ha

Source of	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	0.04511	0.04511	4.17	
rep.*Units* stratum					
cult	2	4.00933	2.00466	185.29	<.001
n level	5	7.3695	1.4739	136.23	<.001
cult.n_level	10	14.04264	1.40426	129.8	<.001
Residual	17	0.18392	0.01082		
Total	35	25.6505			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 1 *units* 8	-0.173	s.e.
rep 1 *units* 10	0.19	s.e.
rep 2 *units* 8	0.173	s.e.
rep 2 *units* 10	-0.19	s.e.

Tables of means

Variate: juice t ha

Grand mean 1.652

cult	p 888	ss 120	ss 27
	1.69	2.04	1.225

n level	1	2	3	4	5	6
	1.193	1.642	1.314	1.869	1.363	2.531

cult	n level	1	2	3	4	5	6
p 888		1.81	1.992	1.742	0.923	1.906	1.767
ss 120		0.579	2.184	1.195	3.295	1.009	3.979
ss 27		1.19	0.749	1.004	1.39	1.173	1.846

Standard errors of differences of means

Table	cult	n_level	cult n level
rep.	12	6	2
d.f.	17	17	17
s.e.d.	0.0425	0.0601	0.104

Least significant differences of means (5% level)

Table	cult	n level	cult n_level
rep.	12	6	2
d.f.	17	17	17
l.s.d.	0.0896	0.1267	0.2194

Stratum standard errors and coefficients of variation

Variate: juice t ha

Stratum	d.f.	s.e.	cv%
rep	1	0.0501	3
rep.*Units*	17	0.104	6.3

528 IF ibalance.eq.0 .OR. ibalance.eq.1

```
 529    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 530    AKEEP [SAVE=  a2save['save']] cult; MEAN=  mean; REP=  rep; VARIANCE=  var; RTERM=  resid
 531    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 532    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=  mean; REPLICATION=  rep; VARIANCE=  var;\
 533   DF=_rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

	Mean	Lower	Upper
cult			
p 888	1.69	1.611	1.769
ss 120	2.04	1.961	2.119
ss 27	1.225	1.146	1.304

```
 534    AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 535   SAVE=_a2save['save']] cult
```

Tukey's 95% confidence intervals

cult

	Mean	
ss 120	2.04	a
p 888	1.69	b
ss 27	1.225	c

```
 536  ENDIF
 537  SET [IN=*]
 543  "Two-way design in randomized blocks"
 544  DELETE [REDEFINE=yes] _ibalance
 545  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=cult,n  level; BLOCKS=rep;\
 546   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=_ibalance]\
 547   juice_t_ha; SAVE=_a2save
```

Analysis of variance

Variate: juice_t_ha

Source of	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	0.04511	0.04511	4.17	
rep.*Units* stratum					
cult	2	4.00933	2.00466	185.29	<.001
n level	5	7.3695	1.4739	136.23	<.001
cult.n_level	10	14.04264	1.40426	129.8	<.001
Residual	17	0.18392	0.01082		

Total	35	25.6505

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 1 *units* 8	-0.173	s.e.
rep 1 *units* 10	0.19	s.e.
rep 2 *units* 8	0.173	s.e.
rep 2 *units* 10	-0.19	s.e.

Tables of means

Variate: juice_t_ha

Grand mean 1.652

cult	p 888	ss 120	ss 27
	1.69	2.04	1.225

n level	1	2	3	4	5	6
	1.193	1.642	1.314	1.869	1.363	2.531

cult	n level	1	2	3	4	5	6
p 888		1.81	1.992	1.742	0.923	1.906	1.767
ss 120		0.579	2.184	1.195	3.295	1.009	3.979
ss 27		1.19	0.749	1.004	1.39	1.173	1.846

Standard errors of differences of means

Table	cult	n_level	cult n level
rep.	12	6	2
d.f.	17	17	17
s.e.d.	0.0425	0.0601	0.104

Least significant differences of means (5% level)

Table	cult	n_level	cult n level
rep.	12	6	2
d.f.	17	17	17
l.s.d.	0.0896	0.1267	0.2194

Stratum standard errors and coefficients of variation

Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
rep	1	0.0501	3
rep.*Units*	17	0.104	6.3

```
548  IF _ibalance.eq.0 .OR. _ibalance.eq.1
549    DELETE [REDEFINE=yes]   mean,   rep,   var,   rdf
550    AKEEP [SAVE=_a2save['save']] n_level; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
551    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
552    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=_mean; REPLICATION=_rep; VARIANCE=_var;\
553   DF=  rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

n level	Mean	Lower	Upper
1	1.193	1.051	1.335
2	1.642	1.499	1.784
3	1.314	1.171	1.456
4	1.869	1.727	2.012
5	1.363	1.22	1.505
6	2.531	2.388	2.673

```
554    AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
555   SAVE=_a2save['save']] n_level
```

Tukey's 95% confidence intervals

n level

	Mean	
6	2.531	a
4	1.869	b
2	1.642	c
5	1.363	d
3	1.314	d
1	1.193	d

```
556  ENDIF
557  SET [IN=*]
563  "Two-way design in randomized blocks"
564  DELETE [REDEFINE=yes] _ibalance
565  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=cult,n_level; BLOCKS=rep;\
566   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=  ibalance]\
```

567 brix_%_ave; SAVE=_a2save
Analysis of variance

Variate: brix % ave

Source of	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	16.4475	16.4475	42.61	
rep.*Units* stratum					
cult	2	66.9704	33.4852	86.74	<.001
n_level	5	64.2436	12.8487	33.28	<.001
cult.n_level	10	75.3784	7.5378	19.53	<.001
Residual	17	6.5627	0.386		
Total	35	229.6027			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 1 *units* 8	1.22	s.e.	0.43
rep 2 *units* 8	-1.22	s.e.	0.43

Tables of means

Variate: brix % ave

Grand mean 13.25

cult	p 888	ss 120	ss 27
	11.33	14.34	14.09

n_level	1	2	3	4	5	6
	10.65	14.5	12.56	13.68	13.62	14.51

cult	n level	1	2	3	4	5	6
p 888		12.53	11.52	10.22	11.16	11.45	11.08
ss 120		10.18	16.3	13.19	13.5	16.18	16.7
ss 27		9.24	15.67	14.27	16.39	13.21	15.73

Standard errors of differences of means

Table	cult	n level	cult n level
rep.	12	6	2
d.f.	17	17	17
s.e.d.	0.254	0.359	0.621

Least significant differences of means (5% level)

Table	cult	n_level	cult n_level
rep.	12	6	2
d.f.	17	17	17
l.s.d.	0.535	0.757	1.311

Stratum standard errors and coefficients of variation

Variate: brix_%_ave

Stratum	d.f.	s.e.	cv%
rep	1	0.956	7.2
rep.*Units*	17	0.621	4.7

```
 568  IF _ibalance.eq.0 .OR. _ibalance.eq.1
 569    DELETE [REDEFINE=yes]  mean,  rep,  var,  rdf
 570    AKEEP [SAVE=_a2save['save']] cult; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
 571    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 572    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=  mean; REPLICATION=  rep; VARIANCE=  var;\
 573   DF=_rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

cult	Mean	Lower	Upper
p 888	11.33	10.86	11.8
ss 120	14.34	13.87	14.81
ss 27	14.09	13.61	14.56

```
 574    AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 575   SAVE=_a2save['save']] cult
```

Tukey's 95% confidence intervals

cult

	Mean	
ss 120	14.34	a
ss 27	14.09	a
p 888	11.33	b

```
 576  ENDIF
 577  SET [IN=*]
 583  "Two-way design in randomized blocks"
 584  DELETE [REDEFINE=yes] _ibalance
 585  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=cult,n_level; BLOCKS=rep;\
 586   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=_ibalance]\
 587   mass  t  ha; SAVE=  a2save
```

Analysis of variance

Variate: mass_t_ha

Source of	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	0.03572	0.03572	0.54	
rep.*Units* stratum					
cult	2	14.38819	7.19409	108.6	<.001
n_level	5	41.73237	8.34647	126	<.001
cult.n level	10	260.90065	26.09006	393.86	<.001
Residual	17	1.12611	0.06624		
Total	35	318.18304			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 1 *units* 5	0.446	s.e.
rep 1 *units* 16	-0.468	s.e.
rep 2 *units* 5	-0.446	s.e.
rep 2 *units* 16	0.468	s.e.

Tables of means

Variate: mass_t_ha

Grand mean 8.296

cult	p 888	ss 120	ss 27
	8.729	8.757	7.402

n level	1	2	3	4	5	6
	8.149	9.39	6.321	9.599	7.937	8.381

cult	n level	1	2	3	4	5	6
p 888		13.181	10.319	6.349	8.033	9.8	4.691
ss 120		1.989	11.45	6.568	13.97	6.951	11.616
ss 27		9.276	6.402	6.045	6.794	7.06	8.837

Standard errors of differences of means

Table	cult	n_level	cult n_level
rep.	12	6	2
d.f.	17	17	17
s.e.d.	0.1051	0.1486	0.2574

Least significant differences of means (5% level)

Table	cult	n_level	cult n_level
rep.	12	6	2
d.f.	17	17	17
l.s.d.	0.2217	0.3135	0.543

Stratum standard errors and coefficients of variation

Variate: mass_t_ha

Stratum	d.f.	s.e.	cv%
rep	1	0.0445	0.5
rep.*Units*	17	0.2574	3.1

```
 588  IF _ibalance.eq.0 .OR. _ibalance.eq.1
 589    DELETE [REDEFINE=yes]   mean,   rep,   var,   rdf
 590    AKEEP [SAVE=_a2save['save']] cult; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
 591    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
 592    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=  mean; REPLICATION=  rep; VARIANCE=  var;\
 593   DF=  rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

	Mean	Lower	Upper
cult			
p 888	8.729	8.533	8.925
ss 120	8.757	8.562	8.953
ss 27	7.402	7.207	7.598

```
 594    AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
 595   SAVE=_a2save['save']] cult
```

Tukey's 95% confidence intervals

cult

	Mean	
ss 120	8.757	a
p 888	8.729	a
ss 27	7.402	b

```
 596  ENDIF
 597  SET [IN=*]
 603  "Two-way design in randomized blocks"
 604  DELETE [REDEFINE=yes]   ibalance
 605  A2WAY [PRINT=aovtable,information,means,%cv; TREATMENTS=cult,n_level; BLOCKS=rep;\
 606   FACTORIAL=2; FPROB=yes; PSE=diff,lsd; LSDLEVEL=5; PLOT=*; COMBINATIONS=present; EXIT=_ibalance]\
 607   juice  t  ha; SAVE=  a2save
```

Analysis of variance

Variate: juice t ha

Source of	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	0.04511	0.04511	4.17	
rep.*Units* stratum					
cult	2	4.00933	2.00466	185.29	<.001
n_level	5	7.3695	1.4739	136.23	<.001
cult.n level	10	14.04264	1.40426	129.8	<.001
Residual	17	0.18392	0.01082		
Total	35	25.6505			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

rep 1 *units* 8	-0.173	s.e.
rep 1 *units* 10	0.19	s.e.
rep 2 *units* 8	0.173	s.e.
rep 2 *units* 10	-0.19	s.e.

Tables of means

Variate: juice t ha

Grand mean 1.652

cult	p 888	ss 120	ss 27

		1.69	2.04	1.225			
	n_level	1	2	3	4	5	6
		1.193	1.642	1.314	1.869	1.363	2.531
cult	n_level	1	2	3	4	5	6
p 888		1.81	1.992	1.742	0.923	1.906	1.767
ss 120		0.579	2.184	1.195	3.295	1.009	3.979
ss 27		1.19	0.749	1.004	1.39	1.173	1.846

Standard errors of differences of means

Table	cult	n_level	cult n_level
rep.	12	6	2
d.f.	17	17	17
s.e.d.	0.0425	0.0601	0.104

Least significant differences of means (5% level)

Table	cult	n_level	cult n_level
rep.	12	6	2
d.f.	17	17	17
l.s.d.	0.0896	0.1267	0.2194

Stratum standard errors and coefficients of variation

Variate: juice_t_ha

Stratum	d.f.	s.e.	cv%
rep	1	0.0501	3
rep.*Units*	17	0.104	6.3

```
608  IF _ibalance.eq.0 .OR. _ibalance.eq.1
609    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
610    AKEEP [SAVE=  a2save['save']] cult; MEAN=  mean; REP=  rep; VARIANCE=  var; RTERM=  resid
611    AKEEP [SAVE=_a2save['save']] #_resid; DF=_rdf
612    CONFIDENCE [METHOD=smm; PROB=0.05] MEANS=  mean; REPLICATION=  rep; VARIANCE=  var;\
613   DF=  rdf
```

Studentized Maximum Modulus 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

	Mean	Lower	Upper
cult			

p 888	1.69	1.611	1.769
ss 120	2.04	1.961	2.119
ss 27	1.225	1.146	1.304

```
614   AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending; PROB=0.05; FACTORIAL=9;\
615   SAVE=_a2save['save']] cult
```

Tukey's 95% confidence intervals

cult

	Mean	
ss 120	2.04	a
p 888	1.69	b
ss 27	1.225	c

```
616  ENDIF
617  SET [IN=*]
```

2016-2017

Potchefstroom

```
Genstat 64-bit Release 18.1 ( PC/Windows 8) 31 October 2017 11:08:02
Copyright 2015, VSN International Ltd.
Registered to: ARC-Grain Crops Institute

                                                  ____________________________________

                                                  Genstat Eighteenth Edition
                                                  Genstat Procedure Library Release PL26.1


  1  SET [WORKINGDIRECTORY='C:/Users/maalis/Documents']
  2  "Data taken from file: 'H:/Wikus/2017 Potch nitro irrig (glass house).xls'"
  3  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
  4  READ [PRINT=*; SETNVALUES=yes] _stitle_
  8  PRINT [IPRINT=*]   stitle  ; JUST=left

Data imported from Excel file: H:\Wikus\2017 Potch nitro irrig (glass house).xls
 on: 31-Oct-2017 11:08:09
 taken from sheet "Sheet1", cells A2:G46

  9  DELETE [REDEFINE=yes] rep,entry,genotype,N  appl  kg  ha,mass  t  ha,brix  %,\
 10  juice_t_ha
 11  UNITS [NVALUES=*]
 12  FACTOR [MODIFY=no; NVALUES=45; LEVELS=3; LABELS=*; REFERENCE=1] rep
 13  READ rep; FREPRESENTATION=ordinal

                                    Identifier                Values               Missing                Levels
                                    rep                           45                     0                     3

 16  VARIATE [NVALUES=45] entry
 17  READ entry

                                    Identifier               Minimum                  Mean               Maximum                Values               Missing
                                    entry                         1                     8                    15                    45                     0

 20  FACTOR [MODIFY=no; NVALUES=45; LEVELS=3; LABELS=!t('HG','ss 007','ss 120')\
 21  ; REFERENCE=1] genotype
 22  READ genotype; FREPRESENTATION=ordinal

                                    Identifier                Values               Missing                Levels
                                    genotype                      45                     0                     3

 25  FACTOR [MODIFY=no; NVALUES=45; LEVELS=!(0,50,100,150,200); LABELS=*\
 26  ; REFERENCE=1] N_appl_kg_ha
 27  READ N_appl_kg_ha; FREPRESENTATION=ordinal

                                    Identifier                Values               Missing                Levels
```

N_appl_kg_ha	45	0	5		

```
 30  VARIATE [NVALUES=45] mass_t_ha
 31  READ mass  t  ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
mass_t_ha	16.45	20.84	28.3	45	0

```
 37  VARIATE [NVALUES=45] brix_%
 38  READ brix  %
```

Identifier	Minimum	Mean	Maximum	Values	Missing
brix_%	9.7	18.08	27.1	45	0

```
 42  VARIATE [NVALUES=45] juice_t_ha
 43  READ juice_t_ha
```

Identifier	Minimum	Mean	Maximum	Values	Missing
juice_t_ha	3.735	7.137	10.17	45	0

```
                                    49
 50  %PostMessage 1129; 0; 100001 "Sheet Update Completed"
 51  "General Analysis of Variance"
 52  BLOCK rep
 53  TREATMENTS genotype*N_appl_kg_ha
 54  COVARIATE "No Covariate"
 55  ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
 56   PSE=diff,lsd; LSDLEVEL=5] brix_%
```

Analysis of variance

Variate: brix_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	79.49	39.74	3.88	
rep.*Units* stratum					
genotype	2	310.42	155.21	15.16	<.001
N_appl_kg_ha	4	105.23	26.31	2.57	0.06
genotype.N_appl_kg_ha	8	111.14	13.89	1.36	0.258
Residual	28	286.64	10.24		
Total	44	892.92			

Tables of means

Variate: brix %

Grand mean 18.08

genotype	HG	ss 007	ss 120
	16.45	21.79	16.01

N_appl_kg_ha	0	50	100	150	200
	15.79	18.44	19.44	16.9	19.83

genotype	N appl kg ha	0	50	100	150
HG		14.17	17.77	17.9	13.7
ss 007		19.4	23.9	24.83	19.07
ss 120		13.8	13.67	15.58	17.93

Standard errors of differences of means

Table	genotype	N_appl_kg_ha	genotype N_appl_kg_ha
rep.	15	9	3
d.f.	28	28	28
s.e.d.	1.168	1.508	2.612

Least significant differences of means (5% level)

Table	genotype	N_appl_kg_ha	genotype N appl kg ha
rep.	15	9	3
d.f.	28	28	28
l.s.d.	2.393	3.09	5.351

Stratum standard errors and coefficients of variation

Variate: brix %

Stratum	d.f.	s.e.	cv%
rep	2	1.628	9
rep.*Units*	28	3.2	17.7

```
 57 DELETE [REDEFINE=yes] mean, rep, var, resid, rdf, scode
 58 SCALAR _scode; VALUE=0
 59 AKEEP [FACTORIAL=9] genotype; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid; STATUS=_scode
 60 IF _scode .in. !(1,2)
 61   AKEEP [FACTORIAL=9] #  resid; DF=  rdf
 62   AMCOMPARISON [PRINT=letter; METHOD=duncan; DIRECTION=descending; PROB=0.05; FACTORIAL=9]\
 63  genotype
```

Duncan's multiple range test

genotype

	Mean	
ss 007	21.79	a
HG	16.45	b

ss 120	16.01	b

```
 64  ELSE
 65    CAPTION !t('Multiple comparisons are available for tests other than',\
 66    'Fisher''s LSD, Bonferroni & Sidak tests, only if all components of the term',\
 67    'are estimated with equal efficiency and in the same stratum.')
 68  ENDIF
 69  ADISPLAY [PRINT=*; FPROB=yes]
 70  DELETE [REDEFINE=yes] _mean, _rep, _var, _resid, _rdf, _scode
 71  SCALAR   scode; VALUE=0
 72  AKEEP [FACTORIAL=9] N_appl_kg_ha; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid;\
 73   STATUS=_scode
 74  IF _scode .in. !(1,2)
 75    AKEEP [FACTORIAL=9] #  resid; DF=  rdf
 76    AMCOMPARISON [PRINT=letter; METHOD=duncan; DIRECTION=descending; PROB=0.05; FACTORIAL=9]\
 77   N_appl_kg_ha
```

Duncan's multiple range test

N_appl_kg_ha

	Mean	
200	19.83	a
100	19.44	a
50	18.44	ab
150	16.9	ab
0	15.79	b

```
 78  ELSE
 79    CAPTION !t('Multiple comparisons are available for tests other than',\
 80    'Fisher''s LSD, Bonferroni & Sidak tests, only if all components of the term',\
 81    'are estimated with equal efficiency and in the same stratum.')
 82  ENDIF
 83  "General Analysis of Variance"
 84  BLOCK rep
 85  TREATMENTS genotype*N_appl_kg_ha
 86  COVARIATE "No Covariate"
 87  ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
 88   PSE=diff,lsd; LSDLEVEL=5] juice_t_ha
```

Analysis of variance

Variate: juice_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	8.4633	4.2316	31.61	
rep.*Units* stratum					
genotype	2	0.8293	0.4147	3.1	0.061
N_appl_kg_ha	4	46.0297	11.5074	85.95	<.001
genotype.N_appl_kg_ha	8	58.541	7.3176	54.65	<.001
Residual	28	3.7489	0.1339		

Total	44	117.6121

Message: the following units have large residuals.

rep 1 *units* 4	0.736	s.e. 0.289
rep 1 *units* 10	0.84	s.e. 0.289

Tables of means

Variate: juice_t_ha

Grand mean 7.137

genotype	HG	ss 007	ss 120		
	7.297	6.965	7.147		

N appl kg ha	0	50	100	150	200
	5.285	8.316	7.147	7.666	7.268

genotype	N appl kg ha	0	50	100	150
HG		5.827	7.072	8.819	9.13
ss 007		4.357	8.68	6.052	6.294
ss 120		5.672	9.194	6.571	7.574

Standard errors of differences of means

Table	genotype	N_appl_kg_ha	genotype N appl kg ha
rep.	15	9	3
d.f.	28	28	28
s.e.d.	0.1336	0.1725	0.2988

Least significant differences of means (5% level)

Table	genotype	N_appl_kg_ha	genotype N appl kg ha
rep.	15	9	3
d.f.	28	28	28
l.s.d.	0.2737	0.3533	0.612

Stratum standard errors and coefficients of variation

Variate: juice t ha

Stratum	d.f.	s.e.	cv%
rep	2	0.5311	7.4

rep.*Units*	28	0.3659	5.1

```
 89  DELETE [REDEFINE=yes] _mean, _rep, _var, _resid, _rdf, _scode
 90  SCALAR   scode; VALUE=0
 91  AKEEP [FACTORIAL=9] genotype; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid; STATUS=_scode
 92  IF _scode .in. !(1,2)
 93    AKEEP [FACTORIAL=9] #_resid; DF=_rdf
 94    AMCOMPARISON [PRINT=letter; METHOD=duncan; DIRECTION=descending; PROB=0.05; FACTORIAL=9]\
 95   genotype
```

Duncan's multiple range test

genotype

	Mean	
HG	7.297	a
ss 120	7.147	ab
ss 007	6.965	b

```
 96  ELSE
 97    CAPTION !t('Multiple comparisons are available for tests other than',\
 98    'Fisher''s LSD, Bonferroni & Sidak tests, only if all components of the term',\
 99    'are estimated with equal efficiency and in the same stratum.')
100  ENDIF
101  ADISPLAY [PRINT=*; FPROB=yes]
102  DELETE [REDEFINE=yes] _mean, _rep, _var, _resid, _rdf, _scode
103  SCALAR   scode; VALUE=0
104  AKEEP [FACTORIAL=9] N_appl_kg_ha; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid;\
105   STATUS=_scode
106  IF   scode .in. !(1,2)
107    AKEEP [FACTORIAL=9] #_resid; DF=_rdf
108    AMCOMPARISON [PRINT=letter; METHOD=duncan; DIRECTION=descending; PROB=0.05; FACTORIAL=9]\
109    N  appl  kg  ha
```

Duncan's multiple range test

N_appl_kg_ha

	Mean	
50	8.316	a
150	7.666	b
200	7.268	c
100	7.147	c
0	5.285	d

```
110  ELSE
111    CAPTION !t('Multiple comparisons are available for tests other than',\
112    'Fisher''s LSD, Bonferroni & Sidak tests, only if all components of the term',\
113    'are estimated with equal efficiency and in the same stratum.')
114  ENDIF
```

```
115  ADISPLAY [PRINT=*; FPROB=yes]
116  DELETE [REDEFINE=yes]  mean,  rep,  var,  resid,  rdf,  scode
117  SCALAR _scode; VALUE=0
118  AKEEP [FACTORIAL=9] genotype; MEAN=  mean; REP=  rep; VARIANCE=  var; RTERM=  resid; STATUS=  scode
119  IF _scode .in. !(1,2)
120    AKEEP [FACTORIAL=9] #_resid; DF=_rdf
121    AMCOMPARISON [PRINT=letter; METHOD=duncan; DIRECTION=descending; PROB=0.05; FACTORIAL=9]\
122   genotype
```

Duncan's multiple range test

genotype

	Mean	
HG	7.297	a
ss 120	7.147	ab
ss 007	6.965	b

```
123  ELSE
124    CAPTION !t('Multiple comparisons are available for tests other than',\
125    'Fisher''s LSD, Bonferroni & Sidak tests, only if all components of the term',\
126    'are estimated with equal efficiency and in the same stratum.')
127  ENDIF
128  "General Analysis of Variance"
129  BLOCK rep
130  TREATMENTS genotype*N_appl_kg_ha
131  COVARIATE "No Covariate"
132  ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7; PCONTRASTS=7; FPROB=yes;\
133   PSE=diff,lsd; LSDLEVEL=5] mass_t_ha
```

Analysis of variance

Variate: mass_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	20.823	10.411	2.08	
rep.*Units* stratum					
genotype	2	43.365	21.683	4.32	0.023
N_appl_kg_ha	4	40.481	10.12	2.02	0.119
genotype.N_appl_kg_ha	8	20.051	2.506	0.5	0.846
Residual	28	140.399	5.014		
Total	44	265.119			

Message: the following units have large residuals.

rep 2 *units* 14	3.82	s.e. 1.77
rep 3 *units* 1	4.2	s.e. 1.77

Tables of means

Variate: mass_t_ha

Grand mean 20.84

genotype	HG	ss 007	ss 120		
	21.3	21.74	19.47		
N appl kg ha	0	50	100	150	200
	20.39	21.2	20.01	20.06	22.53

genotype	N_appl_kg_ha	0	50	100	150
HG		21.84	20.79	20.52	21.45
ss 007		20.48	23.11	20.92	20.22
ss 120		18.84	19.7	18.58	18.5

Standard errors of differences of means

Table	genotype	N_appl_kg_ha	genotype N_appl_kg_ha
rep.	15	9	3
d.f.	28	28	28
s.e.d.	0.818	1.056	1.828

Least significant differences of means (5% level)

Table	genotype	N_appl_kg_ha	genotype N appl kg ha
rep.	15	9	3
d.f.	28	28	28
l.s.d.	1.675	2.162	3.745

Stratum standard errors and coefficients of variation

Variate: mass_t_ha

Stratum	d.f.	s.e.	cv%
rep	2	0.833	4
rep.*Units*	28	2.239	10.7

Appendix K. Distribution tables of climatic conditions across seasons and locations

KEY NOTES FOR DAILY REPORT			
ELEMENT	**DESCRIPTION**	**UNIT**	**STATION TYPE**
Tx	Daily Maximum Temperature	◆C	AWS
Tn	Daily Minimum Temperature	◆C	AWS
Rain	Total Rainfall [Calculated From Hourly Data]	mm	AWS
Rs	Total Radiation [Calculated From Hourly Data]	MJ/m2	AWS
U2	Average Wind Speed [Calculated From Hourly Data]	ms	AWS
RHx	Daily Maximum Relative Humidity	%	AWS
RHn	Daily Minimum Relative Humidity	%	AWS
ET0	Total Relative Evapotranspiration [Calculated From Hourly Data]	mm	AWS
HU	Total Heat Units [Calculated From Hourly Data]	Unitless	AWS
CU	Total Cold Units [Calculated From Hourly Data]	Unitless	AWS
DPCU	Daily Positive Chilling Units [Calculated From Hourly Data]	Unitless	AWS
VP	Vapour Pressure [Calculated From Hourly Data / 06:00 - 18:00]	~~~	AWS
SVP	Saturated Vapour Pressure [Calculated From Hourly Data]	~~~	AWS
VPD	Vapour Pressure Deficit [Calculated From Hourly Data / 06:00 - 18:00]	~~~	AWS
AveT	Average Temperature [[Tx + Tn] / 2]	◆C	AWS
AveRH	Average Relative Humidity [[RHx + RHn] / 2]	%	AWS
UMax	Highest Wind Speed Measurement For The 24 Hour Period	m/s	AWS
UHr	Time of Highest Wind Speed Measurement For The 24 Hour Period	time	AWS

MONTHLY REPORT: Monthly Averages And Totals

Start Year	Start Month	End Year	End Month
2012	1	2017	12

Comp#	Station Name	Latitude	Longitude	Altitude
30142	VAALHARTS	-27,9576	24,8399	1180

Compno	Year	Month	Tx	Tn	Rain	HU
30142	2012	1	36,12	16,19	21,34	483,74
30142	2012	2	32,69	16,84	102,87	392
30142	2012	3	32,96	13,75	34,04	386,43
30142	2012	4	28,02	8,34	11,94	222,18
30142	2012	5	27,71	4,43	0,76	159,06
30142	2012	6	21,68	0,82	9,91	12,94
30142	2012	7	22,06	-0,82	2,29	-3,15
30142	2012	8	24,93	3,96	1,78	129,56
30142	2012	9	27,07	5,95	12,45	190,86
30142	2012	10	32,75	10,61	0,25	361,63
30142	2012	11	34,98	14,27	24,38	441,34
30142	2012	12	32,86	15,71	95,25	419,12
30142	2013	1	36,29	17,83	146,05	495,02
30142	2013	7	22,94	0,96	5,59	35,32
30142	2013	8	23,31	1,9	5,33	73,99
30142	2013	9	28,67	5,7	0,25	209,38
30142	2013	10	31,98	9,33	9,4	330,82
30142	2013	11	34,73	13,64	20,07	421,66
30142	2013	12	32,55	15,9	72,9	422,67
30142	2014	1	36,26	17,64	11,18	442,25
30142	2014	7	23,59	-0,04	0,51	1,38

30142	2014	12	33,86	16,63	109,47	304,77
30142	2015	1	36,16	16,78	35,05	483,15
30142	2015	2	35,23	14,04	19,05	403,53
30142	2015	3	32,27	14,74	50,29	393,98
30142	2015	4	28,46	8,73	14,73	228,76
30142	2015	5	29,37	4,62	2,03	179,01
30142	2015	6	21,73	1,37	28,7	17,13
30142	2015	7	22,19	2,33	4,06	48,91
30142	2015	8	27,57	4,71	1,02	172,49
30142	2015	9	28,32	9,35	19,56	256,6
30142	2015	10	35,19	13,32	8,89	437,28
30142	2015	11	33,71	12,97	40,89	399,13
30142	2015	12	38	16,81	32,77	535,59
30142	2016	1	35,04	18,43	81,79	493,79
30142	2016	2	36,13	17,72	19,56	462,88
30142	2016	3	33,06	14,18	54,86	400,13
30142	2016	4	28,6	10,91	103,38	269,42
30142	2016	5	24,39	5,87	24,89	126,05
30142	2016	6	23,34	1,78	0	49,53
30142	2016	7	21,54	-0,02	17,27	3,3
30142	2016	8	26,15	3,01	0	135,36
30142	2016	9	29,25	7,21	0	246,1
30142	2016	10	33,87	10,23	1,52	369,35
30142	2016	11	35,97	15,88	36,32	449,88
30142	2016	12	36,89	17,72	70,61	505,02
30142	2017	1	32,29	16,34	136,4	420,66
30142	2017	2	31,14	17,65	125,98	364,35
30142	2017	3	33,58	12,67	4,57	383,6
30142	2017	4	28,57	8,8	19,05	233,37
30142	2017	5	26,53	4,48	8,89	124,12
30142	2017	6	24,35	1,19	0	56,72
30142	2017	7	24,79	0,44	0	56,82
30142	2017	8	25,36	2,92	0,25	115,67

30142	2017	9	31,3	8,19	7,87	288,46
30142	2017	10	28,51	12,06	9,4	103,19
30142	2017	11	31,81	12,12	2,79	372,63
30142	2017	12	33,31	15,49	38,61	441,8

Comp#	Station Name	Latitude	Longitude	Altitude
30627	RUSTENBURG SHAFT 10 IMPLANTS: AWS	-25,53271	27,2504	1130

Compno	Year	Month	Tx	Tn	Rain	HU
30627	2012	1	32,16	18,33	58,42	454,73
30627	2012	2	32,9	18,91	49,02	446,7
30627	2012	3	30,97	16,05	85,34	402,68
30627	2012	4	27	10,95	4,06	260,73
30627	2012	5	26,79	8,46	0	220,34
30627	2012	6	21,98	4,45	0,25	80,91
30627	2012	7	23,09	5,15	0	113,38
30627	2012	8	25,59	7,27	0	195,45
30627	2012	9	27,69	10,81	18,54	271,24
30627	2012	10	30	14,52	89,66	365,64
30627	2012	11	31,21	16,62	97,79	407,48
30627	2012	12	29,52	17,32	115,06	394,63
30627	2013	1	32,09	18,84	82,55	462,52
30627	2013	2	33,16	18,42	42,67	422,79
30627	2013	3	30,67	16,46	42,16	398,09
30627	2013	4	27,26	11,92	75,44	272,6
30627	2013	5	25,39	7,74	0	189,5
30627	2013	6	23,67	4,78	0	111,07
30627	2013	7	23,15	5,63	0	118,76
30627	2013	8	24,54	6,57	3,56	165,43
30627	2013	9	30	12,38	1,02	338,41
30627	2013	10	30,7	14,15	69,34	379,44
30627	2013	11	32,63	16,92	29,46	434,09

30627	2013	12	29,26	17,99	103,89	405,4
30627	2014	1	32,73	19,28	66,55	483,92
30627	2014	2	30,1	18,61	241,55	383,79
30627	2014	3	26,84	17,25	245,62	349,46
30627	2014	4	25,83	11,05	34,54	230,41
30627	2014	5	26,13	7,89	1,02	198,05
30627	2014	6	23,19	3,74	0	82,11
30627	2014	7	22,24	3,48	0	69,03
30627	2014	8	25,22	7,48	0	183,87
30627	2014	9	30,58	11,37	0,76	324,88
30627	2014	10	31,3	14,34	3,81	396,66
30627	2014	11	29,03	16,18	117,6	356,93
30627	2014	12	30,38	18	63,5	422,47
30627	2015	1	31,56	18,39	109,47	447,9
30627	2015	2	33,74	17,83	17,27	433,37
30627	2015	3	31,03	17,04	44,2	418,85
30627	2015	4	28,11	13,85	14,48	312,74
30627	2015	5	31,64	3,04	0	140,35
30627	2015	6	41,37	2,86	1,27	339,64
30627	2015	7	36,75	2,85	7,87	260,57
30627	2015	8	26,87	8,45	0	248,19
30627	2015	9	28,76	13,37	57,15	326,63
30627	2015	10	33,8	16,99	2,79	474,31
30627	2015	11	32,56	16,68	0	446,26
30627	2015	12	35,39	20,9	0	555,11
30627	2016	1	32,71	19,65	0	493,93
30627	2016	2	33,81	20,2	0	473,99
30627	2016	3	30,26	17,31	0	417,55
30627	2016	4	29,05	14,65	0	347,77
30627	2016	5	23,61	9,46	0	192,21
30627	2016	6	21,85	6,44	0	109,09
30627	2016	7	21,67	4,7	0	90,63
30627	2016	8	25,8	7,31	0	194,78

30627	2016	9	30,08	12,75	0	346,74
30627	2016	10	32,33	16,06	0	446,45
30627	2016	11	31,44	17,93	23,62	426,46
30627	2016	12	31,69	19,2	75,18	463,98
30627	2017	1	29,53	18,84	195,58	426,51
30627	2017	2	28,28	18,62	211,33	360,96
30627	2017	3	30,1	15,94	26,16	392,77
30627	2017	4	27,02	13,23	36,32	287,04
30627	2017	5	24,71	8,33	25,91	180,57
30627	2017	6	23,13	5,52	0	110,93
30627	2017	7	23,62	5,83	1,27	132,25
30627	2017	8	25,16	7,23	0	187,93
30627	2017	9	30,62	12,55	0	341,5
30627	2017	10	29,66	13,96	64,52	356,81
30627	2017	11	31,49	15,1	72,9	396,2
30627	2017	12	30,76	17,59	76,45	428,85

Comp#	Station Name	Latitude	Longitude	Altitude
30649	POTCHEFSTROOM: OLIESADE	-26,73607	27,07553	1349

Compno	Year	Month	Tx	Tn	Rain	HU
30649	2012	1	30,42	16,22	94,23	391
30649	2012	2	29,11	16,3	100,08	348,05
30649	2012	3	28,72	13,59	88,39	328,15
30649	2012	4	25	8,05	14,99	181,07
30649	2012	5	25	5,16	0	133,21
30649	2012	6	19,79	1,27	17,53	-7,3
30649	2012	7	20,96	0,42	1,78	5,07
30649	2012	8	23,48	4,75	2,54	122,46
30649	2012	9	24,78	7,14	40,64	177,09
30649	2012	10	29,12	12,39	44,45	323,62

30649	2012	11	30,21	14,62	40,64	358,8
30649	2012	12	27,99	15,41	202,95	343,68
30649	2013	1	30,11	16,81	118,36	402,42
30649	2013	2	31,03	15,5	64,01	349,32
30649	2013	3	28,43	14,58	124,21	332,53
30649	2013	4	24,62	8,88	70,87	187,44
30649	2013	5	23,19	4,87	3,05	106,39
30649	2013	6	21,46	0,79	0	13,3
30649	2013	7	21,28	3,6	0	56,1
30649	2013	8	22,17	2,76	0	72,02
30649	2013	9	27,47	7,41	0	226,53
30649	2013	10	29,04	11,48	102,36	309,79
30649	2013	11	30,32	14,04	59,44	356,01
30649	2013	12	27,28	15,49	216,66	331,7
30649	2014	1	30,49	17	81,03	413,08
30649	2014	2	28,4	16,76	116,84	329,57
30649	2014	3	25,75	14,56	182,12	288,86
30649	2014	4	24,48	7,94	6,1	166,1
30649	2014	5	24,24	4,93	3,81	122,44
30649	2014	6	20,76	0,14	0,76	-1,5
30649	2014	7	19,76	-0,23	0	-22,85
30649	2014	8	22,67	4,73	7,62	109,08
30649	2014	9	28,48	8,88	9,4	262,98
30649	2014	10	29,48	11,41	14,48	321,41
30649	2014	11	27,23	13,55	90,17	291,92
30649	2014	12	29,24	16,35	114,55	374,78
30649	2015	1	30,17	16,34	139,19	388,5
30649	2015	2	31,06	14,21	55,63	339,38
30649	2015	3	27,71	14,02	104,65	314,84
30649	2015	4	25,88	10,01	28,96	219,27
30649	2015	5	26,21	5,76	0,76	163,14
30649	2015	6	19,33	1,38	4,06	-7,71
30649	2015	7	20,81	3,05	7,11	39,03

30649	2015	8	26,24	5,49	0	172,68
30649	2015	9	26,31	10,46	71,12	246,4
30649	2015	10	31,68	14,09	30,48	393,94
30649	2015	11	30,45	12,72	36,58	356,75
30649	2015	12	33,44	18,43	64,7	469,04
30649	2016	1	30,84	18,06	94,74	428,5
30649	2016	2	31,59	17,29	76,96	394,62
30649	2016	3	28,7	14,51	60,2	347,55
30649	2016	4	26,46	11,25	76,96	255,67
30649	2016	5	21,98	6,42	42,42	113,38
30649	2016	6	20,45	2,96	11,43	30,46
30649	2016	7	19,55	0,75	59,44	-10,87
30649	2016	8	23,57	3,11	0	96,7
30649	2016	9	27,55	9,53	0	254,38
30649	2016	10	30,17	11,86	55,12	344,05
30649	2016	11	29,67	15,48	94,74	353,59
30649	2016	12	32,62	16,97	93,98	185,7
30649	2017	1	28,42	16,46	29,21	154,79
30649	2017	2	26,51	16,82	225,55	309,21
30649	2017	3	27,93	14,69	33,78	311,1
30649	2017	4	25,42	10,37	46,23	208,41
30649	2017	5	22,51	4,85	10,67	91,15
30649	2017	6	21,91	3,15	0	44,23
30649	2017	7	22,19	3,47	0,25	68,84
30649	2017	8	23,01	3,78	0	104,1
30649	2017	9	28,36	9,23	8,38	241,53
30649	2017	10	26,39	11,36	56,13	250,37
30649	2017	11	29,12	12,67	69,34	313,88
30649	2017	12	29,29	15,69	62,48	368,82

Comp#	Station Name	Latitude	Longitude	Altitude
30655	BETHLEHEM: KLEINGRAANINSTITUUT	-28,16277	28,29733	1721

Compno	Year	Month	Tx	Tn	Rain	HU
30655	2012	1	28,36	13,93	111,75	316,08
30655	2012	2	27,03	13,98	46,23	281,86
30655	2012	3	26,17	11,18	47,5	240,78
30655	2012	4	22,01	5,45	3,05	97,11
30655	2012	5	21,79	2,63	0	44,95
30655	2012	6	15,43	-1,52	32,77	-101,35
30655	2012	7	17,43	-2,37	2,29	-101,41
30655	2012	8	20,54	0,44	0,76	5,55
30655	2012	9	20,28	4,84	42,93	53,16
30655	2012	10	24,51	9,59	34,54	138,06
30655	2012	11	26,19	11,34	29,21	235,56
30655	2012	12	25,06	12,93	125,98	258,23
30655	2013	1	26,57	13,77	178,06	296,35
30655	2013	2	27,35	13,19	46,99	236,49
30655	2013	3	25,48	12,01	26,92	244,75
30655	2013	4	21,21	5,74	69,59	91,32
30655	2013	5	19,39	1,8	13,46	-7,37
30655	2013	6	18,08	-2,45	0	-90,78
30655	2013	7	17,78	0,17	0	-51,6
30655	2013	8	18,91	0,45	4,06	-24,3
30655	2013	9	23,5	4,38	7,87	105,49
30655	2013	10	24,71	7,45	91,95	167,4
30655	2013	11	24,96	10,25	81,54	213,74
30655	2013	12	23,7	12,98	178,81	237,4
30655	2014	1	27,38	14,26	146,56	311,56
30655	2014	2	24,72	14,13	124,97	244,7
30655	2014	3	23,17	12,3	88,89	214,72
30655	2014	4	21,24	5,9	34,29	88,27

30655	2014	5	20,89	2,41	1,52	25,16
30655	2014	6	17,82	-2,72	0	-96,14
30655	2014	7	16,66	-3,77	0	-133,03
30655	2014	8	19,12	1,38	10,67	-5,39
30655	2014	9	25,34	5,17	4,57	153,01
30655	2014	10	25,59	8,04	12,7	188
30655	2014	11	22,94	10,5	186,18	184,29
30655	2014	12	26,26	13,7	102,87	284,31
30655	2015	1	27,48	13,97	134,62	308,12
30655	2015	2	28,04	12,37	29,72	262,92
30655	2015	3	23,99	12,24	145,03	227,77
30655	2015	4	22,18	7,9	27,43	130,16
30655	2015	5	22,33	2,62	0,51	48,7
30655	2015	6	15,91	-0,85	18,03	-94,67
30655	2015	7	16,82	0,24	17,78	-67,58
30655	2015	8	23,17	2,13	0	61,94
30655	2015	9	23,31	6,89	24,89	133,9
30655	2015	10	28,04	10,49	32,77	272,32
30655	2015	11	27,66	9,6	52,58	245,9
30655	2015	12	31,44	14,32	39,37	375,02
30655	2016	1	27,56	14,63	166,12	313,37
30655	2016	2	28,13	13,91	89,92	290,66
30655	2016	3	25,87	11,73	52,83	249,96
30655	2016	4	23,28	8,46	61,21	159,48
30655	2016	5	19,02	4,1	36,58	23,77
30655	2016	6	17,05	1,11	9,91	-40,62
30655	2016	7	15,25	-0,57	66,29	-79,37
30655	2016	8	21,96	2,25	0,51	32,66
30655	2016	9	25,79	4,4	0	33,69
30655	2016	11	27,26	14,05	36,07	88,17
30655	2016	12	27,94	13,63	96,27	305,79
30655	2017	1	26,2	13,32	141,22	283,71
30655	2017	2	25,19	14,5	244,09	244,38

30655	2017	3	27,13	10,64	26,92	242,89
30655	2017	4	24	7,5	24,89	147,88
30655	2017	5	21,82	2,56	9,4	31,58
30655	2017	6	19,7	-1,15	0,25	-58,46
30655	2017	7	20,35	-1,17	0	-44,22
30655	2017	8	20,72	0,01	4,57	-14,71
30655	2017	9	25,88	5,65	26,16	150,22
30655	2017	10	24,61	7,06	43,18	155,53
30655	2017	11	26,76	9,04	94,23	223,71
30655	2017	12	26,66	12,23	114,3	260,01