Evaluation of the effect of selected plant growth regulators on soybean yield parameters in South Africa

GL van Niekerk

orcid.org 0000-0003-3298-8677

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Supervisor:	Prof MJ du Plessis
Co-supervisor:	Mr JS Reynolds
Co-supervisor:	Ms AS de Beer

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Abstract

Soybean is an important protein and oilseed crop throughout the world due to the commercial use of soybean meal, oil and sub-products. South Africa is the largest soybean producer in Africa and produces on average 1.8 t ha^{-1}. Although soybean productivity in South Africa has increased over the past decades, yield gaps can be narrowed by adopting improved agricultural practices that increase yield and decrease yield loss due to genetic barriers and environmental stresses. Available biotechnologies, in particular plant growth regulators (PGRs), have been reported to increase the tolerance of crops to environmental stresses, increase harvestable yield and to enhance growth and yield components. The aims of this study were to evaluate the efficacy of PGRs at different application times and rates to increase soybean yield in South Africa.

To determine the optimal time of application, five field trials were conducted at three localities, *viz.* Bethlehem and Kroonstad in the Free State province and Potchefstroom in the North-West province, South Africa. Two field trials and one pot trial were conducted to investigate application rates at Potchefstroom. The cultivar PAN 1521 R was planted in the field trials while LS 6161 R was used in the pot trial. Foliar treatments consisted of a control (untreated) and three PGRs, to which a specific identification code was assigned, *viz.* A2019 forchlorfenuron (CPPU), B2019 naphthalene acetic acid (NAA) and C2019 gibberellic acid + abscisic acid (GA3 + S-ABA). Plant growth regulators were applied at two different growth stages (R1 and R3). To determine the optimal application time, applications were done at R1 and R3, as well as at both R1 + R3 (double application). Three concentrations of each PGR were applied at growth stages R1 + R3 (double application) to determine the optimal rate of application.

No optimum application time or rate could be determined for any of the PGRs evaluated on soybean in this study. None of the three PGRs evaluated at different application times significantly increased the yield of soybean. Application of PGRs to soybean planted in Bethlehem (cold zone) were more effective in terms of nodes $plant^{-1}$, pods $node^{-1}$ and pods $plant^{-1}$ than PGR applications at Potchefstroom and Kroonstad. Plant growth regulator treatments increased growth parameters of soybean, however these effects varied between Potchefstroom, Bethlehem and Kroonstad and were not consistent. None of the application rates of the respective PGRs, increased any of the growth and yield components in the field trials or the pot trial at Potchefstroom. It could possibly be ascribed to the low application rates used and the warm and dry climatic conditions experienced during this study. Application of PGRs at too low concentrations will have no effect on growth and yield parameters. Although increases in growth and yield parameters were reported for other crops, similar results were

not found during this study. More research on the PGR, rate and application time for soybean is therefore needed in South Africa.

Keywords: abscisic acid, forchlorfenuron, gibberellic acid, *Glycine max*, plant growth regulator, naphthalene acetic acid, South Africa, yield

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Chapter 1

General introduction

1.1 Background of the study

The human population is projected to increase by 2 billion people in the next 30 years and will reach 9.7 billion in 2050 (United Nations Department of Economic and Social Affairs, 2015). World crop production should be increased to meet the increasing demand for food (Ray *et al*., 2013). Legumes are the second most important crops based on area planted and total production (Gepts *et al.,* 2005). Soybean (*Glycine max* (L.) Merr.) is the most economically important oil and protein seed crop due to its high protein and oil content (Stagnari *et al.,* 2017). Soybean contribute significantly to the South African economy as evident from the increase in production and the area planted in recent years (Dlamini *et al.,* 2014). Although soybean production in South Africa has increased over the past few decades (USDA, 2018), the yield gap (difference between maximum achievable crop yield and actual yield) can be reduced through adoption of improved agricultural practices aimed at increasing yield and decreasing yield loss caused by genetic barriers and environmental stresses.

Soybean is mainly cultivated under dryland conditions in South Africa (Liebenberg, 2012). Dryland crops are exposed to several environmental stresses, specifically water and heat stress (Dhopte and Ramteke, 2017). Plants respond to environmental stresses by increasing the level of growth inhibiting hormones and suppressing the level of growth promoting hormones, which results a negative impact on the growth and development of a plant (Dhopte and Ramteke, 2017). However, the physiological response of a plant to its environmental conditions can be altered by chemically synthesised hormones, known as plant growth regulators (PGRs) (Kaya *et al*., 2009). Plant growth regulators can be either natural or synthetic compounds and are applied in low concentrations to mimic or affect production of plant hormones in order to promote, inhibit or modify plant growth and development (Mir *et al*., 2010; Sharma, 2015). Plant growth regulators have successfully been used in agriculture and horticulture as a tool to increase production and to aid in removing or reducing genetic and environmental barriers (Rademacher, 2015).

Studies conducted on tomato (*Solanum lycopersicum*) (Solanaceae) (Prasad *et al.* 2013; Mousawinejad *et al.,* 2014), Okra (*Abelmoschus esculentus*) (Malvaceae) (Shahid *et al*., 2013; Gadade *et al.*, 2017), groundnut (*Arachis hypogaea*) (Fabaceae) (Hasan and Ismail, 2018) and soybean (*Glycine max* (*L.*) Merr.) (Fabaceae) (Leite *et al.,* 2003; Azizi *et al*., 2012; Solanke *et al.,* 2018) indicated PGRs to be effective in increasing the yield of these crops.

In South Africa, studies on the effect of PGRs on soybean are limited. This study was conducted to investigate the potential of PGRs to increase soybean yield under dryland conditions.

1.2 Main objective

To evaluate the efficacy of PGRs at different application rates and times to increase soybean yield in South Africa.

1.3 Specific objective

To determine:

i) the effect of different application times of three different PGRs on soybean yield parameters.
ii) the effect of different application rates of three PGRs on soybean yield parameters.

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Chapter 2

Literature review

2.1 Soybean on a global scale

Soybean (*Glycine max* (*L.*) Merr.) (Fabaceae) is an important protein and oilseed crop throughout the world. Soybean originated in East Asia and was first cultivated by Chinese farmers around the 11th century BC (Dupare *et al*., 2008). It later spread to South-east Asia from where it was introduced into Europe during the 17th and into the United States of America (USA) during the 18th century, respectively (Shurtleff and Aoyagi, 2010). The health benefits and versatile uses of soybean became known by the 20th century (Murray and Pizzorno, 2010). Soybean is known for its high protein and oil content and is often referred to as "poor man's meat" (Anitha *et al*., 2013) or the "golden bean" (Wandkar *et al*., 2012).

The main drivers for the success of soybean production worldwide are the commercial use of soybean meal, oil and its sub-products (Thoenes, 2006). Soybean accounts for 25% of all edible oils and 66% of the world's protein concentrate for animal feedstuff (Darekar and Reddy, 2017). The quantity of high-quality protein meal produced from soybean is more than any other commercial oilseed crop and it is therefore used extensively in commercial feeds for pork, cattle and poultry (Asbridge, 1997). In the food industry, soybean oil is an important source of edible oil and fat, and is used in cooking oils, salad dressings and mayonnaise. Soybean products are also used in pharmaceuticals containing vitamin E and lecithin, as well as in commercial and industrial products such as soap, shampoo, hydraulic fluids and paints (Endres, 2001). It is expected that crops, and specifically soybean will play an increasingly important role as a renewable resource and in strategies to reduce world hunger (Asbridge, 1997; De Beer, 2012).

During the 2016/17 production season, 120.13 million ha of soybean was planted globally which yielded 350.84 million tons with an average productivity of 2 920 kg ha^{-1}, resulting in the largest crop in a decade (Anon, 2017; USDA, 2018). Currently the leading soybean producing countries are USA followed by Brazil, Argentina and China. These countries accounts for almost 90% of the world's total soybean production (FAOSTAT, 2017; Sharma, 2017).

2.2 Soybean production in South Africa

In Africa, South Africa is the largest soybean producer (Anon, 2017), producing on average between 100 000 and 800 000 tons per annum, averaging a yield of 1.7 to 2 t ha^{-1} (Soybean Market Value profile, 2017). Soybean production has over the past two decades increased to such an extent that it exceeded sunflower production in 2012. It is the country's most important oilseed crop, mainly due to the high demand for protein feed in the animal feed industry (Nortjé,

2017). For the 2017/18 season soybean production reached 1.5 million tons (Crop estimates committee, 2018), which is a 15% increase from the previous season (2016/17) when 1.3 million tons were produced (Crop estimates committee, 2017). The increase in production can be ascribed to the increase in area planted, increase in yield per unit area, and investments made to increase the country's soybean crushing capacity from 86 000 tonnes (in 2012) to more than 2.2 million tons. (Sihlobo and Kapuya, 2016; Soybean Market Value profile, 2017; Sihlobo, 2018a). Although South Africa is able to satisfy the local soybean demand it remains a net importer of soybean and oilcake. Imports is however expected to decline by 27% and 17% year-on-year in the 2018/19 production year, respectively, due to the increased local production and favourable domestic prices, especially for soybean processors (Sihlobo and Kapuya, 2016; Sihlobo, 2018a).

The predominant soybean producing provinces in South Africa are Mpumalanga, Free State, and KwaZulu-Natal. Over the past six years, Mpumalanga has contributed 43% of the average National soybean crop (1 058 650 t yr^{-1}), followed by the Free State (34%) and KwaZulu-Natal (8%). Together these three provinces accounted for 84% of the total soybean production in South Africa, while Gauteng (6%), Limpopo (5%), North West (4%) and the Northern Cape (1%) made smaller contributions in the past six years (2013-2018) (Crop estimates committee, 2017).

Soybean yields in the Mpumalanga, Free State and KwaZulu-Natal provinces during the past six years were estimated at 1.9, 1.5 and 2.4 t ha^{-1}, respectively. The Northern Cape province had the highest average yield of 3.5 t ha^{-1} and the Eastern Cape province the lowest of 1.4 t ha^{-1}. In the Limpopo, Gauteng, Western Cape and North West provinces the average yield was estimated at 2.9, 2.4, 1.6 and 1.5 t ha^{-1} respectively (Calculations made using data from the crop estimates committee, for the period 2013-2018).

2.3 Crop description

2.3.1 General description of a soybean plant

Soybean is a bushy and erect annual summer legume grown in warm temperature and short-day conditions. The plants generally grow 40 to 100 cm in height with the first leaves unifoliate, oval and opposite and all other leaves alternate and trifoliate (DAFF, 2010; Hicks, 2012). A large number of self-pollinating, purple or white flowers are produced on short racemes, of which approximately two thirds give rise to a number of small pods with 1-4 seeds (occasionally five) per pod (Acquaah, 2009). The pods are straight or slightly curved, pubescent and are usually black, brown or tan. A soybean plant is anchored to the ground by a taproot that can be as deep as 1.5 to 2.0 m below the soil surface and secondary roots

(lateral roots) that grow in the upper 300 mm of the soil. Nodules may form on the roots as a result of *Rhizobium japonicum* infection (DAFF, 2010).

2.3.2 Vegetative and reproductive stages

Development of a soybean plant is divided into two major growth stages, namely vegetative (V) and reproductive (R) stages. The vegetative stages commence with emergence (VE) which occur when the cotyledons and growing point appear above the soil surface. After emergence, two unifoliate leaves emerge opposite from one another on the first node, unroll and start the cotyledon (VC) stage. The subsequent stages, V1-V(n) are characterised and numbered by the upper, fully developed leaf node (trifoliates) on the main stem above the unifoliate leaves (Endres and Kandel, 2015). Following the period of vegetative growth, the soybean plant enters the reproductive stage that starts when the first flowers appear (at any node) on the main stem (R1). When the plant is in full bloom, it enters the R2 stage. The subsequent reproductive stages include pod development (R3 and R4), seed development (R5 and R6) and plant maturation (R7 and R8) (Endres and Kandel, 2015). A detailed description of these growth stages are provided in Table 2.1. Depending on the soybean cultivar, the vegetative growth period can be between six and eight weeks, while the reproductive growth stage is from seven to twelve weeks (Liebenberg, 2012).

2.3.3 Stem growth habits

In terms of stem growth habits, soybean cultivars can be classified into determinate or indeterminate growth habits (DAFF, 2010). Determinate soybean cultivars are short, complete over 80% of vegetative growth before flowering starts and the growth tips end in a pod-bearing raceme. The pods of determinate varieties usually ripen at the same time (due to simultaneous flowering at all nodes) and can therefore be harvested simultaneously. Indeterminate soybean cultivars are taller and continue to grow vegetatively throughout the flowering and pod set period.

Soybean cultivars with an indeterminate growth habit do better in dryland conditions, especially in regions where moisture stress during early summer is possible. In contrast, determinate cultivars are best grown under irrigation, especially where lush growth can cause soybean to lodge (Liebenberg, 2012). A number of registered determinate and indeterminate cultivars are commercially available to producers in South Africa (DAFF, 2010; De Beer and Bronkhorst, 2018).

Table 2.1 Vegetative (V) and reproductive (R) growth stages of soybean plants (Endres and Kandel, 2015).

Vegetative stages			Reproductive stages		
Stage		**Description**	**Stage**		**Description**
VE	Emergence	Cotyledons rise through soil surface.	R1	Begin flowering	One open flower at any node on the main stem.
VC	Cotyledon	Unifoliate leaves fully expanded.	R2	Full flowering	Open flower on one of the two uppermost nodes on the main stem with a fully developed leaf.
V1	First trifoliate	First trifoliate leaves fully developed and open at unifoliate node.	R3	Beginning pod	Pod 0.5 cm long at one of the four uppermost nodes with a fully developed leaf.
V2	Second node	Two trifoliate leaves fully developed and open at and above the unifoliate node.	R4	Full Pod	Pod 2 cm long at one of the four uppermost nodes with a fully developed leaf.
V3	Third node	Three trifoliate leaves fully developed and open at and above the unifoliate node.	R5	Beginning seed	Seed 3 mm long in the pod at one of the four uppermost nodes with a fully developed leaf.
V(n)	N^{th}-node	Number of fully developed and open leaves at and above the unifoliate node.	R6	Full seed	Pod containing a green seed that fills the pod cavity at one of the four uppermost nodes with a fully developed leaf.
			R7	Beginning maturity	One normal pod on the main stem that obtained the mature colour (brown/tan).
			R8	Full Maturity	95% of pods have reached mature pod colour.

2.4 Growing requirements

2.4.1 Climate

Soybean is a warm-season crop that can be grown in areas with temperate as well as tropical and sub-tropical conditions. Temperature plays an important role in the rate of soybean development. The ideal temperature range for soybean development is considered to be between 20 and 30 °C, with the optimal temperature being 25 °C (Heinemann *et al*., 2006; Liebenberg, 2012). Temperatures higher or lower than the optimal range can lead to poor

growth and development and in some cases, physiological disorders. Temperatures above 30 °C have been reported to reduce the number of flowers, pods per plant, seeds per pod and therefore the total yield (DAFF, 2010; Puteh *et al.*, 2013). Cold temperatures (below 13 °C) inhibit growth, development, flowering and seed formation (DAFF, 2010; Borowski and Michalek, 2014). Compared to maize, soybean is able to tolerate higher temperatures provided that there is sufficient groundwater and moisture present (Liebenberg, 2012).

For effective germination and emergence, soil temperature at planting should be at least 15 °C or higher (Liebenberg, 2012). Planting at low temperatures will negatively affects root and shoot growth, which will slow germination and emergence, delay flowering, reduce the final stand counts and reduce the yield potential (Borowski and Michalek, 2014).

2.4.2 Soil

For successful soybean production, deep, well drained and fertile soil with a good water-holding capacity is required (DAFF, 2010; Liebenberg, 2012). In South Africa, soybean is cultivated on heavier clay due to its ability to utilise water at shallow soil depths. If enough moisture is present in the topsoil to ensure germination and emergence, soybean can be planted in sandy soils. However, sandy soils are traditionally avoided due to the risk of nematode infections, low fertility and its poor water retention capacity. Optimal soil pH values for soybean vary between 5.8 - 7.5. Planting of soybean in soil with a pH lower than 5.2, may impede nitrogen fixation and protein synthesis (Liebenberg, 2012). Planting into excessively compacted soil can reduce the rate and success of seedling emergence and cause yield losses (DAFF, 2010; Calonego *et al.*, 2017).

2.4.3 Rainfall

In South Africa, almost all soybean is grown under rain-fed conditions, with 500 – 900 mm required for optimal soybean growth and yield, depending on the growth conditions (DAFF, 2010). During the vegetative growth stages, soybean is able to tolerate short periods of drought, but prolonged dry conditions can be detrimental since it can result in small plants that are unable to produce high yields. Too much rain early in the season can delay planting, extend the vegetative growth stage or adversely affect seed germination. Soybean requires more water during the flowering, pod formation and seed fill stages in order to increase seed yield (Tewari *et al.,* 2015).

2.5 Cultivation

2.5.1 Planting time

Planting date is an important management practice that has an influence on soybean growth, development and yield (Zhang *et al.,* 2010). Soybean is a short-day plant and the optimal

planting date is therefore mainly determined by day length (photoperiod) and temperature (Kantolic and Slafer, 2007). Since planting dates differ between regions, it affects cultivar choices. Cultivars with a short growing season are generally planted in cool areas whilst cultivars with a longer growing season are planted in warm areas (De Beer, 2012). Early planting can increase the time available for vegetative and reproductive growth, which will lead to bigger plants resulting in higher seed yields (Chen and Wiatrak, 2010; Barnard, 2015a). However, planting too early can result in excessive vegetative growth causing plant lodging. Planting later than the optimal period will shorten the growing period. This will result in insufficient vegetative growth, a low pod height and lower yields per plant (DAFF, 2010; Barnard, 2015b). The recommended planting dates for optimum production in South Africa are provided in Table 2.2 (Anon, 2008; Liebenberg, 2012).

Table 2.2: Planting dates for optimal soybean production in different climatic regions in South Africa (Liebenberg, 2012).

Climatic conditions	**Warm areas**	**Moderate areas**	**Cool areas**
Region	Bushveld and lowveld conditions	North West and Northern Province, northern KwaZulu-Natal and northern Free State	Southern KwaZulu-Natal, eastern Free State, eastern Mpumalanga
Planting date	15 November to 30 December	1 November to 15 December	20 October to 30 November

2.5.2 Row spacing and plant density

Narrow inter-row spacings (18 to 50 cm) produce greater soybean yields than wide inter-row spacings (75 to 100 cm) (Beatty *et al.,* 1982; De Bruin and Pedersen, 2008; Liebenberg, 2012). The increase in yield can be ascribed to earlier canopy formation (which reduce evaporation and weed growth) and increased sunlight interception in narrow inter-row spacings. Narrow inter-row spacings are usually associated with higher yields provided that weeds are sufficiently controlled prior to canopy formation, the plants do not lodge and there is no prevalence of diseases in the dense canopy (Liebenberg, 2012).

Plant density is determined by the yield potential of an area. The higher the yield potential, the higher the plant density (Liebenberg, 2012). Plant density is an important agronomic factor that is used to optimise crop growth and maximise seed yields (Ribeiro *et al*., 2017). A low plant density can result in plants that are short, have many branches and pods that are close

to the soil surface which results in losses during harvest and therefore also yield loss (Liebenberg, 2012; Tanner and Hume, 2012). At low densities yield can also be lowered due to an insufficient number of plants, higher weed infestations and poor radiation use efficiency (Mohammadi *et al.,* 2015). High plant densities can result in tall plants with weak stalks that have a greater tendency to lodge, as well as plants with fewer branches to bear soybean pods (Weber *et al*., 1966; Lueschen and Hicks, 1977).

Gulluoglu *et al.* (2017) reported that plant and bottom pod height decreased significantly as the plant density decreased, due to less competition for sunlight among plants. It may have a negative impact on soybean yield because pods that are too close to the soil surface cannot be harvested. Soybean planted at higher densities produce higher seed yields, although the number of branches per plant and the number of pods per plant are less compared to plants planted at lower densities (Spader and Deschamps, 2015; Gulluoglu *et al.,* 2017). Liebenberg (2012) recommended low plant densities (fewer than 300 000 plants ha^{-1}) under dry climatic conditions, while higher plant densities are recommended for high rainfall areas in South Africa.

Soybean cultivars that have a tendency to lodge or that are inclined to produce more lateral branches when planted early in a growing season, is planted in lower densities (Barnard, 2015b). Soybean planted late in a growing season will have a shorter vegetative development period and will result in plants with fewer nodes and therefore also fewer pods. To compensate for the lower yield per plant, a higher plant density with a narrow inter-row spacing is needed. Several studies have shown that narrow rows and higher seeding rates can reduce yield loss from late plantings (Ball *et al*., 2001; Çalişkan *et al*., 2007; Gulluoglu *et al*., 2016).

2.5.3 Planting depth

The planting depth of soybean influence germination, emergence and growth (Aikins *et al.,* 2011). Soybean is generally planted at a depth of 2 cm in clay soils and 5 cm in sandy soils. Planting too shallow can result in desiccation of the seed, while planting too deep can expose the seedling to diseases and increase the risk of emergence failure due to formation of a soil crust (Liebenberg, 2012). Dehydration and soil crusting can be avoided in sandy soils by increasing the planting depth, but not deeper than 6.25 cm (Lawson *et al*., 2009; Liebenberg, 2012).

2.6 Cultivar selection

Cultivar selection is an important step in achieving maximum soybean yield. The yield potential of soybean cultivars varies between environments and specific cultivars are therefore available for the respective soybean-production areas in South Africa (De Beer, 2012).

Important aspects that should be considered when selecting a soybean cultivar include the growth habit, yield potential, length of growing season (maturity) and tolerance to herbicides. With the exception of yield, the length of the growing season (maturity) is the most important aspect in selecting a cultivar (Carvalho *et al*., 2017).

Soybean development is largely influenced by cultivar-specific day length requirements (photoperiodism) that initiate the development of its reproductive stages. Thus, the period of vegetative and reproductive growth is mainly controlled by the length of the light period soybean plants receive in a 24 hour period. As the number of hours of darkness increase, soybean plants will change from the vegetative growth phase to the reproductive phase (Camara *et al*., 1997). Since day length is a function of latitude, cultivars will mature later and have a longer growing season the further south it is planted in South Africa (De Beer, 2012).

Soybean cultivars can be classified into 13 maturity groups according to their response to daylight and general area of adaption. These groups are designated by roman numerals and range from “000” to “X”. Maturity groups (MG) “000” to “0” represent early maturing cultivars that are adapted to long days and short summers, while MG “VIII” to “X” represent late maturing cultivars that are adapted to shorter days and long summers (Alliprandini *et al.,* 2009). Soybean cultivars in South Africa are grouped into MG IV to VIII (Liebenberg, 2012), where cultivars belonging to MG IV and V are planted in cool production areas, cultivars in MG V-VII in moderate growing areas and cultivars belonging to MG VII-VIII under irrigation in the warmer areas (Anon, 2008; Jarvie, 2008).

2.7 Soybean yield in South Africa

South African soybean imports and exports increased. A further increase in local production is therefore needed to meet the growing local and export demand and subsequently reduce soybean imports over time (Mokone, 2017). Crop production can be increased by either increasing the productivity (yield per unit area) on existing farm land, or by increasing the area under cultivation. Increasing the productivity of existing crops would be the most preferred route to increase yield, because it circumvents greenhouse gas emissions, resource scarcities (e.g. land, water and nutrients) and degradation of existing ecosystems associated with bringing new land into production (Edgerton, 2009). Expanding the area cultivated with soybean could also come at the expense of yellow maize, as both these crops are grown mainly in the eastern parts of South Africa (Sihlobo, 2018a).

Soybean productivity in South Africa has increased over the past few decades to an average of 1.8 t ha^{-1}, but it is still not as high as the yields produced in the USA (3.3 t ha^{-1}), Brazil (3.1 t ha^{-1}) and Argentina (3 t ha^{-1}) (USDA, 2018). Although this can partly be ascribed to the

variability in rainfall, climate and soil fertility (Schultz, 2013), soybean yield can still be further increased by adopting improved agricultural practices that increase yield and decrease yield loss due to genetic barriers and environmental stresses.

The major stresses that affect the growth, development and productivity of crops are drought (water stress), chilling, heat and salinity stress (Bakhsh and Hussain, 2015). Long or short-term exposure to these stresses, especially during sensitive growth stages, can result in yield and quality losses (Shah *et al.,* 2017; Begcy and Dresselhaus, 2018).

Drought and heat stress often occur in combination under field conditions and magnify the harmful effects of each other (cross-synergism) (Prasad *et al.,* 2008). For soybean, drought stress can have adverse effects on the total biomass, number of pods, number of seeds, seed weight and seed yield per plant (Lafet and Ahmad, 2015). Reduction in seed yield and yield components is influenced by the growth stage at which the drought/heat stress occur, as soybean is most susceptible to water and heat stress during its reproductive growth stages (Kpoghomou *et al*., 1990; Oya *et al.,* 2004; Wei *et al.*, 2018). The reduction in seed weight and seed yield are the most severe when drought and heat stress occur at the beginning of seed filling (R5 growth stage) (Maleki *et al.,* 2013).

Cold stress due to chilling (0 - 15 °C) or freezing (<0 °C), is another environmental stress that can affect the productivity of crops (Bevilacqua *et al*., 2015). Soybean is regarded as a chilling sensitive crop and low temperatures can inhibit several aspects of growth, including cell division, photosynthesis, water transport, growth and yield (Hasanuzzaman *et al.,* 2016). Symptoms from cold stress during the reproductive growth of soybean include the inability of flowers to open, seedless pods developing at the top of the plant, deformed pods along the stem and abscission of reproductive structures that can result in yield loss (Gass *et al.,* 1996). Strauss and Van Heerden (2011) reported that soybean cultivation in high altitude regions in South Africa is limited due to low temperatures occurring at night. Night temperatures below 15 °C limit physiological processes, for example, photosynthesis (Jenabiyan *et al.,* 2015), while temperatures below 8 °C reduce pod formation (Van Heerden *et al.,* 2004).

As a moderately salt sensitive crop, soybean production is also affected by salt stress (Yasuta and Kokubun, 2014). Soil salinity is caused by Na^+ salts, particularly NaCl and occurs in many arid and semi-arid regions of the world, including South Africa (Meloni *et al.,* 2004; Materechera, 2011; El-sabagh *et al*., 2015). The accumulation of salts in the soil root environment increases the osmotic potential of soil and reduces the ability of a plant to extract water. Ionic stress is then induced in a plant which results in secondary oxidative stress (Khan *et al.,* 2007a). Several studies have indicated that soybean yield is adversely affected by high salt concentrations (Katerji *et al.,* 2003; Bustingorri and Lavado, 2011; El-sabagh *et al*., 2015).

A substantial reduction in soybean yield attributes, for example number of branches, number of pods, weight per plant and weight of 1000 seeds can occur under salt stress conditions (Bustingorri and Lavado, 2011; El-sabagh *et al*., 2015).

Yield potential can also be constrained by amongst others, pod shattering and lodging (Antwi-Boasiako, 2016). Lodging is a common production problem in high yielding soybean (Antwi-Boasiako, 2016). It can occur because of leggy weak stems and harsh environmental conditions including severe winds, heavy rains and hail (Chen *et al.,* 2011). High available soil nitrogen, abundant water and warm weather can also contribute to lodging. When soybean lodge, the transport of water, nutrients and photosynthetic assimilates are suppressed, which results in a decrease in the number of pods per plant, and smaller seeds per pod (Hicks, 2012). Further losses can occur due to the inability of soybean stems to pass through a combine (Weber and Fehr, 1966; Hicks, 2012). Bhor *et al.* (2014) reported yield loss caused by pod shattering to vary from negligible to significant levels in the range of 34 to 99%. Mature soybean pods are sensitive to shattering, especially under conditions of low humidity, high temperatures, and dry weather conditions followed by wet conditions. Mechanical damage due to wind or harvesting equipment during dry weather conditions can also result in further yield loss (Bhor *et al*., 2014).

Crop productivity is influenced by environmental conditions and stresses. Available biotechnologies, in particular plant growth regulators (PGRs), can be used to increase the tolerance of a soybean plant to environmental stresses and to increase harvestable yield as well as to enhance soybean growth and yield components (Hardy, 2013; Marimuthu and Surendran, 2015; Rademacher, 2015). Application of PGRs is an effective management tool to increase yield and overcome environmental stresses (Khatun *et al.,* 2016b; Tesfahun, 2018).

2.8 Plant growth regulators

Plant hormones are organic substances (signal molecules) that occur naturally within a plant and regulate processes such a growth, differentiation and development (Mir *et al*., 2010). When plant hormones are synthesised chemically, they are termed a PGR (Kaya *et al.,* 2009). Plant growth regulators can be either natural or synthetic compounds and are applied in low concentrations to mimic or affect production of plant hormones in order to promote, inhibit or modify plant growth and development (Mir *et al.,* 2010; Sharma, 2015). Processes such as flowering, fruit formation, ripening, fruit drop, defoliation, or quality traits can also be affected. The term “plant bioregulator” is therefore also often used (Rademacher, 2015).

The value of PGRs in agriculture was first recognised in the 1930s. Since then, PGRs have been used in agriculture and horticulture as a tool to increase production and aid in removing or reducing genetic and environmental barriers (Rademacher, 2015). Plant growth regulators act by influencing the source-sink relationship as well as the translocation of photo-assimilates in plants (Khan *et al.,* 2007b; Amanullah *et al*., 2010). They also interact with important metabolic processes including nucleic acid metabolism and protein synthesis (Sabale *et al*., 2017). Depending on the type or combination of PGRs used, different physiological processes, namely plant metabolism, cell division, cell enlargement, rate of growth, nutrient mobility, abscission, flowering, fruit set and development can be regulated (Rajala, 2004).

The major classes of plant hormones or PGRs are auxins, cytokinins, abscisic acid, gibberellins (GAs) and ethylene. Plant hormones act either simultaneously, or in opposition to each other at various stages of the life cycle to control physiological growth and development. The final biological condition of a plant is therefore, a reflection of the combined interplay of different hormones (Gana, 2011; Wang and Irving, 2011).

2.9 Major classes of plant growth regulators

2.9.1 Auxins

The path to discovering auxins started in the 1880's with Charles Darwin that noted that plants bent towards sunlight. In the book "*The Power of Movement in Plants",* Darwin concluded that when seedlings are illuminated from the side, the tip of a coleoptile is able to perceive phototrophic signals and that "some influence is transmitted from the upper to the lower part, causing the latter to bend". Darwin's observations inspired experiments resulting in the discovery of the first plant hormone, auxin, in 1926 by Fritz Went (Hopkins, 2004). The active compound was determined in the mid-1930 as indole-3-acetic acid (IAA), the principal auxin in plants (Hopkins, 2004; Laxmi *et al*., 2013). In addition to IAA, three other isolates with similar structures and activity occur in plants, namely: indole-3-butyric acid (IBA), 4-chloroindole-3-acetic acid (4-CI-IAA) and Phenylacetic acid (PAA) (Sauer *et al*., 2013). Auxins has a principal role in regulating plant growth through cell enlargement, but are also involved in several other developmental responses, namely cell expansion and division, cell elongation and differentiation, root initiation, phototropism, gravitropism, apical dominance, vascular differentiation and forming an abscission layer in fruit and leaves (Hopkins, 2004; Takatsuka and Umeda, 2014; Aloni *et al.,* 2006; Gana, 2011).

Produced in young leaves, developing seeds and root tips, IAA can be biosynthesised through two mechanisms namely the tryptophan dependent and tryptophan independent pathways. Tryptophan is the main precursor of the production of auxins in plants (Overvoorde *et al.,* 2010; Zhao, 2010; Mashiguchi *et al*., 2011; Ursache *et al*., 2014; Cook and Ross, 2016).

Although the tryptophan independent pathway has been proposed for auxin synthesis, a genetic basis for this pathway still needs to be defined (Mashiguchi *et al*., 2011). In the tryptophan dependent pathway, IAA is synthesised in three steps. Firstly, the oxidative transamination of tryptophan to indole-3-pyruvic acid (IPA), followed by the decarboxylation of IPA to indole-3-acetaldehyde (IAAld) via IPA decarboxylase and oxidation to IAA through IAAld dehydrogenase (Hopkins, 2004; Sardar and Kempken, 2018). Auxin biosynthesis takes place at a site different from the site of action.

Auxins are transported from the site of synthesis to the site of action through two distinct pathways (Friml and Palme, 2002; Michniewicz *et al.,* 2007; Petrášek and Friml, 2009). The predominant mechanism of transport occurs via a polar gradient from the shoot meristem and young leaves, downward to the root tips. This pathway is slow, regulated and cell-to-cell that mediates short range auxin movement in different tissues. Occurring at the same time, auxins is also translocated in the phloem from photosynthetic sources (leaves) to photosynthetic sinks (fruit, roots and meristematic regions) in a non-directional way. Both these pathways are essential for proper plant development (Friml and Palme, 2002; Michniewicz *et al.,* 2007; Petrášek and Friml, 2009).

There are two categories of auxins within plants, namely free and bound (Goss, 2013; Sharma, 2015). Free auxins such as IAA, are forms that after being diffused out of tissue are immediately available to regulate physiological processes in plants. Bound auxins are not freely available but become available after being subjected to hydrolysis, enzymolysis or autolysis. Bound auxins are generally considered as storage and detoxification reserves from which IAA can be released (Vivanco and Flores, 2000; Arteca, 2013). In contrast to the formation of auxins, deactivation occur through two pathways. One being the conjugation of sugars, alcohols and amino acids, and the second by complete oxidation of IAA (De Klerk, 2002).

In addition to natural occurring auxins, synthetic auxins have been developed to mimic the physiological responses of natural auxins (Sauer *et al*., 2013). Examples of the commonly known synthetic auxin analogues used in the horticultural and field crops are 1-naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 3,6-dichloro-2-methoxybenzoic acid (dicamba) and 4-amino-3,5,6-trichloropicolinic acid (tordon or picloram) (Sauer *et al*., 2013). Unlike natural auxins, the metabolic turnover of synthetic auxins is slower and therefore more stable (Sauer *et al*., 2013).

2.9.2 Effect of auxins on crop growth and yield

Auxin applied to crops influences a diverse range of growth and development processes. Indole-acetic acid exogenously applied to okra (*Abelmoschus esculentus*) (Malvaceae) (Khandaker *et al*., 2018), cowpea (*Vigna unguiculate*) (Fabaceae) (El-Saeid *et al*., 2010), faba bean (*Vicia faba*) (Fabaceae) (Ibrahim *et al*., 2007) and mung bean (*Vigna radiata*) (Fabaceae) (Rumman Shafi Quaderi *et al*., 2006) increased the height of plants, number of leaves and flowers per plant as well as yield.

In crops, dry matter production is determined by the source-sink relationship, where the source are assimilate-producing plant parts such as leaves and the sink, the plant parts that has the potential to store or metabolise assimilates, such as seed and fruits. Source-sink relationships are important for determining crop yields, as a small sink or source capacity will limit higher yields (Fageria *et al*., 2006; Bijanzadeh and Emam, 2010). The interaction between photo-assimilate and PGR levels determines the fate of individual yield components and source sink manipulations (Aminullah *et al.,* 2000). Plant growth regulators can influence the source-sink relationship by acting either on the source, sink or the transport system between the two. When a PGR stimulate metabolic activity in the sink it will improve its utilisation and thus stimulate source activity. Auxins have been shown to regulate photo-assimilate partitioning in reproductive organs and are therefore an effective method to increase seed yield (Aminullah *et al.,* 2000).

Aslam *et al*. (2010) reported the importance of the time of NAA application to chickpea (*Cicer arietinum*) (Fabaceae). They succeeded to increase the number of pods per plant by 11.5%, 100 seed weight by 9.5% and crop growth rate by 5%. All these contributed to an increase in biological yield by 2.87%, seed yield by 17.17% and harvest index by 11.07%, compared to the untreated control plants. In China, a NAA application to soybean cultivars (Nannong 99-6 and Kefeng 1) before exposure to drought stress for 10 days, improved the drought tolerance which resulted in higher plant biomass and yield. This was attributed to an increase in the ability to antioxidise and the decreased lipid peroxidation triggered by NAA (Xing *et al*., 2016). Total chlorophyll content, soluble protein, nitrate reductase and therefore pod yield in soybean were also improved by an application of 40 ppm NAA (Senthil *et al*., 2003). Mouhib (1981) reported that application of NAA (0.033 kg ha^{-1}) at growth stage R2, significantly decreased lodging and can be due to the large and more rigid stems caused by the hyperauxin effect on growth.

The dehiscence (shattering) of pods, prior to harvest causes yield losses in soybean. According to Chauvaux *et al.* (1997) pods shatter at maturity due to the loss of cellular cohesion in the dehiscence zone (layer of cells sealing the separation layer). It can be

attributed to elevated ethylene production that causes enzymatic breakdown of the middle lamella. Auxins control the enzymatic activity involved in cell separation in the dehiscence zone of soybean (Chauvaux *et al*., 1997). Decreased auxin content in the valve margins correlates with an increase in β-1,4- glucanase activity, which triggers cell wall degradation (Ogutcen *et al*., 2018). The activity of β -1,4-glucanase in oilseed pods treated with an auxin growth regulator, 4-CPA can delay pod shattering by 10 days (Chauvaux *et al*., 1997).

2.9.3 Gibberellins

During the late 19th and early 20th centuries, Japanese farmers noted that certain rice seedlings in their fields were growing excessively tall and spindly, causing them to lodge and produce little to no seed. They named this disease “bakane” meaning, foolish seedling disease (Hopkins, 2004). Japanese plant pathologists discovered that the disease was caused by a fungus *Gibberella fujikoroi* that secreted gibberellin A (Vivanco and Flores, 2000; Hopkins, 2004). When scientist applied gibberellic acid (GA) to the roots of rice seedlings, it was found to stimulate plant growth (Vivanco and Flores, 2000). Today more than 135 forms of GAs have been identified, but only a few are biologically active, whereas the rest are either intermediates or products of inactivation (Hopkins, 2004).

All GAs are diterpenes, consisting of four isoprenoid subunits with either 19 or 20 carbon atoms. Naturally occurring GAs are abbreviated by an “A” number, which is assigned in order of its discovery (Hopkins, 2004; Hedden and Thomas, 2012). The most biologically active forms of GAs are GA_1, GA_3, GA_4 and GA_7, of which GA_3 (gibberellic acid) is the most common commercially available form (Hedden and Phillips, 2000; Sun, 2008).

In the biosynthesis of GAs, melavonic acid is firstly converted to the gibberellic acid precursor molecule, geranylgeranyl diphosphate (GGPP). This molecule is then converted in a two-step cyclization reaction to copalyl diphosphate (CPP) and then *ent*-kaurene through the enzymes *ent*-copalyl diphosphate synthase (CPS) and *ent*-kaurene synthase (KS), respectively. *Ent*-kaurene are then transferred from the plastids to the endoplasmic reticulum where it is catalysed by *ent*-kaurene oxidase (KO) and *ent*-kaurenoic acid oxidase (KAO) to produce GA_{12}-aldehyde. As the precursor to all other GAs, GA_{12} can be further converted to intermediate and bioactive Ga’s (Hedden and Proebsting, 1999; Sun, 2008).

Gibberellins are mainly produced in growing meristematic tissues such as developing seeds, fruits, shoots and the apical regions of roots. In contrast to auxins, GAs are transported through non-polar pathways (phloem, xylem or cell to cell), from its site of synthesis to the sink (Hopkins, 2004). The primary hormonal function of GAs in plants is to control plant height by enhancing cell elongation and, cell division. It also plays a role in processes such as root

growth, stem elongation, leaf expansion, floral induction and fruit and seed development (Gao *et al*., 2017).

From the 136 different GA molecules discovered, only gibberellic acid (GA_3) and to a lesser extent GA_4 and GA_7 are commercially used (Petracek *et al*., 2003; Gao *et al.,* 2017). At present, GAs are used in viticulture, horticulture and agriculture. Examples are to increase the size of grapes or the production of seedless grapes, to improve citrus fruit quality by delaying rind senescence, to increase the growth and yield of sugarcane and to increase crop yields in pears and apples (Brueckner *et al*., 2012). GAs are also used in berry thinning of grapes and in accelerating seed germination (Rademacher, 2015).

2.9.4 Effect of gibberellins on crop growth and yield

Gibberellins are the most widely commercially used group of hormones (Noor *et al*., 2017). The role and influence of GAs on different crops have been extensively studied and reported to influence a variety of plant responses (Gustafson, 1960; Rood, 1985; Kumar *et al*., 2001; Shah and Ahmad, 2006; Emongor, 2007; Naghashzadeh *et al.*, 2009; Dheeba *et al*., 2015; Lien *et al*., 2016; Jaques *et al.,* 2019). Seed germination and seedling growth are considered to be critical in order to produce a successful crop (Bora and Sarma, 2006). To overcome seed dormancy and improve seed germination and emergence, crop seeds are often primed with GAs to activate embryo growth, mobilise reserves and to weaken the endosperm layer (Ma *et al*., 2018). The yield contributing factors of field planted soybean, namely the number of pods per plant, number of seeds per pod, seed weight, seed yield and biological yield were reported to be increased by GA_3 seed priming prior to planting (Agawane and Parhe, 2015). Priming of chickpea seeds with GA_3, also resulted in more pods, higher seed yield per plant and protein content (Mazid, 2014).

Since cell division, enlargement and elongation were reported to be stimulated by GA_3 (Metraux, 1987; Sauter and Kende, 1992; Beall *et al.,* 1996; De Souza and MacAdam, 2001; Bultynck and Lambers, 2004), foliar application of GA_3 improve growth, yield and yield components of many plants (Beltrano *et al.,* 1994; Batlang *et al.,* 2006; Ghodrat *et al*., 2012). Yield is enhanced by GA_3 through improved utilisation of photosynthates and metabolic components (Khan *et al*. 2002).

The length of the first two internodes of soybean was reported by Mislevy *et al*. (1989) to increase with early application of GA_3 when the hypocotyl cracks the soil, but the total plant height at maturity remains similar to that of untreated plants. Harvestable seed yield is not affected, but the stem elongates causing a greater portion of seeds to be produced above 80 mm, which is important to increase accessibility for commercial combining. The length of

lateral branches, number of pods per plant, seeds per pod, 1000 seed weight, economic seed yield, biological yield and harvest index of different soybean genotypes can all be increased with an application of GA_3 at growth stage V3-V4 (Azizi *et al*., 2012).

GAs have vital roles in plant responses to environmental stress conditions (Wigoda *et al*., 2006; Ko *et al.,* 2007). Crops that are grown in saline soil conditions are exposed to high dissolved salts concentrations that can inhibit growth and development paramaters such as leaf area and length, as well as root and shoot dry weight (Hamada and Al-Hakimi, 2002). These paramaters are reduced due to a reduction in the formation of carbohydrates, caused by a lack of activity of ribose 1, 5-biphosphate carboxylase (El-Shihaby *et al.,* 2002). Hormones such as GA_3 have been reported to improve the antioxidant capasity of plants and induced plant stress responses (Lien *et al*., 2016).

Drought stress is known for inhibiting growth and development of plants. In cotton (*Gossypium hirsutum*) (Malvaceae), GA_3 reverse these effects, and increases the net photosynthetic rate, stomatal conductance and transpiration rate (Kumar *et al*., 2001). The yield of groundnut (*Arachis hypogaea*) (Fabaceae) was also reported to increase in both wet and dry seasons by foliar applied GA_3 (Yakubu *et al.,* 2013).

2.9.5 Cytokinins

Cytokinins are a class of plant growth hormones with the primary function to promote cell division and cell differentiation. The term is derived from the words “cyto”, meaning cell and “kinin”, meaning division. Naturally occurring cytokinins are all adenine derivatives with either an isoprene-related side chain (isoprenoid cytokinins) or an aromatic side chain (aromatic cytokinins) (Mok and Mok, 2001; Giron *et al*., 2013). In the 1910’s, G. Haberlandt demonstrated that phloem sap from different plants has the ability to cause non-dividing, parenchymatous potato tuber tissue to revert to an actively dividing meristematic state (Haberlandt, 1913; Hopkins, 2004). Although Hamberlandt discovered cytokinins, it was F. Skoog, a professor at the University of Wisconsin that identified the active material from autoclaved herring sperm DNA as cytokinins in the 1950’s (Miller *et al*., 1955; Hopkins, 2004; Feng *et al*., 2017). Since then, several other compounds with cytokinin activities were isolated from various plant species (Feng *et al*., 2017). As a key growth-promoting phytohormone, cytokinin are involved in regulating various processes in plant growth and development (Mok and Mok, 2001), including embryo development, seed germination, transduction of nutritional signals, vascular development, shoot apical meristem development, photomorphogenesis, leaf senescence and floral development (Wang and Irving, 2011; Jameson and Song, 2015; Osugi and Sakakibara, 2015; Feng *et al*., 2017).

A major site of cytokinin biosynthesis in higher plants is the roots. High cytokinin levels are usually found in the active root tips and xylem sap of roots as well as in other meristematic regions such as young leaves, developing fruits and seeds (Hopkins, 2004; Osugi and Sakakibara, 2015). It is generally concluded that roots are the principal source of cytokinins in plants and that they are transported from the roots to the shoots through the xylem. Cytokinins in plants are mainly produced through the de novo pathway. At first isopentyl pyrophosphate and adenosine monophosphate (AMP) is converted into isopentenyl AMP, through the enzyme adenosine phosphate-isopentenyl transferase (IPT). The product is isopentenyladenosine-5-monophosphate (iPRMP) which is then transformed through a series of metabolic steps to eventually produce active cytokinins (Zazimalova and Kaminek, 1999; Hopkins, 2004).

Cytokinins are present in plant cells in either free or conjugated forms. Conjugated cytokinins can be generated through various ways, such as glucosides or alanine conjugates. The biological activity of glucoside conjugates is to act as a storage form and to assist in transport of certain cytokinins, whereas alanine conjugates assist in the detoxification mechanisms of plants. Conversely, free cytokinins are characterised by isopentenyl adenine and zeatin, which are the most common and naturally occurring cytokinins in plants (Vivanco and Flores, 2000). Commercially available cytokinins containing agrochemicals are used in agriculture to enhance growth, stimulate germination, lateral branching and the release of buds from apical dominance and to improve yield. They also play important roles in responses to biotic and abiotic stresses, external factors such as light reception in the shoots and the availability of nutrients and water to the roots (Stirk and Van Staden, 2010; Koprna *et al*. 2016). Some of the commonly known commercial cytokinins used are synthetic phenylurea type cytokinins (e.g. forchlorfenuron, diphenylurea and thidiazuron), and adenine compounds (e.g. benzyladenine and kinetin) (Karanov *et al*., 1992; Mok and Mok, 2001).

2.9.6 Effect of cytokinins on crop growth and yield

An essential factor that determine soybean seed yield is the number of pods per plant (Yashima *et al*., 2005). The number of flowers that give rise to pods is an important agronomic trait that influence soybean yield (Zhang *et al*., 2010). Although soybean produces an abundance of flowers, a large number are aborted naturally during development (Nonokawa *et al.,* 2007). Rates of flower and pod abscission could be as high as 75% under normal growing conditions (Wiebold *et al*., 1981; Peterson *et al*., 1990). Thus, by reducing the abortion of flowers and pods, it might be possible to increase the yield (Nonokawa *et al.,* 2007). The formation and abortion of reproductive organs are influenced by, amongst others, phytohormones, specifically a decrease of cytokinin in the flowers (Yashima *et al*., 2005).

Therefore, the application of growth regulators could reduce the rate of flower and pod abortion, increase their sink strength and stimulate their development (Nagel *et al.,* 2001; Nonokawa *et al.,* 2007).

The rate of flower abscission varies with the position on the plant. Higher rates of abscission occur on the branches, bottom part of the main stem and in the top nodes of the main stem of soybean (Kokubun, 2011). With regard to soybean racemes, distal flowers on the rachis are more likely to abscise than proximal flowers. These abscissions correlate with the lower levels of cytokinin during the reproductive stages and the proximity of the flowers on the plant (Kokubun and Honda, 2000).

2.9.7 **Abscisic acid**

Plant material frequently contains substances that interfere with the response of auxin and acts as inhibitors of growth (Hopkins, 2004). Shoots and seeds of some plants become dormant in the winter and senescing leaves and mature fruits abscise. Based on these observations, extracts from several plant parts were tested and found to contain an inhibitory substance, inhibitor β (Bennet-Clark and Kefford, 1953). Meanwhile other scientists, through fractionation and bioassays discovered similar inhibitory substances. In 1963 “abscissin II” were discovered from fruits and leaves of cotton (Ohkuma *et al.,* 1963) and in 1965, “dormin” was discovered from leaves of sycamore trees (Cornforth *et al*., 1966). Because all three substances proved to be chemically identical it was renamed to abscisic acid (ABA) (Srivastava, 2002; Hopkins, 2004).

Unlike auxins, GAs and cytokinins, ABA is represented by a single 15-carbon sesquiterpene that originates from isoprene known as IPP (Srivastava, 2002; Hopkins, 2004). Like other plant hormones, ABA controls various physiological processes in plants and is best known for protecting plants against abiotic stresses (Hopkins, 2004; Sah *et al.,* 2016; Vishwakarma *et al.*, 2017). Abscisic acid acts as an inhibitory chemical compound that influence developmental processes, such as bud growth, seed and bud dormancy, senescence, initiation of secondary roots, germination and maturation. By inducing bud and seed dormancy, ABA prevents premature growth or germination during unfavourable environmental conditions (Hopkins, 2004; Gana, 2011). Under stress conditions, especially stresses associated with dehydration (salinity, cold, drought), ABA is diffused from chloroplasts into the cytoplasm of mesophyll cells, and from the mesophyll cells into the guard cells where it inhibits the influx of potassium ions and efflux of hydrogen ions into the guard cells (Yamburenko *et al*., 2013). This causes guard cells to lose their turgidity and partially close their stomata, thereby preventing water loss by transpiration (Luan, 2002).

Abscisic acid is synthesised through a direct or indirect pathway. The direct pathway only occurs in pathogenic fungi where IPP is synthesised from the mevalonate pathway, while the indirect pathway occurs in plants and uses the methylerythritol phosphate (MEP) pathway as a source of IPP (Srivastava, 2002). The indirect pathways begin in the chloroplast where carotenoid pigments are produced. The carotenoid violaxanthin is cleaved by the enzyme nine-cis-expoycarotenoid dioxygenase (NCED) and produces a 15-carbon product, xanthoxin and a 25-carbon "by product". Thereafter xanthoxin is converted to abscisic aldehyde (via an alcohol dehydrogenase), which is then oxidised to ABA through abscisic aldehyde oxidase (Hopkins, 2004). Abscisic acid is mainly synthesised in mature leaves, stems, developing seeds and fruits and roots and are transported in both xylem and phloem tissues (Srivastava, 2002). Unlike auxins, ABA does not exhibit any polarity, and can therefore move freely through the plant (Peter, 2009).

Abscisic acid occurs in different structural forms. The naturally occurring enantiomorph is (S)-ABA, and is optically active, having one centre of asymmetry at C-1. Synthetic ABA on the other hand, is a racemic mixture of (S)-and (R)-enantiomers. The synthetic (R)-ABA accounts for half of the racemic mixtures of ABA and has the same biological activity to that of natural (S)- ABA (Naqvi, 2001). Commercially ABA is used to slow plant growth prior to transplanting, reduce stress from transplanting, increase stress tolerance, improve crop establishment, slow crop growth and improve crop quality (Racsko *et al*., 2014).

2.9.8 Effect of abscisic acid on crop growth and crop yield

Plants are commonly exposed to various environmental stresses. ABA is the main hormone involved in response signalling when plants are under stress. ABA concentrations vary according to the stressful conditions (Vishwakarma *et al.*, 2017), with synthesis and concentration of ABA which change in plant tissues (Hu *et al*., 2016). Abscisic acid acts as a mediator in plant responses during periods of stress (Christmann *et al*., 2006; Ramachandran *et al.,* 2017), since it was found that the ABA in leaves increased under water stress conditions but, after rehydration, the ABA concentration in leaves reduced to control values. These results are in accordance with other studies that reported declining ABA levels following relief of water stress (Pierce and Raschke, 1981; Henson *et al*., 1984).

Abscisic acid also plays a role in growth promotion and development in plants (Finkelstein and Rock, 2002; Liu *et al*., 2006). Travaglia *et al*. (2009) reported a foliar application of ABA at the V7 and R2 phenological stages of soybean to increase dry matter accumulation and favour vegetative growth. Water stressed wheat plants also produced higher shoot biomass after treatment with ABA (Travaglia *et al*, 2007). Similar results were reported under controlled growing conditions, where an increase in ABA levels (through exogenous application) resulted

in better growth than plants with lower (endogenous) levels of ABA (Sansberro *et al.,* 2004; Finkelstein and Rock, 2002). Abscisic acid therefore plays an important role in regulating cell and whole-plant water status, which can be due to a higher water potential and greater turgor, enabling optimal cellular expansion (Munns and Sharp, 1993; Sansberro *et al.,* 2004; Ramachandran *et al.,* 2017). Exogenous ABA application also improves the photosynthetic activity of soybean (Travaglia *et al*., 2009) and wheat (Travaglia *et al*., 2010), since total dry matter content and chlorophyll levels are increased. Abscisic acid, therefore, also plays a role in protecting plants against photo-inhibition and early senescence under conditions of stress, allowing the plants to produce more dry matter and higher chlorophyll levels (Yordanov, 1995; Travaglia *et al.,* 2009).

Moreover, it is well known that closure of stomata is also regulated by ABA through altering of ion fluxes in guard cells. During periods of drought stress the closure of stomata has been reported to reduce shoot growth (Sauter *et al*., 2001), while regulating the balance between transpirational water loss, CO_2 uptake and assimilation (Travaglia *et al.,* 2010; Pirasteh-Anosheh *et al*., 2016). Treatment of crops with ABA before or during periods of drought have been shown to maintain ostiolar aperture and therefore maintain a higher leaf conductance (Reinoso *et al.,* 2011). Foliar applied ABA to holley, *Ilex paraguariensis* (Aquifoliaceae) under different photosynthetic photon flux densities reduced diurnal water stress and improve growth and dry matter content in stems and leaves (Sansberro *et al.* 2004). Plants require an appropriate level of ABA to grow under stress conditions (Travaglia *et al.,* 2010), but an abrupt increase in ABA concentrations can lead to a reduced growth rate (Egamberdieva *et al*., 2017).

Several studies have reported the contribution of ABA in enhancing the source-sink activity, which usually correlates with an increase in yield (Yang *et al.,* 2003; Travaglia *et al.,* 2007; 2010). Foliar application of S-ABA to soybean under water stress conditions during the flowering and pod filling stages, increases seed yield, number of seeds and pods under both optimum and mild water stress conditions (Kamal *et al.,* 1998). However, under severe water stress conditions, S-ABA was not effective in increasing yield (Kamal *et al.,* 1998).

2.10 References

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Chapter 3

Application time and rate of selected plant growth regulators on soybean yield parameters

3.1 Abstract

The effect of three Plant growth regulators (PGRs) [A2019 forchlorfenuron (CPPU), B2019 naphthalene acetic acid (NAA) and C2019 gibberellic acid + abscisic acid (GA3 + S-ABA)] applied at different times (R1, R3 and R1 + R3) and rates were evaluated on soybean (*Glycine max* (*L.*) Merr) in this study. Application time trials were conducted in the field at three localities *viz.* Potchefstroom, Bethlehem and Kroonstad. To evaluate the application rate of PGRs, field trials and a pot trial were conducted at Potchefstroom. A soybean cultivar with an indeterminate growth habit (PAN 1521R) were used for the field trials, while a determinate growth habit (LS 6161 R) was used for the pot trial. The experimental design for all the field trials was a complete randomized block, with 60 trial plots. Plant growth regulators were applied at two different growth stages (R1 and R3). To determine the optimal time, applications were done separately at R1 and R3, as well as at both R1 + R3. Three concentrations of each PGR (low, medium and high) were applied at growth stages R1 and R3 to determine the optimal rate of application.

No optimum time of application of A2019, B2019 and C2019 on plant growth and yield parameters were found in this study. Application of PGRs to soybean planted in Bethlehem (cold zone) were more effective in terms of nodes plant^{-1}, pods node^{-1} and pods plant^{-1} than PGR applications at Potchefstroom and Kroonstad. Plant growth regulator treatments increased growth parameters of soybean, however these effects varied between Potchefstroom, Bethlehem and Kroonstad and were not consistent. No increase in 1000 seed mass and yield was caused by the PGRs applied at different times in the field trials at Potchefstroom, Bethlehem and Kroonstad. Plant growth regulators applied at different application rates in the field and pot trial at Potchefstroom did not significantly increase the height of the lowest pods above soil surface, seed pod^{-1}, nodes plant^{-1}, pods node^{-1} pods plant^{-1}, 1000 seed mass and yield.

The variable response of soybean to different application times and rates could be ascribed to low application rates used as well as the warm and dry climatic conditions during the 2018/19 growing season. Therefore, no general recommendations can be made and further research is needed to evaluate PGR application on soybean growth and yield parameters under South African growing conditions.

3.2 Introduction

The main objective of any crop production system is to increase the yield per unit area according to the availability of light, water and nutrients on each portion of a field (Reinoso *et al*., 2011). For soybean (*Glycine max* (L.) Merrill.), the number of seed per unit area and seed weight are the two main components determining yield (Van Roekel and Purcell, 2016). The number of seed per plant is a difficult component to estimate since it is influenced by the number of nodes per plant and number of fertile flowers per node which determine the number of pods (with seed) per node (Kobraei *et al*., 2011; Basuchaudhuri, 2016a; Egli, 2017). These factors are determined during the critical period which begins around flowering and extends through pod set, including the beginning of seed-filling (R1-R6) (Gutierrez Boem *et al*., 2007; Kantolic and Slafer, 2007). The post-flowering phases of soybean (pod formation, development and filling) are influenced by the availability of assimilates and the balance of endogenous plant growth regulators (Basuchaudhuri, 2016a). This is also the period that crops are the most sensitive to environmental stresses such as drought and extreme temperatures, as the strength of both source and sink is affected (Saini and Westgate, 1999; Basuchaudhuri, 2016a; Senapati *et al.,* 2018). Response to stress conditions changes the hormonal balance in plants and alters the concentration of phytohormones participating in growth processes (auxins, cytokinins, gibberellins) as well as increase the concentration of hormones that are involved in the control of stress responses (ABA, ethylene, jasmonic and salicylic acid). Plant growth is consequently decreased and crop productivity limited (Shakirova *et al.,* 2012; Wani *et al.,* 2016; Egamberdieva *et al*., 2017).

The use of Plant growth regulators (PGRs) to enhance crop productivity has become of great interest in agriculture and horticulture (Rodriguez-Furlán *et al*., 2016). Plant growth regulators should be able to increase yield or crop quality to have value in the agricultural industry (Harms and Oplinger, 1988). Application of PGRs improves the source-sink relationship and enhance effective partitioning of accumulates, which contributes to effective flower formation as well as fruit and seed development (Marimuthu and Surendran, 2015; Basuchaudhuri, 2016a). The effectiveness of PGRs are influenced by environmental conditions, the plant growth stage during application, the rate of application, method of application (seed priming, drenching, pre-plant dipping, foliar application), cultivar planted, and the physiological and nutritional status of the plant (Shakirova *et al.,* 2012; Sajjad *et al.,* 2017).

Studies were conducted on the effect of PGRs applied to soybean (Khatun *et al.,* 2016b; Solanke *et al*., 2018), groundnut (*Arachis hypogaea*) (Fabaceae) (Faldu *et al.,* 2018), maize (*Zea mays*) (Gramineae) (Shekoofa and Emam, 2008; Ghodrat *et al.,* 2012) and pigeon pea (*Cajanus cajan*) (Fabaceae) (Sumathi *et al*., 2018). These studies indicated PGR application during the critical period of crop development to enhance the sink size in plants and to cause

an increase in yield. However, PGRs have a relatively narrow concentration range that are suitable to attain a desired effect. Too high application rates can inhibit growth or injure the crop while very low rates may have no effect (Ikiba, 2017). Studies investigating the role of PGR applications at different growth stages and different application rates on soybean are limited (Khatun *et al.,* 2016b; Solanke *et al*., 2018).

The aims of this study were therefore to determine the optimum application time and rate of selected PGRs to maximize yield parameters of dryland soybean.

3.3 Material and methods

Plant growth regulators (treatments) were assigned an identification code, *viz*. A2019, B2019, and C2019. PGR A2019 is a cytokinin-based product consisting of forchlorfenuron (CPPU). PGR B2019 is an auxin-based product consisting of NAA and PGR C2019 is a combination of GA_3 and S-ABA. Five field trials were conducted to determine the optimum time of PGR application, while two field trials and one pot trial were conducted for evaluation of different PGR application rates to soybean. A non-ionic wetting agent containing the active ingredient, isotridecanol (alkylpolyethylene glycol ether), was added to all PGR treatments at a rate of 6 ml $100L^{-1}$. A 16 L knapsack sprayer fitted with a Hypro f110-04 flat fan nozzle was used to apply the treatments at a spray volume of 270 L ha^{-1}. Control treatments consisted of water and the wetting agent only.

3.3.1 Effect of plant growth regulators applied at different plant growth stages on plant growth and yield parameters

Field trials were conducted under dryland conditions during the 2018/19 growing season at three localities, *viz*. Bethlehem (28°09'47.3"S 28°18'25.1"E) and Kroonstad (27°36'41.7"S 27°13'22.6"E) in the Free State province, and at the ARC-Grain Crops, Potchefstroom (26°44'04.2"S 27°04'41.1"E), North-West province. At Potchefstroom (Figure 3.1a) and Bethlehem (Figure 3.1b) two trials were planted at different planting dates, while only one trial was planted at Kroonstad (Figure 3.1c) (Table 3.1). The three localities represented a cold (Bethlehem), and two temperate zones (Kroonstad and Potchefstroom). The respective planting and harvest dates, as well as agronomic practices applied at each locality are provided in Table 3.1.

Before planting commenced each field was ploughed once. Seedbed preparations were done with a disc harrow, and the surface crust of the soil was broken with a cultivator. At each locality soil samples were taken at random from different sampling points and soil analyses were done. The results obtained were used as a guide for rates of superphosphate and limestone ammonium nitrate (LAN) applied (Table 3.1). Weeds were controlled with pre- and post-emergence herbicides (Table 3.1). Due to early season drought, the field trials at

Potchefstroom and Bethlehem received supplementary irrigation during the four weeks after planting to ensure successful germination and growth. No supplementary irrigation was available and provided at the Kroonstad trial site. To prevent damage to emerging seedlings by pigeons and guinea fowls at Potchefstroom and Bethlehem, field trials were covered with frost cover for the first three weeks after planting (Figure 3.2).

Figure 3.1: Soybean field trials planted at a) Potchefstroom, b) Bethlehem and c) Kroonstad to determine the optimum time for plant growth regulator application.

Figure 3.2: Trials covered with frost cover to prevent damage to seedlings by birds.

The soybean cultivar, PAN 1521R was planted in all the trials. This cultivar has an indeterminate growth habit and continues to grow vegetatively throughout the flowering and pod set period. Selection of this cultivar was based on its performance [yield (t ha^{-1})], and yield potential in cold, moderate and warm climate areas in South Africa according to the National soybean cultivar recommendations (De Beer and Bronkhorst, 2018). The recommendations indicated this cultivar to perform well in all three climatic zones.

The experimental design for all the field trials was a complete randomized block, with 60 trial plots. The treatment design contained four treatments (three PGR products + control) and three application times at plant growth stage R1, R3 and R1 + R3 (double application). All applications for the three PGRs, at the three application times were replicated six times, while the control treatment was replicated six times only. Each trial plot consisted of four rows of 5 m in length, with an inter-row spacing of 0.75 m and intra-row spacing of 4 cm. At planting, seeds were inoculated with *Bradyrhizobium japonicum* bacteria.

The soil form (Avalon) and properties at Bethlehem and Kroonstad were similar, with pH 6.49, 15% clay, 78% sand, 7% silt at Bethlehem and pH 6.85, 16% clay, 79% sand, 7% silt at Kroonstad. At Potchefstroom the soil type was Hutton, with pH 7.02, 34% clay, 53% sand and 13% silt.

Table 3.1: Planting and harvest dates at the respective trial sites in Bethlehem, Kroonstad and Potchefstroom as well as the agronomic practices applied in soybean trials for evaluation of the application time of plant growth regulators.

Agronomic practices	**Locality**		
	Bethlehem	**Kroonstad**	**Potchefstroom**
Planting date:			
1st Trial	8 November 2018	20 December 2018	29 November 2018
2nd Trial	27 November 2018		13 December 2018
Harvest date:			
1st Trial	30 April 2019	14 May 2019	9 April 2019
2nd Trial	30 April 2019		2 May 2019
Fertilizers applied:			
LAN (28)/plot (kg)	18	16	1
Superphosphate/plot (kg)	24	22	13
Weed control:			
Pre-emergence	Alachlor (chloroacetanilide)		
Post-emergence	Diclosulam (triazolopyrimidine sulfonanilide)		
	Glyphosate		
	Mechanical		

PGR A2019 (CPPU) was applied at a rate of 50 ml ha^{-1} (1.85 ppm). PGR B2019 (NAA) was applied at a rate of 30 ml ha^{-1} (3.89 ppm) and PGR C2019 (GA_3 + S-ABA) at a rate of 10 g ha^{-1} (14.82 ppm GA_3 and 1.15 ppm S-ABA).

Plant growth parameters

At physiological maturity (R7) (Figure 3.3), five randomly selected plants from the center two rows of each replicate were harvested to collect plant growth parameter data *viz.* height of the lowest pods above soil level (cm), number of pods $node^{-1}$, number of pods $plant^{-1}$, number of seed pod^{-1} and number of nodes $plant^{-1}$. The soybean plant growth parameters were determined from the trials planted first at Potchefstroom, Bethlehem and the trial at Kroonstad only.

Yield parameters

At plant maturity (R8) (Figure 3.4), soybean plants in 4 m of each of the two center rows per plot of each replicate were harvested and mechanically threshed to determine the mass of a

1000 seed and to calculate yield (kg ha^{-1}). The soybean yield parameters were determined for both trials (two planting dates) at Potchefstroom and Bethlehem and the trial at Kroonstad.

All plant material was manually separated from the seed after threshing using a sieve. The seed moisture content was determined separately for each replicate, using a moisture meter. The total seed mass per replicate and 1000 seed mass were determined. The yield for each replicate was determined by adjusting the seed moisture to 12.5% and using the following equation:

$$Yield = \frac{(100 - seed\ moisture\ \%)}{(100 - 12.5)} \times seed\ mass$$

The seed yield per replicate was converted to kg ha^{-1}, taking into account the number of plants that were harvested per replicate.

Figure 3.3: Physiological mature soybean plants in the trial at Potchefstroom.

Figure 3.4: Mature soybean plants in the trial at Bethlehem, prior to harvesting.

3.3.2 Effect of plant growth regulators applied at different rates on plant growth and yield parameters

Two dryland field trials were conducted during the 2018/19 growing season at the ARC - Grain Crops, Potchefstroom (26°44'04.2"S 27°04'41.1"E) (Figure 3.5). The respective planting and harvesting dates as well as agronomic practices applied were the same as for the application time trials planted in Potchefstroom described above and details provided in Table 3.1.

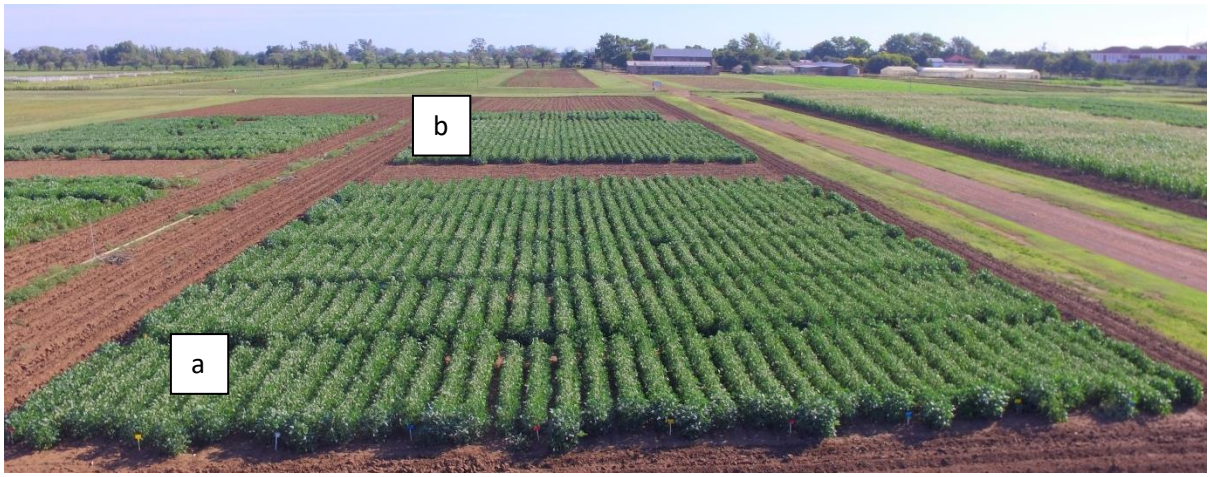

Figure 3.5: Two application rate trials were planted at Potchefstroom, a) first planting date and b) second planting date.

There were four treatments (three PGR products + control) and three application rates at plant growth stages R1 and R3 (double application). PGR A2019 (CPPU) was applied at a rate of 25 ml ha^{-1} (1.25 ppm), 50 ml ha^{-1} (2.5 ppm) and 100 ml ha^{-1} (5 ppm). PGR B2019 (NAA) was applied at a rate of 15 ml ha^{-1} (2.63 ppm), 30 ml ha^{-1} (5.25 ppm) and 60 ml ha^{-1} (10.5 ppm). PGR C2019 (GA_3 + S-ABA) was applied at a rate of 5 g ha^{-1} (10 ppm GA3; 0.78 ppm S-ABA), 10 g ha^{-1} (20 ppm GA3; 1.56 ppm S-ABA) and 20 g ha^{-1} (40 ppm GA3; 3.12 ppm S-ABA). All applications for the three PGRs with three application rates were replicated six times, while the control treatment was replicated six times only. Harvesting procedure and data collection were similar to the application time trials.

3.3.3 Effect of plant growth regulators on plant growth and yield parameters in a pot trial

The pot trial was conducted under a shade net structure at the North-West University, Potchefstroom (26°67'42.7"S 27°10'64.5"E), North-West province (Figure 3.6). A total of 80 pots (40 L), each filled with sandy loam soil were planted with six soybean seeds (LS 6161 R) and thinned to one plant per pot after three weeks. The cultivar, LS 6161 R has a determinate growth habit and completes more than 80% of the vegetative growth before flowering commences. At planting, seeds were inoculated with *Bradyrhizobium japonicum* bacteria. Pots

were watered three times a week during the first three weeks. From the fourth week after planting pots were watered only when necessary to maintain adequate soil moisture. Throughout the study period weeds were removed by hand.

There were four treatments (three PGRs + control) and three application rates at plant growth stage R1 and R3 (double application). The application rates were the same as applied in the field experiment (see 3.3.2). All applications for the three PGRs with three application rates were replicated eight times, while the control treatment was replicated eight times only.

Figure 3.6: Pot trial to evaluate different application rates of plant growth regulators applied at growth stages R1, R3 and R1 + R3 (double application).

Plant height was measured on the day prior to PGR application and again at physiological maturity of the plants to calculate the increase in plant height after application of PGR's. Plant height was measured from the soil surface to the growing point of the soybean plant. At physiological maturity, each soybean plant was harvested and the height of the lowest pods above soil level (cm), number of pods node^{-1}, number of pods plant^{-1}, number of seed pod^{-1}, number of nodes plant^{-1} and mass of 1000 seeds were determined, and yield plant^{-1} was calculated. For the field trials, plant growth parameters were determined from the trial planted first, but yield parameters were determined from both trials.

3.3.4 Statistical analysis

The effect of application time or application rate of the PGRs were analyzed by means of one-way ANOVA's, followed by Tukey's unequal N HSD post-hoc test. The effect of the two planting dates as well as the effect of locality (Potchefstroom and Bethlehem), for the second planting date trials on mass of a 1000 seed and yield were compared by means of independent-samples t-tests. All analyses were performed with TIBCO Statistica^{TM} 13.3 (TIBCO Software, Inc, 2017).

3.4 Results

The monthly rainfall at Potchefstroom, Bethlehem and Kroonstad during the 2018/19 crop production season as well as the long-term average rainfall (2004-2019) are shown in Figure 3.7. Rainfall (mm) in October to March at Potchefstroom and October to February in Bethlehem and Kroonstad was lower than the long term mean rainfall. However, the mean rainfall was higher than the long term mean rainfall in August and September 2018 at all three localities (Data not shown). Rainfall in March at Bethlehem and Kroonstad was above the long-term mean. During April, all three localities received rainfall above the long-term mean. It did, however, not have any effect on the results of these trials, since the soybean plants were already mature, and ready to be harvested.

The mean daily minimum and maximum temperatures from October 2018 to April 2019 at Potchefstroom, Bethlehem and Kroonstad are shown in Figure 3.8 and 3.9 respectively. During the months of October to February, the mean minimum temperatures at all three localities, were slightly lower or the same as the long-term mean. The mean minimum temperature for March and April were higher than the long-term mean.

The mean maximum temperatures at all three localities were almost similar to the long term mean for the period October to April. Overall, the mean minimum and maximum temperatures were within an acceptable range for soybean production. This range is between 20 – 30 °C, with the optimal temperature at 25 °C (Heinemann *et al*., 2006; Liebenberg, 2012).

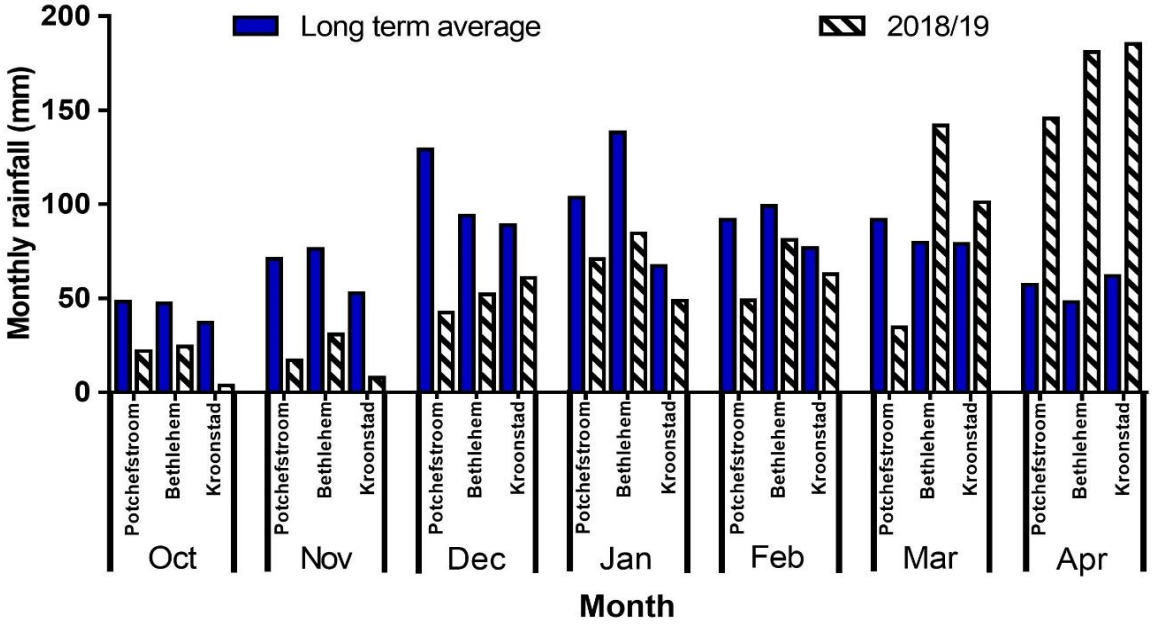

Figure 3.7: Total monthly rainfall (mm) from October 2018 to April 2019 and the long-term monthly rainfall at Potchefstroom, Bethlehem and Kroonstad (Data from ARC-ISCW).

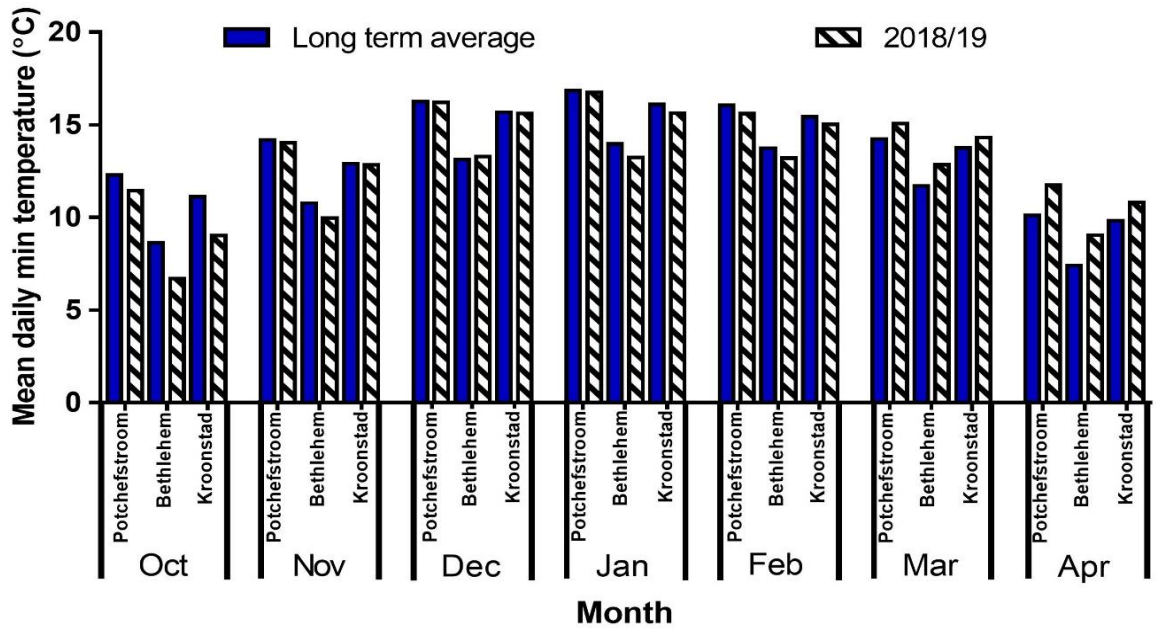

Figure 3.8: Mean daily minimum temperature (˚C) from October 2018 to April 2019 and the long-term mean daily minimum temperature at Potchefstroom, Bethlehem and Kroonstad (Data from ARC-ISCW).

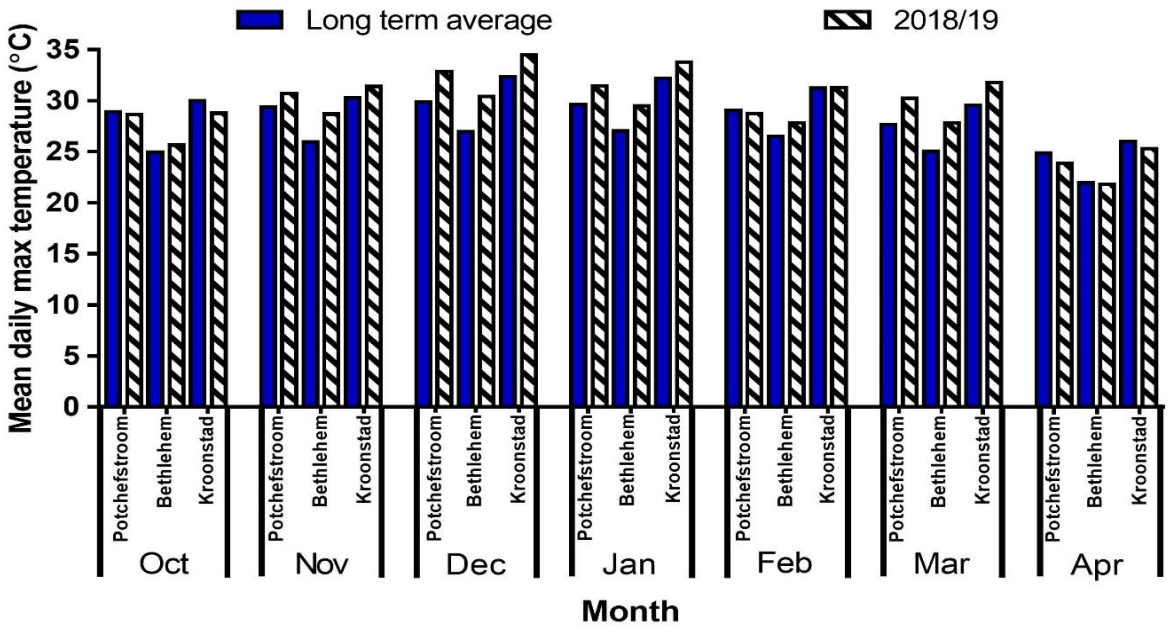

Figure 3.9: Mean daily maximum temperature (˚C) from October 2018 to April 2019 and the long-term mean daily maximum temperature at Potchefstroom, Bethlehem and Kroonstad (Data from ARC-ISCW).

3.4.1 Effect of plant growth regulators applied at different plant growth stages on plant growth and yield parameters

Height of the lowest pod above the soil surface

At Potchefstroom, the height of the lowest pods differed significantly between the treatments ($F_{9,290}$=3.6; P<0.001) and ranged between 9 and 11.95 cm above the soil surface (Table 3.2). However, the height of the lowest pods from plants treated with PGRs, did not differ significantly from the lowest pod height of plants in the control treatment. The height of the lowest pod of plants treated with B2019 at R1 was significantly higher than from plants treated with A2019 applied at R3 and C2019 applied at R3 and R1 + R3. At Bethlehem, the height of the lowest pods ranged between 9.00 and 11.62 cm above the soil surface (Table 3.2) and did not differed significantly between the treatments ($F_{9,260}$=1.6; P=0.11). At Kroonstad, the height of the lowest pods differed significantly between treatments ($F_{9,290}$=3.0; P<0.01) and ranged between 7.53 and 10.67 cm above the soil surface. Lowest pod height from plants treated with C2019 at R1, was significantly higher (>41%) compared to plants in the control, B2019 (R3) and B2019 (R1 + R3) treatments. Locality had a significant effect on the height of the lowest pods above the soil surface ($F_{2,87}$=8.2; P<0.001). The height of the lowest pods ranged between 7.53 and 10.68 cm above the soil surface in the control, with the lowest pods significantly lower in the trial at Kroonstad compared to those in the Potchefstroom and Bethlehem trials (Table 3.3).

Number of pods per node

The number of pods node^{-1} was not significantly influenced by the respective PGRs applied to soybean at Potchefstroom ($F_{9,290}$=1.7; P=0.1), where it varied between 2.79 and 3.54 and between 2.63 and 3.18 pods node^{-1} at Kroonstad ($F_{9,290}$=0.9; P=0.55) (Table 3.4). At Bethlehem, the number of pods node^{-1} differed significantly between the treatments ($F_{9,260}$=2.8; P<0.01) and ranged between 3.54 and 5.19 (Table 3.4). Plants treated with B2019 at R1 and R3 produced significantly more (>40%) pods node^{-1} than plants in the control treatment. The number of pods node^{-1} from these plants, did however, not differ significantly from plants where the same PGR was applied twice (at R1 + R3) and from those where any of the A2019 and C2019 treatments were applied (Table 3.4). Locality did not affect the number of pods node^{-1} significantly ($F_{2,87}$=3.0; P=0.05). The number of pods node^{-1} in the control treatments of the three localities varied between 2.86 and 3.54 (Table 3.3).

Table 3.2: The effect of plant growth regulators applied at different growth stages on the mean height of the lowest pods above the soil surface (cm) at Potchefstroom, Bethlehem and Kroonstad.

Treatment	Mean height of lowest pod above the soil surface (cm) ± SE		
	Locality		
	Potchefstroom	Bethlehem	Kroonstad
A2019 (R1)	11.02 ± 0.54abc	11.10 ± 0.61a	8.37 ± 0.56ab
A2019 (R3)	9.00 ± 0.55a	9.85 ± 0.52a	9.83 ± 0.67ab
A2019 (R1 + R3)	11.17 ± 0.60abc	11.40 ± 0.65a	8.73 ± 0.59ab
B2019 (R1)	11.95 ± 0.42c	11.28 ± 0.63a	9.00 ± 0.54ab
B2019 (R3)	10.60 ± 0.58abc	10.97 ± 0.64a	7.53 ± 0.53a
B2019 (R1 + R3)	10.77 ± 0.65abc	11.16 ± 0.63a	8.00 ± 0.61a
C2019 (R1)	11.78 ± 0.44bc	11.32 ± 0.64a	10.67 ± 0.61b
C2019 (R3)	9.02 ± 0.48a	9.00 ± 0.54a	9.33 ± 0.53ab
C2019 (R1 + R3)	9.40 ± 0.41ab	11.62 ± 0.79a	8.23 ± 0.58ab
Control	10.18 ± 0.75abc	10.68 ± 51a	7.53 ± 0.45a

Means within a column followed by the same letter are not significantly different at P<0.05 (Tukey's Unequal N HSD).

Table 3.3: Plant growth and yield parameters as affected by localities (Potchefstroom, Bethlehem and Kroonstad) during the first planting date (Application time trials).

Growth and yield parameters	Mean values of control treatments at each locality ± SE		
	Locality		
	Potchefstroom	Bethlehem	Kroonstad
Height of lowest pod	10.18 ± 0.75a	10.68 ± 0.51a	7.53 ± 0.45b
Pods node^{-1}	3.16 ± 0.21a	3.54 ± 0.22a	2.86 ± 0.14a
Pods plant^{-1}	46.47 ± 3.16a	55.17 ± 3.78a	38.37 ± 2.22b
Seed pod^{-1}	2.09 ± 0.04a	2.10 ± 0.05a	2.12 ± 0.04a
Nodes plant^{-1}	14.67 ± 0.26a	15.50 ± 0.31a	13.33 ± 0.18b
Mass of 1000 seed (g)	135.63 ± 3.83b	181.71 ± 3.21a	195.98 ± 6.84a
Yield (kg ha^{-1})	1018.39 ± 81.10a	2829.46 ± 149.91b	2257.35 ± 181.76ab

Means within a row followed by the same letter are not significantly different at P<0.05 (Tukey's HSD).

Table 3.4: The effect of plant growth regulators applied at different growth stages on the mean number of pods per node at Potchefstroom, Bethlehem and Kroonstad.

Treatment	Mean number of pods node^{-1} ± SE		
	Locality		
	Potchefstroom	Bethlehem	Kroonstad
A2019 (R1)	3.37 ± 0.21a	4.02 ± 29ab	2.97 ± 0.16a
A2019 (R3)	2.79 ± 0.15a	4.88 ± 0.27ab	2.77 ± 0.17a
A2019 (R1 + R3)	3.08 ± 0.17a	3.94 ± 0.28ab	3.18 ± 0.18a
B2019 (R1)	3.24 ± 0.18a	5.19 ± 0.50a	2.73 ± 0.14a
B2019 (R3)	3.22 ± 0.17a	5.12 ± 0.37a	2.88 ± 0.15a
B2019 (R1 + R3)	3.48 ± 0.19a	4.90 ± 0.31ab	2.96 ± 0.17a
C2019 (R1)	3.54 ± 0.22a	4.90 ± 0.31ab	2.93 ± 0.19a
C2019 (R3)	3.00 ± 0.13a	4.62 ± 0.27ab	2.63 ± 0.16a
C2019 (R1 + R3)	2.98 ± 0.15a	4.43 ± 0.45ab	2.85 ± 0.13a
Control	3.16 ± 0.21a	3.54 ± 0.22b	2.86 ± 0.14a

Means within a column followed by the same letter are not significantly different at P<0.05 (Tukey's Unequal N HSD).

Number of pods per plant

The application time of the respective PGRs [(B2019, A2019 and C2019)] to soybean plants at Potchefstroom ($F_{9,290}$=1.8; P=0.07) and Kroonstad ($F_{9,290}$=1.7; P=0.31) did not significantly influence the number of pods formed per plant. The number of pods plant^{-1} ranged between 41.43 to 54.13 at Potchefstroom and 34.73 and 44.23 at Kroonstad (Table 3.5). At Bethlehem, significantly more pods were produced by plants (> 50% more) to which B2019 was applied, regardless of the application time, and also A2019 applied at R3 compared to plants in the control plots ($F_{9,260}$=3.4; P<0.0001) (Table 3.5). The number of pods plant^{-1} ranged between 55.17 and 91.87. The number of pods plant^{-1} to which any of the PGRs were applied, and regardless of the application time, did not differ significantly at this locality. Locality had a significant effect on the number of pods plant^{-1} ($F_{2,87}$=7.0; P<0.01). The number of pods plant^{-1} in the control at Kroonstad was significantly fewer than for plants in the control treatments at Potchefstroom and Bethlehem and ranged between 38.37 and 55.17 at the three sites (Table 3.3).

Table 3.5: The effect of plant growth regulators applied at different growth stages on the mean number of pods per plant at Potchefstroom, Bethlehem and Kroonstad.

Treatment	Mean number of pods plant^{-1} ± SE		
	Locality		
	Potchefstroom	Bethlehem	Kroonstad
A2019 (R1)	53.97 ± 3.47a	66.76 ± 5.60ab	40.80 ± 2.50a
A2019 (R3)	41.43 ± 2.37a	84.20 ± 5.34a	35.93 ± 2.28a
A2019 (R1 + R3)	47.53 ± 2.62a	77.84 ± 4.93ab	44.23 ± 2.82a
B2019 (R1)	51.67 ± 3.44a	90.16 ± 8.97a	37.53 ± 2.27a
B2019 (R3)	49.97 ± 2.88a	91.87 ± 7.90a	38.90 ± 2.58a
B2019 (R1 + R3)	52.27 ± 3.05a	87.24 ± 5.95a	41.10 ± 3.05a
C2019 (R1)	54.13 ± 3.92a	73.97 ± 6.81ab	41.77 ± 3.03a
C2019 (R3)	44.67 ± 2.37a	65.96 ± 4.41ab	34.73 ± 2.51a
C2019 (R1 + R3)	49.03 ± 2.72a	74.80 ± 8.63ab	39.07 ±2.36a
Control	46.47 ± 3.16a	55.17 ± 3.78b	38.37 ± 2.22a

Means within a column followed by the same letter are not significantly different at P<0.05 (Tukey's Unequal N HSD).

Number of seed per pod

The application of the PGRs did not influence the mean number of soybean seed pod^{-1} significantly, regardless of the PGR treatment or time of application at Bethlehem ($F_{9,260}$=1.3; P=0.24) and Kroonstad ($F_{9,290}$=0.9; P=0.53), respectively (Table 3.6). The mean number of seed pod^{-1} ranged between 2.05 and 3.05 at Bethlehem, and between 1.99 and 2.13 at Kroonstad. The mean number of seed per pod produced by plants that were sprayed with the respective PGRs at different times, differed significantly at Potchefstroom ($F_{9,290}$=2.2; P<0.05). Significantly more seed pod^{-1} were produced by plants treated with B2019 at R1 compared to plants that were treated with C2019 at R3 (Table 3.6). However, the mean number of seed pod^{-1} from plants treated PGRs, did not differ significantly from the mean number of seed pod^{-1} in the control treatment. Locality did not have a significant effect on the number of seed pod^{-1} ($F_{2,87}$=0.2; P=0.82). The number of seed pod^{-1} in the control treatments of the trials at Potchefstroom, Bethlehem and Kroonstad ranged between 2.09 and 2.12 (Table 3.3).

Table 3.6: The effect of plant growth regulators applied at different growth stages on the mean number of seed per pod at Potchefstroom, Bethlehem and Kroonstad.

Treatment	Mean number of seed pod^{-1} ± SE		
	Locality		
	Potchefstroom	Bethlehem	Kroonstad
A2019 (R1)	2.00 ± 0.04ab	2.17 ± 0.04a	2.12 ±0.05a
A2019 (R3)	1.94 ± 0.04ab	2.06 ± 0.03a	2.07 ± 0.05a
A2019 (R1 + R3)	1.98 ± 0.03ab	2.13 ± 0.03a	2.08 ± 0.05a
B2019 (R1)	2.12 ± 0.07b	2.21 ± 0.11a	2.10 ± 0.04a
B2019 (R3)	2.04 ± 0.04ab	2.05 ± 0.03a	2.09 ± 0.04a
B2019 (R1 + R3)	2.05 ± 0.05ab	2.10 ± 0.03a	2.13 ± 0.04a
C2019 (R1)	2.05 ± 0.03ab	2.06 ± 0.03a	2.09 ± 0.03a
C2019 (R3)	1.92 ± 0.05a	2.10 ± 0.04a	2.08 ± 0.03a
C2019 (R1 + R3)	2.02 ± 0.03ab	3.05 ± 0.85a	1.99 ± 0.04a
Control	2.09 ± 0.04ab	2.10 ± 0.05a	2.12 ± 0.04a

Means within a column followed by the same letter are not significantly different at P<0.05 (Tukey's Unequal N HSD).

Number of nodes per plant

At Potchefstroom, the number of nodes plant^{-1} to which the respective PGRs were applied, differed significantly between the treatments ($F_{9,290}$=4.7; P<0.0001) and ranged between 14.67 and 16.34 (Table 3.7). Plants treated with C2019 at R1 + R3 and A2019 at R1 had significantly more (>8%) nodes plant^{-1} than plants in the control treatment, as well as plants treated with A2019 at R3 and C2019 at R3. Plants treated with a double application of C2019 (R1 + R3) also had significant more nodes plant^{-1} than plants treated with a single application of C2019 (R1) and C2019 (R3). The number of nodes plant^{-1} treated with the respective PGRs at different times, differed significantly at Bethlehem also ($F_{9,260}$=2.9; P=<0.01). The mean number of nodes plant^{-1} ranged between 17.68 and 15.50 (Table 3.7). Plants treated with B2019 at R1 + R3 and R3 had significantly more (>11%) nodes plant^{-1} than plants in the control treatment. There was no significant difference in mean number of nodes plant^{-1} in the PGR and control treatments at Kroonstad ($F_{9,290}$=1.9; P=0.06), where it varied between 12.97 and 14.10 (Table 3.7). Location significantly affected the number of nodes plant^{-1} ($F_{2,87}$=17.2; P<0.001). The number of nodes plant^{-1} ranged between 13.3 and 14.67 with significantly fewer nodes plant^{-1} in the control plots at Kroonstad compared to plants in the control plots of the Potchefstroom and Bethlehem trials (Table 3.3).

Table 3.7: The effect of plant growth regulators applied at different growth stages on the mean number of nodes per plant at Potchefstroom, Bethlehem and Kroonstad.

Treatment	Mean number of nodes plant^{-1} ± SE		
	Locality		
	Potchefstroom	Bethlehem	Kroonstad
A2019 (R1)	15.97 ± 0.22bc	16.24 ± 0.33ab	13.57 ± 0.23a
A2019 (R3)	14.80 ± 0.17a	17.05 ± 0.33ab	12.97 ± 0.24a
A2019 (R1 + R3)	15.47 ± 0.26abc	16.76 ± 0.23ab	13.77 ± 0.21a
B2019 (R1)	15.65 ± 0.24abc	17.20 ± 0.43ab	13.57 ± 0.23a
B2019 (R3)	15.45 ± 0.26abc	17.47 ± 0.35a	13.30 ± 0.26a
B2019 (R1 + R3)	14.95 ± 0.29ab	17.68 ± 0.22a	13.60 ± 0.30a
C2019 (R1)	15.07 ± 0.25ab	16.90 ± 0.44ab	14.10 ± 0.32a
C2019 (R3)	14.83 ± 0.31a	16.84 ± 0.38ab	12.97 ± 0.22a
C2019 (R1 + R3)	16.34 ± 0.20c	16.64 ± 0.52ab	13.50 ± 0.26a
Control	14.67 ± 0.26a	15.50 ± 0.31b	13.33 ± 0.18a

Means within a column followed by the same letter are not significantly different at P<0.05 (Tukey's Unequal N HSD).

Mass of a 1000 seed

The mass of a 1 000 seed from plants treated with the respective PGRs and control treatments did not differ significantly at Potchefstroom ($F_{9,50}$=0.6; P=0.1), Bethlehem ($F_{9,44}$=1.0; P=0.45) and Kroonstad ($F_{9,50}$=0.72; P=0.69) in the early planted trials as well as the late planted trials at Potchefstroom ($F_{9,40}$=0.8; P=0.63) and Bethlehem ($F_{9,48}$=0.9; P=0.55). The mass of a 1000 seed ranged between 133.92 g and 144.29 g at Potchefstroom, 173.50 g and 181.71 g at Bethlehem and 192.97 and 206.32 g at Kroonstad in the early planted trials. In the late planted trials, the mass of a 1000 seed ranged between 139.43 and 159.34 at Potchefstroom and 176.45 and 183.93 at Bethlehem (Table 3.8).

Locality had a significant effect on the mass of a 1000 seed in the early planting date trials ($F_{2,26}$=35.0; P<0.001). The mass of 1000 seed in the control at the three localities, ranged between 135.63 and 195.98 g (Table 3.3). The mass of a 1000 seed of control plants at Potchefstroom was significantly lower than at Bethlehem and Kroonstad. Locality also had a significant effect on the mass of a 1000 seed in the second planting date trials (t=-4.18; P<0.01), with the mass of a 1 000 seed significantly higher at Bethlehem. The mass of 1000

seed in the control at Potchefstroom and Bethlehem ranged between 148.41 and 179.78 (Table 3.9).

Table 3.8: The effect of plant growth regulators applied at different growth stages on the mean mass of a 1 000 seed (g) at Potchefstroom, Bethlehem and Kroonstad.

Treatment	Mean mass of a 1000 seed (g) ± SE				
	Locality				
	Planting date 1			Planting date 2	
	Potchefstroom	Bethlehem	Kroonstad	Potchefstroom	Bethlehem
A2019 (R1)	144.29 ± 4.43a	178.25 ± 2.21a	203.00 ± 8.12a	139.47 ± 5.56a	183.93 ± 4.13a
A2019 (R3)	142.17 ± 2.25a	177.26 ± 2.42a	206.32 ± 0.99a	144.96 ± 4.03a	188.76 ± 1.80a
A2019 (R1 + R3)	138.31 ± 9.10a	173.50 ± 0.76a	204.76 ± 3.08a	147.65 ± 5.79a	176.45 ± 3.26a
B2019 (R1)	133.92 ± 4.06a	174.33 ± 1.92a	206.13 ± 4.89a	139.43 ± 5.07a	177.20 ± 3.29a
B2019 (R3)	139.69 ± 3.90a	178.15 ± 2.42a	205.13 ± 3.73a	159.34 ± 5.72a	178.37 ± 2.73a
B2019 (R1 + R3)	139.62 ± 4.08a	180.05 ± 0.93a	200.64 ± 2.89a	143.98 ± 6.08a	177.54 ± 2.42a
C2019 (R1)	135.13 ± 7.57a	179.01 ± 2.51a	202.83 ± 3.43a	150.57 ± 7.11a	181.57 ± 3.04a
C2019 (R3)	136.93 ± 7.11a	181.11 ± 2.65a	192.97 ± 3.22a	148.43 ± 5.85a	179.19 ± 5.10a
C2019 (R1 + R3)	135.99 ± 5.12a	179.72 ± 2.76a	203.74 ± 6.98a	144.64 ± 5.75a	181.15 ± 3.53a
Control	135.63 ± 9.38a	181.71 ± 3.21a	195.98 ± 16.75a	148.41 ± 5.87a	179.77 ± 3.52a

Means within a column followed by the same letter are not significantly different at P<0.05 (Tukey's Unequal N HSD.

Table 3.9: Plant yield parameters as affected by localities, (Potchefstroom and Bethlehem), during the second planting date (Application time trials).

Growth and yield parameters	Mean values of control treatments at each locality ± SE	
	Locality	
	Potchefstroom	Bethlehem
Mass of 1000 seed (g)	148.41 ± 5.87a	179.78 ± 3.52b
Yield (kg ha^{-1})	1963.75 ± 261.20a	2625.63 ±115.63a

Means within a row followed by the same letter are not significantly different at P<0.05 (Independent-samples t-test).

There was no significant difference in the mass of a 1000 seed in the control of trials from the two planting dates at Potchefstroom (t=-1.66; P=0.13) and Bethlehem (t=0.37; P=0.72). The mass of a 1000 seed between the two planting dates ranged between 135.63 and 148.41 g at Potchefstroom and 179.77 and 181.71 at Bethlehem (Table 3.10).

Table 3.10: Plant yield parameters as affected by different planting dates at Potchefstroom and Bethlehem (Application time trials).

Locality	Growth and yield parameters	Mean values of control treatments at each planting date ± SE	
		1st planting date	2nd planting date
Potchefstroom	Mass of 1000 seed (g)	135.63 ± 3.83a	148.41 ± 5.87a
	Yield (kg ha^{-1})	1018.39 ± 81.10a	1963.75 ± 261.20b
Bethlehem	Mass of 1000 seed (g)	181.71 ± 3.21a	179.77 ± 3.52a
	Yield (kg ha^{-1})	2829.46 ± 149.91a	2625.63 ± 115.63a

Means within a row followed by the same letter are not significantly different at P<0.05 (Independent-samples t-test)

Yield

Soybean yield was not significantly affected by any of the PGRs applied as well as by application time, at any of the three localities for the trials planted at the first planting date, *viz*. at Potchefstroom ($F_{9,50}$=0.4; P=0.90), Bethlehem ($F_{9,44}$=0.5; P=0.84) and Kroonstad ($F_{9,50}$=1.0; P=0.41). The yield ranged between 2201.84 and 2791.79 kg ha^{-1} at Potchefstroom, 2487.22 and 2857.86 kg ha^{-1} at Bethlehem and 2201.84 and 2791.79 kg ha^{-1} at Kroonstad (Table 3.11). There was also no significant difference in yield from plants treated with any of the PGRs applied at the respective application times in the second planting date trial at Potchefstroom ($F_{9,40}$=0.9; P=0.53) and Bethlehem ($F_{9,48}$=0.8; P=0.58). The yield ranged between 1196.10 and 1968.57 kg ha^{-1} at Potchefstroom and 2625.63 and 3162.43 kg ha^{-1} at Bethlehem (Table 3.11)

Locality had a significant effect on yield for the early planted soybean trials at Potchefstroom, Bethlehem and Kroonstad ($F_{2,26}$=9.0; P<0.01). Yield in the control at the three sites ranged between 1018.39 and 2829.46 kg ha^{-1} (Table 3.3), with significantly higher soybean yield at Bethlehem and Kroonstad. Locality (Potchefstroom and Bethlehem) had no significant effect on soybean yield in trials planted at the second planting date (t=-2.12; P=0.06). The mean soybean yield in the second planting date trial at Potchefstroom was 1963.75 kg ha^{-1}, and 2625.63 kg ha^{-1} in Bethlehem (Table 3.9). The yield differed significantly between the two planting dates of the control treatments at Potchefstroom (t=-3.16; P<0.01). Yield in the control plots of trials from the two planting dates was 1018.39 kg ha^{-1} (first planting date) and 1963.75 kg ha^{-1} (second planting date) (Table 3.10), with significantly higher yield recorded in the trial planted at the second planting date. Planting date had no significant effect on yield in trials planted at Bethlehem (t=0.98; P=0.34), where it varied between 2625.63 and 2829.46 kg ha^{-1} for the two planting dates (Table 3.10).

Table 3.11: The effect of plant growth regulators applied at different growth stages on the mean yield ha^{-1} (kg) at Potchefstroom, Bethlehem and Kroonstad in trials planted at two planting dates.

Treatment	Mean yield ha^{-1} (kg) ± SE				
	Locality				
	Planting date 1			Planting date 2	
	Potchefstroom	Bethlehem	Kroonstad	Potchefstroom	Bethlehem
A2019 (R1)	2326.70 ± 225.93a	2857.86 ± 273.94a	2326.70 ± 225.93a	1756.07 ± 250.08a	2838.40 ± 124.84a
A2019 (R3)	2564.71 ± 199.36a	2693.00 ± 212.54a	2564.71 ± 199.36a	1738.34 ± 158.91a	2873.96 ± 169.26a
A2019 (R1 + R3)	2702.66 ± 212.39a	2523.17 ± 78.61a	2702.66 ± 212.39a	1547.38 ± 116.99a	2627.50 ± 164.91a
B2019 (R1)	2791.79 ± 188.48a	2487.22 ± 64.22a	2791.79 ± 188.48a	1509.29 ± 89.21a	3009.20 ± 220.86a
B2019 (R3)	2787.51 ± 202.54a	2607.72 ± 75.37a	2787.51 ± 202.54a	1732.45 ± 269.12a	3162.43 ± 195.00a
B2019 (R1 + R3)	2589.12 ± 198.56a	2621.55 ± 166.15a	2589.12 ± 198.56a	1968.57 ± 393.08a	2683.74 ± 225.21a
C2019 (R1)	2646.39 ± 173.74a	2637.76 ± 62.02a	2646.39 ± 173.74a	1196.10 ± 155.44a	2993.13 ± 113.27a
C2019 (R3)	2201.84 ± 199.56a	2606.50 ± 98.96a	2201.84 ± 199.56a	1739.70 ± 133.87a	2908.35 ± 159.89a
C2019 (R1 + R3)	2658.42 ± 144.22a	2770.32 ± 164.83a	2658.42 ± 144.22a	1362.88 ± 58.28a	2858.07 ± 186.06a
Control	2257.35 ± 181.76a	2829.46 ± 149.91a	2257.35 ± 181.76a	1963.75 ± 261.20a	2625.63 ± 115.63a

Means within a column followed by the same letter are not significantly different at $P<0.05$ (Tukey's Unequal N HSD).

3.4.2 Effect of plant growth regulators applied at different rates on plant growth and yield parameters

Height of the lowest pod

The height of the lowest pod above the soil surface differed significantly between the respective treatments ($F_{9,290}$=3.9; P<0.0001), and ranged between 10.03 and 12.72 cm from the soil surface (Table 3.12). Pods of plants treated with A2019 (50 ml ha^{-1}) were significantly higher above the soil surface compared to pods from plants treated with 30 ml ha^{-1} B2019 (30 ml ha^{-1}), A2019 (100 ml ha^{-1}), C2019 (10 g ha^{-1}) and the control treatment. There was no significant difference in lowest pod height above the soil surface of plants in the control compared to that of plants treated with any of the PGRs applied at the respective rates, except for A2019 (50 ml ha^{-1}).

Mean number of pods per node

The number of pods $node^{-1}$ ranged between 3.13 and 3.96 and did not differ significantly between the treatments ($F_{9,290}$=2.3; P=0.05) (Table 3.12).

Mean number of pods per plant

None of the PGRs applied at the respective application rates affected the mean number of soybean pods $plant^{-1}$ significantly ($F_{9,290}$=1.8; P=0.07). The mean number of pods $plant^{-1}$ ranged between 49.70 and 64.47 (Table 3.12).

Mean number of seed per pod

No increase in the number of seed pod^{-1} was induced by any of the PGRs applied at any of the rates applied when compared to the mean number of seed per pod in the control (Table 3.12).

Mean number of nodes per plant

The mean number of nodes $plant^{-1}$ differed significantly between the respective treatments ($F_{9,290}$=7.1; P=<0.0001), and ranged between 15.23 and 17.40 (Table 3.12). The nodes $plant^{-1}$ treated with 25 ml ha^{-1} and 50 ml ha^{-1} A2019 were significantly fewer than that of the plants in the control and plants treated with 100 ml ha^{-1} A2019, B2019 (15 ml ha^{-1} and 30 ml ha^{-1}) as well as C2019 (5 g ha^{-1} and 10 g ha^{-1}). Soybean treated with 60 ml ha^{-1} B2019 had significant fewer nodes $plant^{-1}$ than plants treated with 15 ml ha^{-1} and 30 ml ha^{-1} B2019. No PGR treatments were able to significantly increase the number of nodes $plant^{-1}$ compared to the control treatment.

Table 3.12: The effect of plant growth regulators applied at different rates on plant growth parameters of soybean plants at Potchefstroom.

Treatment	Mean height of lowest pods above soil surface ± SE	Mean number of pods node^{-1} ± SE	Mean number of pods plant^{-1} ± SE	Mean number of seed pod^{-1} ± SE	Mean number of nodes plant^{-1} ± SE
A2019 (25 ml ha^{-1})	11.67 ± 0.48ab	3.83 ± 0.18a	58.90 ± 3.47a	2.07 ± 0.03abc	15.27 ± 0.38d
A2019 (50 ml ha^{-1})	12.72 ± 0.41b	3.21 ± 0.18a	49.70 ± 3.63a	2.02 ± 0.02abc	15.23 ± 0.42d
A2019 (100 ml ha^{-1})	10.45 ± 0.60a	3.80 ± 0.15a	63.60 ± 2.70a	2.11 ± 0.03ab	16.70 ± 0.23abc
B2019 (15 ml ha^{-1})	11.95 ± 0.54ab	3.63 ± 0.18a	62.67 ± 3.67a	2.04 ± 0.03abc	17.03 ± 0.26ab
B2019 (30 ml ha^{-1})	10.03 ± 0.37a	3.48 ± 0.21a	61.13 ± 4.12a	2.01 ± 0.03abc	17.40 ± 0.26b
B2019 (60 ml ha^{-1})	10.55 ± 0.53ab	3.96 ± 0.23a	61.37 ± 3.65a	2.10 ± 0.02ab	15.63 ± 0.35cd
C2019 (5 g ha^{-1})	11.52 ± 0.42ab	3.13 ± 0.19a	52.53 ± 3.35a	2.06 ± 0.03abc	16.67 ± 0.23abc
C2019 (10 g ha^{-1})	10.07 ± 0.29a	3.28 ± 0.18a	56.40 ± 3.59a	1.98 ± 0.04ac	17.03 ± 0.22ab
C2019 (20 g ha^{-1})	10.48 ± 0.66ab	3.59 ± 0.22a	58.10 ± 3.99a	1.94 ± 0.04c	16.03 ± 0.24acd
Control	10.25 ± 0.54a	3.81 ± 0.15a	64.47 ± 3.08a	2.12 ± 0.02b	16.77 ± 0.20abc

Means within a column followed by the same letter are not significantly different at P<0.05 (Tukey's Unequal N HSD).

Mass of a 1000 seed

The mean mass of a 1000 seed did not differ significantly, regardless of the rate and PGRs applied to plants in the trial planted first in Potchefstroom ($F_{9,50}$=0.1; P=0.5) and ranged between 140.44 and 151.66 g (Table 3.13). Although there were significant differences in the mean mass of a 1000 seed between the respective PGR treatments of the trial planted later ($F_{9,39}$=2.8; P<0.01), none differed significantly from the 1 000 seed mass of plants in the control treatment (Table 3.13). In this trial, the mean mass of a 1000 seed ranged between 129.90 and 160.98 g. The mass of a 1000 seed of soybean plants treated with 5 g ha^{-1} C2019 was significantly lower compared to the mass of a 1 000 seed from plants treated with A2019 (100 ml ha^{-1}) and the control treatment. The mass of a 1000 seed in the control plots of the two planting dates did also not significantly differ (t=-1.88; P=0.09) and varied between 146.04 and 158.28 g (Table 3.14).

Table 3.13: The effect of plant growth regulators applied at different rates on plant yield parameters of soybean plants at Potchefstroom.

Treatment	Mean mass of a 1000 seed (g) ± SE		Mean yield ha^{-1} (kg) ± SE	
	Planting date 1	Planting date 2	Planting date 1	Planting date 2
A2019 (25 ml ha^{-1})	147.92 ± 4.24a	145.45 ± 5.34ab	1566.09 ± 140.99a	1767.36 ± 22.35a
A2019 (50 ml ha^{-1})	145.35 ± 2.60a	143.60 ± 3.71ab	1523.88 ± 116.50a	1833.55 ± 168.47a
A2019 (100 ml ha^{-1})	151.66 ± 3.73a	160.98 ± 7.06a	1854.11 ± 94.50a	1951.18 ± 126.53a
B2019 (15 ml ha^{-1})	141.09 ± 2.18a	147.72 ± 1.62ab	1539.37 ± 132.49a	1948.67 ± 89.39a
B2019 (30 ml ha^{-1})	148.65 ± 4.40a	146.18 ± 3.40ab	1535.35 ± 158.41a	2055.26 ± 221.50a
B2019 (60 ml ha^{-1})	142.55 ± 2.41a	141.42 ± 4.77ab	1607.58 ± 158.98a	1753.75 ± 188.90a
C2019 (5 g ha^{-1})	148.55 ± 1.98a	129.90 ± 1.89b	1677.37 ± 116.19a	1600.71 ± 142.53a
C2019 (10 g ha^{-1})	140.44 ± 4.21a	154.50 ± 7.15ab	1384.28 ± 156.95a	1751.56 ± 76.48a
C2019 (20 g ha^{-1})	149.11 ± 4.14a	152.98 ± 5.41ab	1357.57 ± 196.02a	2372.64 ± 363.33a
Control	146.04 ± 7.89a	158.28 ± 5.18a	1721.40 ± 131.69a	2267.79 ± 327.06a

Means within a column followed by the same letter are not significantly different at P<0.05 (Tukey's Unequal N HSD).

Table 3.14: Plant yield parameters as affected by different planting dates at Potchefstroom (Application rate trials).

Growth and yield parameters	Mean values of control treatments at Potchefstroom at each planting date ± SE	
	Locality	
	1st planting date	2nd planting date
Mass of 1000 seed (g)	146.04 ± 3.22a	158.28 ± 5.18a
Yield (kg ha^{-1})	1721.40 ± 131.70a	2267.79 ± 327.06a

Means within a row followed by the same letter are not significantly different at P<0.05 (Independent samples t-test)

Yield

Regardless of planting date and PGR application rate, none of the PGR affected soybean yield significantly. Yield in the trial planted first, ranged between 1357.57 and 1854.11 kg ha^{-1} ($F_{9,50}$=0.9; P=0.52) and in the trial planted second, between 1600.71 and 2372.64 kg ha^{-1} ($F_{9,39}$=1.1; P=0.36) (Table 3.13). The yield in the control treatment of the trials planted at both the planting dates did also not differ significantly (t=-1.49; P=0.17) and ranged between 1721.40 and 2267.79 kg ha^{-1} (Table 3.14).

3.4.3 Effect of plant growth regulators on plant growth and yield parameters in a pot trial

None of the PGRs applied to soybeans, at any of the application rates in the pot trial had a significant effect on any of the growth and yield parameters (Table 3.15). It did also not differ significantly from the plant growth and yield parameters of plants in the control.

Table 3.15: The effect of plant growth regulators applied at different application rates on plant growth and yield parameters in a pot trial at Potchefstroom.

Treatment	Increase in plant height after application of PGR (cm) ± SE	Mean height of lowest pods above soil surface ± SE	Mean number of pods node^{-1} ± SE	Mean number of pods plant^{-1} ± SE	Mean number of seed pod^{-1} ± SE	Mean number of nodes plant^{-1} ± SE	Mean mass of a 1000 seed (g) ± SE	Mean yield plant^{-1} (g) ± SE
A2019 (25 ml ha^{-1})	20.14 ± 1.46a	11.0 ± 1.62a	7.70 ± 0.48a	160.86 ± 5.16a	2.75 ± 0.05a	21.29 ± 0.98a	166.64 ± 3.85a	73.93 ± 3.96a
A2019 (50 ml ha^{-1})	15.71 ± 2.26a	14.29 ± 1.00a	7.09 ± 0.18a	144.71 ± 4.23a	2.87 ± 0.04a	20.43 ± 0.49a	188.57 ± 7.72a	78.06 ± 2.92a
A2019 (100 ml ha^{-1})	17.14 ± 1.81a	11.14 ± 0.82a	7.04 ± 0.24a	149.00 ± 5.91a	2.78 ± 0.05a	21.14 ± 0.37a	178.04 ± 9.17a	72.81 ± 2.79a
B2019 (15 ml ha^{-1})	19.63 ± 1.10a	12.50 ± 0.71a	8.05 ± 0.66a	161.25 ± 4.74a	2.75 ± 0.04a	20.13 ± 1.17a	181.39 ± 5.71a	80.37 ± 3.26a
B2019 (30 ml ha^{-1})	18.88 ± 1.80a	15.13 ± 1.11a	6.98 ± 0.22a	150.13 ± 5.03a	2.80 ± 0.04a	21.50 ± 0.31a	167.76 ± 6.41a	70.22 ± 2.93a
B2019 (60 ml ha^{-1})	17.00 ± 1.56a	12.25 ± 0.82a	6.56 ± 0.30a	144.38 ± 5.14a	2.80 ± 0.05a	22.13 ± 0.45a	168.49 ± 8.13a	67.73 ± 3.16a
C2019 (5 g ha^{-1})	17.33 ± 1.74a	15.17 ± 1.19a	6.87 ± 0.14a	146.33 ± 2.66a	2.79 ± 0.06a	21.33 ± 0.38a	167.45 ± 7.82a	68.45 ± 3.91a
C2019 (10 g ha^{-1})	16.75 ± 3.01a	13.63 ± 1.75a	7.18 ± 0.38a	151.00 ± 8.99a	2.85 ± 0.05a	21.00 ± 0.47a	171.98 ± 7.39a	73.56 ± 4.74a
C2019 (20 g ha^{-1})	15.25 ± 1.21a	12.38 ± 0.58a	8.02 ± 0.37a	167.13 ± 7.44a	2.73 ± 0.06a	20.88 ± 0.28a	176.73 ± 7.23a	79.72 ± 3.61a
Control	17.82 ± 1.90a	10.55 ± 1.08a	6.74 ± 0.15a	145.91 ± 4.56a	2.82 ± 0.04a	21.64 ± 0.35a	188.41 ± 7.71a	76.66 ± 2.84a
F-P Value	$F_{9,68}$=0.6; P=0.8	$F_{9,68}$=1.9; P=0.06	$F_{9,68}$=2.0; P=0.06	$F_{9,68}$=1.7; P=0.1	$F_{9,68}$=0.8; P=0.7	$F_{9,68}$=0.9; P=0.6	$F_{9,68}$=1.2; P=0.3	$F_{9,68}$=1.5; P=0.2

Means within a column followed by the same letter are not significantly different at P<0.05 (Tukey's Unequal N HSD).

3.5 Discussion

The PGRs [A2019 (CPPU) (cytokinin), B2019 (NAA) (auxin), and C2019 (GA3 + S-ABA) (gibberellin)] were applied to increase the concentration of phytohormones participating in growth processes, to consequently increase soybean productivity. Auxin, gibberellins and cytokinins play an important role in developmental processes in plants (Rastogi *et al*., 2013; Reyes-Olalde *et al.,* 2017). Auxins primarily control growth by stimulating cell elongation (especially of shoots), apical dominance and root development, while gibberellins promote cell growth of stem, leaves and other aerial parts through cell elongation, and increase in internodal length (Rastogi *et al*., 2013). Cytokinins promote cell division and cell differentiation in meristematic tissues, delay leaf senescence and attract assimilates into sinks (Rademacher, 2015). Due to the physiological function of these hormones in plants it was expected that exogenous application of CPPU (A2019), NAA (B2019), and GA_3 (C2019) would increase growth and yield components of soybean.

Although no effect of the time of application of A2019 B2019 and C2019 on plant growth and yield parameters was found in this study, Sarkar *et al*. (2002) reported a single PGR application to soybean at 42 days after planting to be more effective than application at 20 days after planting. A double spray application (20 and 42 days after sowing) resulted in the highest increase in yield of soybean (Sarkar *et al*., 2002; Khatun *et al.,* 2016a; Solanke *et al.,* 2018). Multiple PGR applications are in most cases more effective than single applications (Sajjad *et al.*, 2017).

Height of the lowest pods above the soil level of a soybean plant is important to lower losses during harvesting of a crop. This height should be sufficient to allow for a mechanical harvester to harvest a maximum number of pods (Ramteke *et al*., 2012). Application of PGRs [A2019 (50 ml ha^{-1}), B2019 (30 ml ha^{-1}), C2019 (10 g ha^{-1})] at two reproductive stages, *viz*. R1 and R3 as well as an application at both the R1 and R3 stage at Potchefstroom and Bethlehem were unable to significantly increase the height of the lowest pods above soil surface. However, at Kroonstad, an application of C2019 (10 g ha^{-1}) at R1 caused a significant increase in height of the lowest pods above the soil surface. The height of the lowest pods was also not increased by the respective PGRs applied to soybean at different rates, except for plants treated with A2019 applied at 50 ml ha^{-1}. This result was, however not found in the pot trial and in the trial where the application time for A2019 was investigated.

It is, however, known that plant and pod height can be increased by GA_3 due to its effect on increasing cell elongation, division and internodal elongation (Emongor, 2007). This was demonstrated by Emongor (2007) who reported cowpea (*Vigna unguiculate*) (Fabaceae) plant height and height of the first node to be significantly increased by GA_3. Similarly, GA_3 increased

the lowest pod height of common bean (*Phaseolus vulgaris*) (Fabaceae) (Pavlista *et al.,* 2013). Similar results were not found in this study where A2019, B2019 and C2019, at different application times and application rates, with the exception of C2019 (10 g ha^{-1}) applied at R1, at Kroonstad only, did not increase the height of the lowest pods of soybean.

The number of seed pod^{-1} was also not increased by the application of PGRs regardless of the PGR treatment, time or rate of application. However, C2019 applied at growth stage R1 + R3 at 10 g ha^{-1} and 20 g ha^{-1} significantly reduced the seed pod^{-1} of soybean compared to the control treatment. This reduction in seed pod^{-1} were only found in the trial planted at the second planting date of the application rate trials. The same results were not found in the trials planted first or in the pot trials. Although the number of seed pod^{-1} can be affected by environmental conditions, it is largely under genetic control (Zhu and Sun, 2006; Liu *et al.,* 2015), but can be influenced by PGRs (Emongor, 2007; Agawane and Parhe, 2015; Krishnaveni *et al.,* 2016). GA_3 significantly increased the number of seed pod^{-1} of soybean (Azizi *et al.,* 2012) and cowpea (Emongor, 2007). Similar to these reports, the number of seed pod^{-1} was also reported to increase with NAA applications in soybean (Kalyankar *et al.,* 2008) and chickpea (*Cicer arietinum*) (Fabaceae) (Bangal *et al*., 1982; Muhammad *et al*., 2010) respectively.

An increase in the number of nodes per plant, results in more pods $node^{-1}$ and therefore also more pods $plant^{-1}$ (Nico *et al.,* 2016; Allen *et al.,* 2018). In this study, this effect was only found in the trials planted in the cold zone, Bethlehem. No increase in the number of nodes $plant^{-1}$, pods $node^{-1}$ and pods $plant^{-1}$ was induced by the PGR treatments in the field trial at Kroonstad (temperate zone). At Potchefstroom, C2019 applied at R1 + R3 at 10 g ha^{-1} and A2019 applied at R1 at 50 ml ha^{-1} increased only the number of nodes $plant^{-1}$ of soybean. At Bethlehem, plants treated with B2019 (30 ml ha^{-1}) at R3 significantly increased the nodes $plant^{-1}$, pods $node^{-1}$ and pods $plant^{-1}$. Application of B2019 (30 ml ha^{-1}) at R1 + R3 also significantly increased the nodes $plant^{-1}$ and pods $plant^{-1}$, but the increase in pods $node^{-1}$ were not significant. Similarly, B2019 (30 ml ha^{-1}) application at R1 significantly increased the pods $node^{-1}$ and pods $plant^{-1}$, but not the nodes $plant^{-1}$. The increase in pods $plant^{-1}$ caused by B2019 could be explained by increased photosynthetic processes and improved partitioning of accumulates from source to sink (Khan *et al.*, 2002; Bora and Sharma, 2006). NAA creates a high source-sink potential that accelerates the translocation of assimilates from source to sink and manifests in increased yield components (Basuchaudhuri, 2016b). Exogenous application of NAA has been reported to increase the number of pods $plant^{-1}$ of soybean (Kalyankar *et al*., 2008), chickpea (Muhammad *et al.,* 2010), groundnut (Devasenapathy *et al.,* 1987) and common beans (*Phaseolus vulgaris* L.) (Fabaceae) (Mohtashami *et al*., 2016).

A2019 applied at 25 ml ha^{-1} and 50 ml ha^{-1} under field conditions significantly reduced the nodes $plant^{-1}$, but no significant response was recorded in the pot trial. The reason for the reduced number of nodes $plant^{-1}$ in the field trial could be ascribed to natural variation within the trial, since it occurred only at the lower concentrations and is in contrast with results from the pot trial.

In contrast to studies done on GA_3 (Sarkar *et al.,* 2002; Bora and Sharma, 2006; Emongor, 2007; Ayyub *et al.,* 2013; Rathod *et al.,* 2015), C2019 did not significantly increase the number of pods $plant^{-1}$ of soybean at different application times and rates. The number of pods $plant^{-1}$ at Bethlehem was increased only by A2019 application at R3. No literature on the use of CPPU applied to legumes could be found. However, other synthetic cytokinins such as 6-benzylaminopurine (BA) have been shown to increase the number of pods $plant^{-1}$ (Carlson *et al.*, 1987; Nagel *et al.*, 2001; Nonokawa *et al*., 2007).

All PGR treatments during both planting dates at different application times and application rates did not increase the 1000 seed mass and yield of soybean. The lower mass of a 1 000 seed at Potchefstroom (second planting date), caused by C2019 applied at 5 g ha^{-1} (low GA_3 level), compared to the control cannot be explained. Results from application time and application rate trials are in contradiction with previous studies, that showed that NAA (Table 3.17), CPPU (Table 3.18) and GA_3 (Table 3.19) are able to increase yield of crops.

Planting date and environmental conditions affected soybean growth, development and yield (Hu and Wiatrak, 2012). The effect of planting time on soybean yield and other traits vary between locations (Rehman *et al.,* 2014). Changes in photoperiod, temperature and precipitation affect the duration of vegetative and reproductive growth and therefore yield (Hu and Wiatrak, 2012).

Trials in the current study were planted at different planting dates according to the climatic conditions which allowed for planting at the respective trial sites. In South Africa, an earlier planting date (20 October- 30 November), is recommended for soybean planting in cold areas such as Bethlehem. The recommended planting time for soybean in moderate areas such as Potchefstroom and Kroonstad is 1 November – 15 December (Liebenberg, 2012). The different planting dates at the three localities, resulted in different vegetative and reproductive growth periods at the respective localities. Due to rainfall that occurred late during the 2018/19 growing season, the last trial that was planted, was at Kroonstad on 20 December 2018, later than the recommended planting date. The growth period for plants in the trial at Kroonstad was therefore shorter and the height of the lowest pods above soil surface, number of nodes $plant^{-1}$ and pods $plant^{-1}$ were significantly lower than the control treatment of soybean planted at Bethlehem and Potchefstroom. A shorter vegetative growth period was reported by Rehman

et al. (2014) to result in shorter stems, less reproductive nodes and a shorter reproductive phase.

The growth parameters in the control treatment of soybean planted in the temperate zone, at Potchefstroom and Bethlehem (cold zone), were similar. The yield parameters, did, however, differ significantly between the zones. It could be ascribed to the climatic conditions at Potchefstroom during the study period. Trials at Potchefstroom were exposed to high temperatures and received little rain during the reproductive stages, R5 to R7. Drought stress during reproductive stages can reduce the carbon dioxide exchange rate, photosynthesis, sugar production and flow of metabolites to the expanding cells. This can decrease the duration of seed filling, seed size and seed yield of soybean (Hu and Wiatrak, 2012).

Possible reasons for the inconsistency of plant growth regulators in the application rate and application time trials is that the application rate of each of the PGRs used during this study were lower than what was reported to be effective on various crops for NAA (Table 3.17), CPPU (Table 3.18) and GA_3 (Table 3.19). This could possibly explain why inconsistent effects on growth and yield parameters were found. Auxins such as NAA can improve plant growth and yield parameters by increasing cell division, differentiation and expansion (Tantasawat *et al.,* 2015; Parveen *et al.,* 2017). NAA assists in translocating assimilates from source to sink, which in turn can increase crop yield (Basuchaudhuri, 2016b). Increases in yield of baby corn (*Zea mays* L.) (Gramineae) (Muthukumar *et al.*, 2007), tomato (*Solanum lycopersicum*) (Solanaceae) (Prasad *et al*., 2013), chili (*Capsicum annuum* L.) (Solanaceae) (Raj *et al.*, 2016), cucumber (*Cucumis sativus*) (Cucurbitaceae) (Tantasawat *et al.*, 2015), blueberry (*Vaccinium corymbosum*) (Ericaceae) (Milić *et al.*, 2018), okra (*Abelmoschus esculentus*) (Malvaceae) (Shahid *et al*., 2013; Gadade *et al.*, 2017)) and bitter gourd (*Momordica charantia*) (Cucurbitaceae) (Nagamani *et al.*, 2015) were reported resulting from NAA applications (Table 3.17). NAA concentrations that were reported to be effective in these studies (Table 3.17) were notably higher than what was used in the current study [15 ml ha^{-1} (1.95 ppm), 30 ml ha^{-1} (3.89 ppm), 60 ml ha^{-1} (7.78 ppm)] and were therefore possibly too low to increase growth and yield parameters.

CPPU are used on crops to increase yield by promoting fruit setting and fruit enlargement (Zeng *et al.,* 2016). Increased yield resulting from CPPU applications was reported for blueberry (NeSmith, 2002; Retamales *et al.*, 2014) and tomato (Mousawinejad *et al.*, 2014) (Table 3.18) by increasing cell division and cell elongation which enables fruit to expand to a larger size. The CPPU concentrations (25 ml ha^{-1} (0.93 ppm), 50 ml ha^{-1} (1.85 ppm), 100 ml ha^{-1} (3.70 ppm)) applied in this study was possibly too low to initiate a yield increase.

Similarly, GA_3 concentrations used in this study [5 g ha^{-1} (7.41 ppm), 10 g ha^{-1} (14.82 ppm), 20 g ha^{-1} (29.64 ppm)] were lower than what was reported to be effective on bitter gourd (Nagamani *et al.*, 2015), tomato (Prasad *et al.*, 2013; Akand *et al.*, 2015), groundnut (Hasan and Ismail, 2018), cowpea (Emongor, 2007), okra (Gadade *et al.*, 2017), strawberry (*Fragaria ananassa*) (Rosaceae) (Saha *et al.*, 2019) and soybean (Leite *et al.*, 2003; Azizi *et al*., 2012; Solanke *et al.*, 2018) (Table 3.19). In cowpea, the application of GA_3 increased yield through enhanced vegetative growth, dry matter accumulation and increased yield components (pod $plant^{-1}$, seed pod^{-1}, 100-seed weight) (Emongor, 2007). In the current study, yield components and yield were not increased by GA_3 applications. The low concentrations applied in the current study, might be the reason for the lack of effect on yield parameters and yield. Kumar *et al.* (2010) reported a GA_3 application at a low concentration (25 ppm) to be the least effective in increasing growth and yield of marigold plants, while 50, 100 and 200 ppm were more effective.

Table 3.17: Effective application rates of NAA on crops.

Crop	Dosage rate	Effect	Reference
Baby corn	40 ppm	Increase protein content and cob yield (kg ha^{-1})	Muthukumar *et al.* (2007)
Tomato	100 ppm	Increase plant height and yield	Prasad *et al.* (2013)
Chilli	75 ppm	Increase yield per plant and yield per hectare	Raj *et al.* (2016)
Cucumber	100 ppm	Increase number of branches, fruit length, fruit weight, fruit yield	Tantasawat *et al.* (2015)
Blueberry	20 ppm	Increase vegetative growth, berry size, fruit set and yield.	Milić *et al.* (2018)
Okra	150 ppm	Increase plant height, number of branches per plant, and yield per plant	Gadade *et al.* (2017)
Okra	100 ppm	Increase leaves per plant, branches per plant, plant height, pods per plant, pod length, pod fresh weight, seed yield.	Shahid *et al*. (2013)
Bitter gourd	200 ppm	Enhance vegetative growth, fruit and seed yield (Increase vine length, branches per plant, nodes per plant, fruit per plant, fruit weight, number and weight of filed seed, seed germination and vigour).	Nagamani *et al.* (2015)

Table 3.18: Effective application rates of CPPU on crops.

Crop	Dosage rate	Effect	Reference
Blueberry	10 ppm	Increase fruit set, and berry size	NeSmith, 2002
Blueberry	10 ppm	Increase fruit diameter and yield.	Retamales *et al.* (2014)
Tomato	20 ppm	Increase fruit mass, volume, density, length and width.	Mousawinejad *et al.* (2014)

Table 3.19: Effective application rates of GA_3 on crops.

Crop	Dosage rate	Effect	Reference
Bitter gourd	50 ppm	Enhance vegetative growth, fruit and seed yield (Increase vine length, branches per plant, nodes per plant, fruit per plant, fruit weight, number and weight of filed seed, seed germination and vigour).	Nagamani *et al.* (2015)
Tomato	80 ppm	Increase plant height and yield	Prasad *et al.* (2013)
Tomato	125 ppm	plant height, number of leaves, number of branches per plant, number of fruits, number of flowers, fruit clusters, diameter of fruit, yield per plant (kg) and yield per hectare.	Akand *et al.* (2015)
Groundnut	150 ppm	Increase plant height, number of branches per plant, total dry weight, number of pods per plant, pod yield, 100 seed weight, % shelling, oil content, protein content, seed moisture and germination percentage.	Hasan and Ismail (2018)
Cowpea	90 ppm	Increase cowpea plant height, first node height, leaf area and leaf number plant^{-1}, nodulation, plant dry matter accumulation, pod length, pod number plant^{-1}, seed number pod^{-1}, 100 seed weight, harvest index and seed yield ha^{-1}.	Emongor (2007)
Okra	100 ppm	Increase plant height, number of branches per plant, and yield per plant.	Gadade *et al.* (2017)
Strawberry	40 ppm	Improved vegetative growth, flowering, fruiting parameters, productivity and fruit quality.	Saha *et al.* (2019)
Soybean	100 ppm	Increased plant height, first node height, stem diameter, leaf area and dry matter production.	Leite *et al.* (2003)
Soybean	100 ppm	Increased plant height, chlorophyll content, dry weight, and seed yield of soybean.	Solanke *et al.* (2018)
Soybean	125 ppm	Increase lateral branches, pods per plant, seeds per pod, 1000 seed weight, economic seed yield, biological yield and harvest index.	Azizi *et al*. (2012)

The climatic conditions at the localities could also have contributed to the inconsistency in results obtained with the PGRs. Rainfall at all three localities was below the long-term average

rainfall. Plants in the trials were therefore exposed to water stress during critical growth stages (germination, flowering, pod set and seed filling). Environmental and growing conditions could influence the efficacy of PGRs, with negative or ineffective responses been reported (Foster *et al.,* 1991; Matysiak, 2006). Application of ethephon to barley (*Hordeum vulgare*) (Poaceae) only increased the tiller numbers during seasons with above average rainfall (Foster *et al.,* 1991). Similarly, the influence of trinexwas apac-ethyl (a GA inhibitor) on winter wheat (*Triticum aestivum*) (Poaceae) was more effective in increasing the yield potential (increase in seed per year) during seasons with abundant rainfall (Matysiak, 2006). In South Africa, PGR application (GA_3 and a combination of GA_3 and NAA) to sweet pepper (*Capsicum annuum*) (Solanaceae) did not increase yield when plants were grown at high temperatures (36- 45 °C) in a tunnel without temperature control. However, when these plants were grown under lower temperatures, 23 - 34 °C in a temperature-controlled tunnel, the PGRs caused an increased the yield (Maboko and Du Plooy, 2015).

Soybean that is planted earlier, will have a longer growing season, and therefore a higher leaf area per plant (Shegro *et al.,* 2010). Trials at Bethlehem where planted earlier than trials at Potchefstroom and Kroonstad. A bigger leaf area that accumulated more of the applied PGRs, along with higher rainfall and lower temperatures, could possibly explain why PGRs where more effective at Bethlehem than at Potchefstroom and Kroonstad.

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Chapter 4

Summary and General conclusion

Plant growth regulators (PGRs) are natural or synthetic compounds, primarily used to promote, inhibit or modify plant growth and development (Mir *et al*., 2010; Sharma, 2015). Plant growth regulators have successfully been used in agriculture and horticulture as a tool to increase production and aid in removing or reducing genetic and environmental barriers (Rademacher, 2015). In South Africa, studies investigating the role of PGR applications on commercial soybean cultivars are limited. Due to the potential to increase soybean yield with the application of PGRs under dryland conditions, a study was conducted to investigate the potential of PGRs to increase soybean yield. The objective of the study was to evaluate the efficacy of PGRs at different application times and rates to increase soybean yield in South Africa.

To determine the optimal time of application, two field trials with different planting dates were planted at Potchefstroom and Bethlehem, and one field trial was planted at Kroonstad. To investigate the optimal rate for PGR application, two field trials with different planting dates and one pot trial were conducted at Potchefstroom. The cultivar PAN 1521 R was planted in the field trials while LS 6161 R was used in the pot trial. Foliar treatments consisted of a control (untreated) and three PGRs. Plant growth regulators were applied at different growth stages (R1, R3, R1 + R3) to determine the optimal time of application, while three concentrations of each PGR were applied at growth stage R1 and R3 to determine the optimal rate of application.

Based on the results of this study, none of the PGRs (A2019, B2019 and C2019) affected plant growth parameters. No recommendation with regard to best time of application could therefore be made. Application of PGRs to soybean planted in Bethlehem (cold zone) was more effective than PGR applications at Potchefstroom and Kroonstad. It could possibly be ascribed to the earlier planting date, higher rainfall and lower environmental temperature in Bethlehem.

Although the PGR treatments had an effect on some of the growth parameters of soybean, none of these effects were consistent between the trials at the different localities. None of the PGR treatments at different application times increased the yield parameters (1000 seed mass and yield) of soybean, at Potchefstroom, Bethlehem and Kroonstad. None of the application rates of the respective PGRs, increased any of the growth and yield components in the field trials or the pot trial at Potchefstroom. These results on the application time and rates trials are not in accordance with those from previous studies showing that CPPU, NAA and GA_3 are able to increase yield of crops.

Possible reasons for the inconsistency of PGRs in the application rate and application time trials is that the application rate of each of the PGRs used during this study were lower than what was reported to be effective in previous studies. Trials at all three localities received below average rainfall and were therefore exposed to water stress during critical growth stages (germination, flowering, pod set and seed filling). Environmental and plant growth conditions could influence the efficacy of PGRs, with negative or ineffective responses been reported.

Differences in growth parameters were found between the control treatment of trials planted at Potchefstroom, Bethlehem and Kroonstad. The trial planted at Kroonstad was planted later than trials planted at Potchefstroom and Bethlehem. The shorter growth period at Kroonstad caused soybean to have a significant lower pod height above soil surface, number of nodes plant^{-1} and pods plant^{-1}. The yield parameters of the control treatment in the trials at Potchefstroom were significantly lower than at Bethlehem. It could be ascribed to the high temperature and low rainfall during the reproductive stages, (R5-R7) of the crop.

Future research recommendations:

- A concentration series of the respective PGRs should be evaluated to determine the range suitable for application to soybean in South Africa.
- Different cultivars can respond differently to PGRs, therefore more than one commercial soybean cultivar should be used in experimental trials.
- Since plants respond differently to PGRs under different growing conditions, the effectiveness of PGRs on dryland and irrigated soybean crops should be compared.
- Once the optimum application rates are determined, a cost analysis study should be conducted to determine if it is economically feasible to apply PGRs to commercial soybean in South Africa.

4.1 References

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