Effect of O_3 fumigation on photosynthesis and growth of quinoa and its interaction with drought and elevated CO_2

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ABSTRACT

South Africa is a water scarce country frequently experiencing drought and has an extremely energy intensive economy contributing to the escalating levels of air pollutants which are threatening food production and agriculture. The effects of water stress and elevated levels of O_3 and CO_2 were investigated on *Chenopodium quinoa* Willd. The physiological response of quinoa was evaluated by subjecting quinoa to severe drought (10% field capacity), moderate drought (20% field capacity), watered (75% field capacity) and well-watered (90% field capacity) conditions in the ambient environment. In a separate set of experiments well-watered and drought-induced quinoa plants were fumigated with 80 ppb O_3, 120 ppb O_3, 700 ppm CO_2 and 700 ppm CO_2 + 80 ppb O_3 in open-top chambers. Ozone exposure-thresholds for damage, elevated CO_2 and water stress effects were assessed by prompt chlorophyll *a* fluorescence induction kinetics. Chlorophyll *a* fluorescence data for both drought-induced and ozone treated plants exhibited a marked decrease in photochemical efficiency, active photosystem II (PSII) reaction centres per leaf cross section and increase in non-photochemical dissipation. Drought-induced plants had higher fluorescence intensity at the J-phase compared to the well-watered plants, indicating a decrease in the electron transport further than reduced plastoquinone (Q_A^-). The exhibition of a positive ΔV_K-band and ΔV_L-band by the plants under severe and moderate drought stress indicate that PSII was susceptible to drought stress. The functional antenna size of absorption (ABS/RC) and heat dissipation (DI_o/RC) was increased and energetic grouping of PSII units was decreased as drought stress progressed. Severe drought stress also decreased the amount of active PSII reaction centres (RC) per excited cross section (RC/CS). Exposure to elevated levels of CO_2 resulted in a significant increase in PI_{total} of the well-watered plants compared to drought-induced plants, which is an indication of the potential increase in photosynthesis. Drought-induced plants exposed to elevated CO_2, 80 ppb O_3 and CO_2 + O_3 had a higher energetic grouping and stability of PSII compared to well-watered plants from 14 to 21 days fumigation. The exhibition of a positive ΔV_L-band from the start in plants treated with 120 ppb O_3 indicates a strong concentration-dependent O_3-induced inhibition and decrease in parameters like reduction of end electron acceptors per reaction centre (RE/RC) and photosynthetic performance index (PI_{ABS} and PI_{total}). O_3 fumigation increased the stomatal conductance (g_{H2O}) under well-watered and drought-induced conditions indicating that the stomata

were possibly severely damaged by O_3. Exposure to severe drought stress and 120 ppb O_3 resulted in delayed flowering date, abortion of flowers and potential decrease in photosynthesis, biomass accumulation, total leaf area, plant height, and grain yield. Elevated CO_2 potentially increase photosynthesis and ameliorated the negative effects of O_3 in all variables.

Keywords: Chlorophyll fluorescence, crop yield, drought stress, elevated CO_2, JIP-test, O_3, quinoa, stomatal conductance (g_{H2O})

OPSOMMING

Suid-Afrika is ’n waterskaars land wat dikwels droogte ervaar. As gevolg van ’n baie energie-intensiewe ekonomie, wat bydra tot stygende vlakke van lugbesoedeling, word voedsel en landbouproduksie bedreig. Die uitwerking van watertekort en verhoogde vlakke van O_3 en CO_2 is in *Chenopodium quinoa* Willd ondersoek. In ‘n afsonderlike eksperiment, is die fisiologiese effek van verskillende watertoestande op *C. quinoa* geëvalueer, deur die plante bloot te stel aan ernstige droogte (10% veldkapasiteit), matige droogte (20% veldkapasiteit), waterryke (75% veldkapasiteit) en waterversadigde (90% veldkapasiteit) kondisies. Daarna is plante wat blootgestel is aan droë of waterryke kondisies begas met 80 dpb O_3, 120 dpb O_3, 700 dpm CO_2 en 700 dpm CO_2 + 80 dpb O_3 in ooptopkamers. Die invloed van osoon-blootstellingsvlakke, verhoogde CO_2 en water stres is geëvalueer deur spesifieke chlorofil *a* fluoressensie induksie kinetika. Chlorofil *a* fluoressensie data, vir beide droogte en osoon behandelde plante, het ’n merkbare afname in fotochemiese doeltreffendheid en aktiewe fotosisteem II (PSII) reaksiesentrums per blaar deursnee getoon en ’n gevolglike toename in nie-fotochemiese (hitte) kwytraking. Plante onderworpe aan ’n watertekort het ’n hoër fluoressensie intensiteit getoon by die J-fase, in vergelyking met waterryke plante. Dit dui op ’n afname in elektronoordrag stroomaf van plastokinoon (Q_A^-). Die verskyning van 'n positiewe ΔV_K-band en ΔV_L-band, in die fluoressensiekromme van plante onder matige en erge vogstremming, dui aan dat quinoa PSII reaksiesentrum sensitief is vir droogte. Die funksionele antennagrootte van absorpsie (ABS/RC) en hitte kwytraking (DI_o/RC) het verhoog en die energieke groepering van PSII-eenhede het afgeneem tydens verhoogde watertekort. Erge droogte verminder ook die fraksie aktiewe PSII reaksie sentrums (RC) per deursnit (RC/CS). Blootstelling aan verhoogde CO_2 vlakke, het gelei tot ’n betekenisvolle toename in die PI_{total} van die waterryke plante, in vergelyking met plante onder waterstremming, wat dus op hoër fotosintese dui. Plante onderworpe aan droogte en begassing met verhoogde CO_2, 80 dpb O_3 en verhoogde CO_2 + O_3 van 14 to 21 dae, het 'n hoër energieke groepering en stabiliteit van PSII getoon in vergelyking met waterryke plante. Die voorkoms van ’n positiewe ΔV_L-band van die begin af, in plante wat behandel is met 120 dpb O_3, dui op 'n sterk konsentrasie-afhanklike O_3 inhibisie en verlaging in parameters reduksie van eind elektron akseptors per reaksie sentrum (RE/RC) en fotosintetiese uitslag indekse (PI_{ABS} en PI_{total}). Begassing met O_3 het die huidmondjiegeleiding (g_{H2O}) verhoog onder waterryke en

droogte kondisies, wat moontlik kan dui op ernstige beskadiging deur O_3. Blootstelling aan ernstige watertekort en 120 dpb O_3 lei tot ’n vertraging in blomtyd, afspeen van blomme en ’n afname in fotosintese, opeenhoping van biomassa, totale blaaroppervlakte, plant hoogte en graanopbrengs. Dus, ’n hoër CO_2 blootstelling veroorsaak ’n potensiële toename in fotosintese en vererger die negatiewe gevolge van O_3 in alle veranderlikes.

Sleutelwoorde: Chlorofil fluoressensie, droogte, graanopbrengs, huidmondjiegeleiding (g_{H2O}), JIP-toets, O_3, quinoa, verhoogde CO_2

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LIST OF ABBREVIATIONS

ABS/CS	Phenomenological energy flux (per excited cross section of leaf) for light absorption
ABS/RC	The specific energy flux (per PSII reaction centre) for light absorption
CS	Excited cross section of leaf
δ	Probability for formation of end electron acceptors
δ_{Ro}	Efficiency of electron transfer from reduced plastoquinone (Q_A^-) to the PSI end electron acceptors
ET	Energy flux for electron transport
ET_o/CS_m	Phenomenological energy flux (per excited cross section of leaf) for electron transport
ET_o/RC	Specific energy flux (per PSII reaction centre) for electron transport
F_o	$F_{50\ \mu s}$, fluorescence intensity at 50 µs
$F_{100\ \mu s}$	Fluorescence intensity at 100 µs
$F_{300\ \mu s}$	Fluorescence intensity at 300 µs
F_J	Fluorescence intensity at the J-step (at 2 ms)
F_I	Fluorescence intensity at the I-step (at 30 ms)
F_M	Maximal fluorescence intensity
F_v/F_m or φ_{Po}	Quantum yield of primary photochemistry
g_{H2O}	Stomatal conductance
K_N	Non-photochemical de-excitation rate constant
K_P	Photochemical de-excitation rate constant
OEC	Oxygen Evolving Complex
OTC	Open-top chamber
PEA	Plant Efficiency Analyser
PI_{ABS}	Performance Index (potential) for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors
PI_{total}	Performance Index (potential) for energy conservation from photons absorbed by PSII to the reduction of PSI end acceptors
ppb	parts per billion
ppm	parts per million

PQH_2	Plastoquinol
PSI	Photosystem I
PSII	Photosystem II
φ_{Eo}	Probability that an absorbed photon will move an electron into the electron transport chain or Quantum yield of electron transport
ψ_{Eo}	Probability that a photon trapped by the PSII RC enters the electron transport chain
ψ_o	Efficiency with which a trapped exciton can move an electron into the electron transport chain
Q_A	Primary bound quinone
Q_A-	Primary bound quinone in reduced state
RC	Reaction center
RC/ABS	The density of active PSII reaction centres on a chlorophyll basis
RC/CS	The density of active PSII reaction centres per excited cross section
RE_o/RC	Electron transport from plastoquinol (PQH_2) to the reduction of PSI end electron acceptors
t_{FM}	Time to reach F_M (ms)
TR	Energy flux for trapping
TR_0/CS_m	The phenomenological energy flux (per excited cross section of leaf) for trapping
TR_o/RC	The specific energy flux (per PSII reaction centre) for trapping
V_J	Relative variable fluorescence at the J-step = $(F_{2ms} - F_o)/(F_M - F_o)$

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CHAPTER 1: INTRODUCTION

1.1 Introduction

Stress is a deleterious effect that limits plant growth and yield globally. Frequent exposure to biotic and abiotic stress factors on sensitive plants may lead to plant death. Abiotic stressors like drought and ozone have become increasingly worrying due to their negative impact on crop productivity (Kimball, 2004; Hopkins and Hüner, 2006; Kudoyarova *et al*., 2013; Scheepers *et al*., 2013).

South Africa is a water scarce country and is frequently experiencing drought. Drought stress is a key environmental factor limiting global crop production as one third of the global land is classified as arid and semi-arid (Bohnert *et al*., 1995; Dai, 2011). The increasing global human population, food demand and multiple effects of water scarcity in agriculture is a major concern (Millar and Roots, 2012; Shabala, 2013) as crop growth and yield decline in drought stressed plants (Souza *et al*., 2004).

More than 80% of South African arable land is semi-arid receiving an average rainfall of less than 500 mm annually. Drought resulting from climate change is projected to become more frequent and intensified in many regions including Southern Africa. Drought has been responsible for some of the catastrophic famines in the world. Current water usage trends indicate that the water demand might exceed the availability for irrigated agriculture by 2025, due to a rapid increase in the population, industrialisation and urbanisation (IPCC, 2001; IPCC, 2013). Given that there is severe shortage of water resources and frequent drought in South Africa, the expansion of arable land is expected to be limited. Increasing the plant water use efficiency and venturing into the use of arid and semi-arid regions for further expansion of irrigation is important for food production to support a growing human population (Deng *et al*., 2003b).

Air pollution is the major environmental problem throughout the world. South Africa is committed on supplying affordable energy to grow its economy. The extensive use of the coal-fired power stations made it the 13th largest emitter of greenhouse gases in the world due to high use of fossil fuel (Heyneke *et al*., 2012; U.S. EIA, 2013; Hanneman *et al*., 2016). High levels of pollution are recorded in the Highveld regions where 90% of

South Africa's electricity is generated by more than 10 coal-fired power plants, of which five are among the largest in the world (Josipovic *et al*., 2010). There are large agronomic practices in this area and houses the country's main producers of grain crops like maize, sorghum and soybean (Tyson *et al*., 1996).

The elevated levels of tropospheric O_3 and CO_2 concentrations have prompted numerous studies evaluating the effect of these gases on the plant growth, physiology and ecosystem. Concentrations of these gases have been increasing intensely since the inception of the industrial revolution and are projected to escalate by the end of 21st century (Watson *et al*., 1990; IPCC, 2007; Fuhrer, 2009). These greenhouse gases can increase the mean air temperature and decreases precipitation, which forms a complex set of the possible future climate (IPCC, 2001). Drought may become common under a projected future climate (Krupa and Kickert, 1989). Most emissions are anthropogenic from biomass burning and combustion of fossil fuels (Fowler *et al*., 1999; Keeling *et al*., 2009). At the same time, these tropospheric gases are increasing in developing countries (Ghude *et al*., 2008). These gases contribute heavily to the greenhouse effect and have a direct impact on plant physiology and crop production (Bowes, 1993; IPCC, 2007).

The climate decisively affects agricultural productivity and potential yield changes can cause adverse economic consequences on the economy which is based on agricultural production (van Dingenen *et al*., 2009). The effects of climate change on agricultural yields vary on crops and regions as the effect and concentration of environmental stressors can vary regionally and may act antagonistically. Maize and wheat yields have been negatively affected in many regions and with medium confidence on a global scale. Soybean and rice yield losses has been smaller in major production regions and globally (IPCC, 2013).

Recent studies indicate that approximately 7% of the global human population will be exposed to reduced renewable water resources of at least 20% for every increase in degree Celsius of global warming. In addition, the renewable surface water and ground water found in the subtropical regions are expected to be severely reduced, which will intensify the competition for water in ecosystems, settlements, agriculture, industry and energy production (IPCC, 2013). It is, therefore, important to identify plants that are

resilient and that can withstand various environmental stressors while still being productive.

Anthropogenic CO_2 emissions are estimated to increase and intensify the frequency of droughts in many parts of the world (IPCC, 2013). In addition, climatic variations are also estimated to exceed pre-industrial conditions by 2040 (Mora *et al*., 2013). Agricultural yields are expected to be negatively affected due to the unpredictable growing conditions caused by these climatic variations. Furthermore, elevated levels of CO_2 are capable of escalating the photosynthetic rate and reducing stomatal conductance (Ainsworth and Long, 2004).

However, high levels of CO_2 mitigate the effects of O_3 damage and temperature (Parry *et al*., 2004; Rai and Agrawal, 2008). The effects of elevated CO_2 concentrations on the crop yield is underestimated, due to various limitations such as the availability of nutrients that become more important over time in various environments (Kimball, 2004).

Studies also indicate that increased atmospheric CO_2 concentrations can also reduce the stomatal conductance and improve the intrinsic water use efficiency of the plants. Reduction in the inherent water use efficiency of the plants will result in an augmented plant biomass and productivity (Hamerlynck *et al*., 2002; Kimball, 2004; Kumar *et al*., 2014).

C_3 plants respond positively to the increases in the CO_2 concentrations by increasing the photosynthetic rate and reducing the stomatal conductance. This response has the potential to reduce the sensitivity of the C_3 plants to changes in the water availability (Ainsworth and Long, 2004; Leakey *et al*., 2009).

Plants with the C_4 pathway, for example, maize show smaller response to elevated CO_2 levels when compared with the C_3 plants. Elevated CO_2 levels stimulate a reduction in the transpiration of the C_4 plants. As a result, the water use efficiency is primarily controlled by transpiration (Cousins *et al*., 2001).

Elevated CO_2 ameliorates the negative effects of O_3 damage on the plant photosynthesis and growth (Krupa and Kickert, 1989). Drought and elevated CO_2-

induced stomatal closure can limit the influx of O_3 into the plant, therefore, reducing O_3-induced yield losses (McLaughlin *et al*., 2007; Xu *et al*., 2009). At the same time, severe drought stress can drastically constrain and suppress the ability of elevated CO_2 to stimulate plant growth and productivity (Smith *et al*., 2000; Morgan *et al*., 2001; Xu *et al*., 2007b; Leakey *et al*., 2012). However, O_3 sensitive plants that are experiencing drought can fail to close their stomata completely which can result in an excessive water loss, a high O_3 flux into the plants and an overall poor plant performance (McLaughlin *et al*., 2007; Wilkinson and Davies, 2009, 2010).

Escalating levels of O_3 are a threat to food production and agriculture as a whole, as plant adaptation strategies to O_3 are not yet well understood. Surface O_3 has been observed as one of the most serious air pollutants causing severe damage to health and ecosystems. This gas is the most widespread and phototoxic produced gas that often exceeds World Health Organisation (WHO) air quality guidelines for agricultural crops across the globe (Fuhrer and Booker, 2003). It is also viewed as one of the major phytotoxic air pollutants on the crop yield (IPPC, 1992; Pleijel, 2011; Teixeira *et al*., 2011; Wilkinson *et al*., 2011).

Climatic changes are expected to further contribute to the escalation of surface O_3 (Wu *et al*., 2012). It is produced as a result of photochemical reactions in the troposphere by catalytic oxidation of nitrogen oxides (NO_x), carbon monoxide (CO), methane (CH_4) and volatile organic compounds (VOCs) (Felzer *et al*., 2007). O_3 production is more prominent during summer when there is a high temperature, solar radiation and pressure systems due to elevated levels of NO_x and VOCs emissions (Mauzerall and Wang, 2001).

Crop damage that is O_3-induced is expected to offset a significant chunk of the growth domestic product (GDP) growth rate, especially in the countries with the economy based in agriculture. Agriculture has been one of the major pillars to the South African economy for decades. Global yield reductions are estimated to range from 2.2 to 5.5% for maize, 3.9 to 15% for wheat and 8.5 to 14% for soybean in the year 2000 (The Royal Society, 2008; van Dingenen *et al*., 2009; Avnery *et al*., 2011a; Avnery *et al*., 2013).

The consequent O_3 damage on the plants will lead to the reduction of photosynthesis, biochemical and important physiological functions, but will also vary according to the

species (Reich, 1987; Felzer *et al*., 2007). Global yields are predicted to decrease further as the concentrations of surface O_3 is still increasing by an average of 0.3 ppb per year. O_3-induced global yield losses are projected to range between 4 and 26% for wheat, between 9.5 and 19% for soybean and between 2.5 and 8% for maize by the year 2030 which will be worth 35 billion US$ (Avnery *et al*., 2011b).

O_3 episodes are frequently coupled with climatic conditions that also induce soil drying. The frequency of episodes are also expected to escalate, as polluted areas can have up to 400 ppb of O_3 during peak time and is expected to be intensified in Southern Asia and Africa (The Royal Society, 2008). These generally threaten food supply and agricultural sustainability, because ambient O_3 concentrations can prevent complete stomata closure of O_3 sensitive species. The latter can expose plants to other detrimental environmental stressors like drought stress and high vapour pressure deficit. The challenge remains huge, as farmers may be unaware of the O_3 cumulative effects on biomass accumulation and grain filling as they are only measured post-harvest (Singh and Agrawal, 2010). It is of great concern to know that O_3 concentrations are predicted to rise to the highest levels in areas where the population is rapidly increasing and water is likely the scarcest commodity (Bates *et al*., 2008; The Royal Society, 2008). South Africa is a water scarce country frequently experiencing drought and has an extremely energy intensive economy contributing to an average of 60 ppb O_3 in the Highveld region (Josipovic, 2010). This region is an economic hub of South Africa which also contributes immensely to food security as main grain producers are located in this region (Heyneke *et al*., 2012). Urgent intervention is required in reducing ozone precursor emissions and management methods need to be developed in this region.

Quinoa (*Chenopodium quinoa*) has been identified as a C_3 crop of great value, due to its resilience to climatic changes. Currently, the cultivation of quinoa in temperate and tropical regions has gained global attention, due to its ability to thrive in various stressed conditions and because of its high nutritive value (Risi and Galwey, 1991; Jacobsen *et al*., 1996; Jacobsen *et al*., 2003; Bhargava *et al*., 2007). Apart from these, its grains contain a 14 to 18% protein content, and a wide range of minerals, vitamins, oil and antioxidants (Koziol, 1992; Repo-Carrasco *et al*., 2003; Comai *et al*., 2007; FAO, 2013).

A study by Geerts *et al*. (2008) showed that deficit or supplementary irrigation in semi-arid regions can be beneficial in stabilizing quinoa production while increasing water productivity. In addition, Lanino (2006) also showed that irrigation is not required in the areas that receive between 150 mm to 170 mm annual rainfall. More so, quinoa is capable of producing high protein grains under drought, cold and salinity conditions which necessitate its importance for crop diversification in dry areas for future agricultural systems (Bhargava, 2003a).

On account of its nutritional quality and its ability to thrive under harsh environments, quinoa was identified as one of the crops which can play a huge role in fighting malnutrition globally and securing food security by Food and Agriculture Organisation, which has also designated it as food of the year in 2013 (Bazile *et al*., 2015). Quinoa can be potentially explored in South Africa as an all year round cash crop, because it can easily adapt to diverse habitats (Jacobsen, 2001; Bhargava *et al*., 2003a).

1.2 Problem statement (s)

Nearly 3 million child deaths globally are linked to undernutrition which is a result of low birth weight, protein-energy deficiency and deficiencies of vitamins and minerals. In addition, 25% of South Africa's children suffer from undernutrition, which significantly influences their health, physical and intellectual development and economic productivity at a later stage (www.unicef.org/publications/index.html).

South Africa is a water scarce country, which is frequently experiencing drought. Water shortage poses a great challenge to plant production due to low rainfall, high evapotranspiration rate and poor soils with low water retaining capacity. Annual precipitation in subtropical regions is expected to decrease due to climate change in the next decade. Drought is one of the main constraints in agriculture worldwide as one third of the global land is classified as arid and semiarid. Agricultural food production needs to increase by 50 to 70% by 2050 to match the 9.3 billion projected population growths. Available arable land will not be able to meet the demand and there is about 5.2 billion hectors of dry land that is used for agriculture.

High levels of pollution are recorded in the Highveld regions where 90% of South Africa electricity is generated and main grain producers are located. The average O_3

concentration range between 40 to 60 ppb in this region (Josipovic, 2010). The threshold O_3 concentration of 40 ppb is considered to represent a risk for crops like maize, wheat and soybean (Zunckel *et al*., 2004). These three major crops that are grown in this region suffer great losses (from 2.2 to 5.5% for maize, 3.9 to 15% for wheat and 8.5 to 14% for soybean) when exposed to high levels of O_3 (van Dingenen *et al*., 2009; Avnery *et al*., 2011a; Avnery *et al*., 2013).

1.3 Motivation

Subtropical regions renewable surface water and ground water are expected to be severely reduced, which will intensify competition for water in ecosystems, settlements, agriculture, and industry and energy production. Therefore, it is important to identify plants that are resilient and that can withstand various environmental stressors while still being productive. Furthermore, new O_3-tolerant plant species and varieties that can address malnutrition and food security must be introduced.

Quinoa has the ability to thrive in various stress conditions and has a high nutritive value, which can help South Africa to address malnutrition. It has the potential to produce high protein grains under drought, cold and salinity conditions which necessitate its importance for crop diversification in dry areas for future agricultural systems. In addition, quinoa can be potentially explored as an alternative crop in South Africa as it can easily adapt to diverse habitats.

There are no studies that have been conducted investigating the combined effects of water and O_3 stress and elevated CO_2 on quinoa. Elevated levels of CO_2 directly promote plant growth, or indirectly by allowing effective photosynthesis at a reduced stomatal conductance while enhancing water use efficiency, especially in the C_3 plants such as quinoa. It can also ameliorate the negative effects of O_3. These warrant the assessment of the crops response based on the South African conditions, as there is no study done on quinoa. The assessment on the physiological response of quinoa to drought and O_3 stress and elevated CO_2 will provide valuable information to the quinoa producers and the entire quinoa industry. The assessment of drought stress and ozone exposure-threshold levels for damage by analysing the physiological state of the photosynthetic machinery by means of chlorophyll *a* fluorescence will stretch the understanding on how quinoa tolerates various environmental stressors. Phenological

advancement of quinoa under these conditions is vital, as different cropping cycle length could hinder planning of good agricultural practices and labour.

1.4 Hypothesis

- Exposure of quinoa to drought and O_3 stress, elevated CO_2 and elevated CO_2 + O_3 treatments cause changes in the flowering date, decrease photosynthesis, biomass accumulation, total leaf area, plant height, and grain yield.
- PSII fluorescence parameters are more suitable than PSI fluorescence parameters as an indicator of drought and O_3 stress.
- Elevated CO_2 could prevent O_3-induced damage in the photosynthetic efficiency of quinoa.

1.5 Objective(s)

The main objectives of this study was to test whether drought stress and elevated CO_2 would offer quinoa a better protection against elevated levels of O_3. To quantify the effects of different water regimes (WR) and a combination of drought and O_3 stress and elevated CO_2. To determined the physiological responses and tolerance threshold levels of quinoa at different phenological stages by analysing and comparing changes in the PSII photochemistry parameters derived from the fast chlorophyll *a* fluorescence data using JIP-test.

1.6 Goals

- To determine if severe water stress (WR1) could induce down-regulation of the photochemical activity in quinoa by deactivating PSII RCs.
- To evaluate JIP-test as a measure to identify differences in the PSII and PSI behaviour of drought stressed quinoa.
- To ascertain the extent of O_3 damage and drought stress and the effects of elevated CO_2 and CO_2 + O_3 on the stomatal conductance, photochemical efficiency, flowering, biomass accumulation, total leaf area, plant height, and grain yield.
- To determine if elevated CO_2 could prevent O_3-induced damage in the photosynthetic efficiency of quinoa.

CHAPTER 2: LITERATURE REVIEW

2.1 Drought stress

Drought reduces the soil water potential, which creates a cumbersome condition for plants to maintain their water balance. However, plants withstand these conditions by reducing transpirational losses through partial stomatal closure and reduced stomatal conductance. The turgor pressure of the guard cells regulates the stomatal opening and accumulation of registered active osmotic substances in the guard cells, which provides a high turgor pressure and cell water holding capacity (Roelfsema and Hedrich, 2005). The reduced leaf water potential cannot substantially change stomata cell volume which decreases the role of hydropassive closure. Thus, osmosensors triggers the stomata to respond directly to the changes in the cell membrane tension, while inducing their closure after a cascade of reactions (Luan, 2002).

Plants generally respond to water stress by closing their stomata and restricting water loss by means of maintaining the water content within the cell tissue and increasing their capacity to absorb water. Stomatal closure induced by the water stress is triggered via a reduction in the hydrostatic pressure of the guard cell walls. When a plant contains a high water potential, a decrease in the stomatal conductance and transpiration rate becomes a critical physiological response for the plants to save water (Franca *et al.*, 2000; Hopkins and Hüner, 2006; Ma *et al*., 2008).

2.2 Soil water potential

Soil moisture determines the plant productivity and the water status, as it represents the availability of a water resource. Available soil water controls the plant growth, the water use, leaf area expansion and the stomata patterns (Liu *et al*., 2007; Jacobsen *et al*., 2009). Different soil types may influence the rate of the stomata closure. Prolonged water stress induces the reduction in the rate of leaf expansion, the photosynthetic surface area and it may cause the process photosynthesis to cease completely. Photosynthesis is highly limited by the stomatal closure that hinders the CO_2 supply due to water stress (Hopkins and Hüner, 2006; Shahnazari *et al*., 2008).

The gradient of water potential facilitates the plant water transport and maintains water evaporation, as it exists between soil and atmosphere. The equilibrium between leaf and soil water potential induce partial stomatal closure, thus minimizing water loss due to transpiration at the expense of photoinhibition of the photosynthesis. More so, increases in transpirational water loss through the stomatal opening will result in the reductions of the leaf water potential and relative leaf water content (Kudoyarova *et al*., 2013).

During soil drying stomata respond differently to moderate and severe water stress. Quinoa tolerates drought through its extensive root system and intensify it through a reflective and hygroscopic white papillae found on the leaf cuticula. The reduced leaf water potential induces rapid stomatal closure, a reduced transpiration rate and photosynthetic rate. However, soil drying in the quinoa plants is safe guarded by a sensitive stomatal closure that enables the plants to maintain a high leaf water potential and a high photosynthetic rate, therefore, increasing the water use efficiency. In addition, oxalic acid is converted to CO_2 to enable plants to maintain a high photosynthetic rate when the stomata are closed, thus allowing a high water use efficiency (Sen *et al*., 1971; Jacobsen *et al*., 2009).

Water stress and a reduced soil water content also induces the increase of the soluble sugars that are among compatible metabolites and osmolytes. Plants with a low water potential reduce the osmotic potential as the soluble sugars accumulate in the roots and shoots (Rosa *et al.*, 2009). The accumulation and increase of the soluble sugars enables drought-induced plants to maintain leaf turgidity and it also protects the plants from protein and cell membrane dehydration (Ji *et al*., 2009).

The photosynthetic efficiency is reduced as the plants respond to water stress and elevated CO_2 by increasing and accumulating soluble sugars in the leaves (Wullschleger *et al*., 2002; Xu *et al*., 2007). High concentrations of compatible solutes reduces the water potential and ameliorates oxidative damage, therefore, maintaining the protein and membrane structure under moderate dehydration during drought spells (Erdei *et al*., 2002). More so, mild water stress can also increase the soluble sugar's concentration, thereby activating the plant osmotic adjustment (Zhou and Yu, 2009).

The photosynthetic machinery of quinoa is protected against oxidative stress in the developing leaves through osmotic adjustment which is induced by the accumulation of organic osmolytes (Shabala *et al*., 2012). However, the accumulation of the osmolytes requires a lot of energy, but it also reflects their osmoprotective role which differs in the plant age and the physiological competence of the specific tissue (Hariadi *et al*., 2011).

2.3 Carbon dioxide

Carbon dioxide (CO_2) is the first major variable gas component with an atmospheric background of 0.039% in clean air. Despite its relatively small concentration, CO_2 is the third most abundant gaseous component of the earth's atmosphere after nitrogen and oxygen and contributes in regulating the earth's surface temperature (Ballantyne *et al*., 2012; Dlugokencky and Tans, 2016). This greenhouse gas can increase the mean air temperature and decreases precipitation, which form a complex set of possible future climates (IPCC, 2001). Burning of fossil fuels and industrial processes have increased emissions of CO_2 from approximately 315 parts per million (ppm) over the past 50 years (Keeling *et al*., 2009) to a current atmospheric average of approximately 404.70 ppm (IPCC, 2013; Dlugokencky and Tans, 2016).

Global CO_2 concentrations are expected to continue rising to approximately 500 to 1000 ppm by the year 2100 (Watson *et al*., 1990; IPCC, 1992). Population growth and economic activities are the most important drivers of the escalating levels of CO_2 that result from combustion of carbon based fuels. The remaining net CO_2 emissions are contributed by terrestrial biota through respiration and clearing and burning of forest, which have drastically contributed to the current global atmospheric CO_2 concentrations (Stern *et al*., 1992; Holtz-Eakin and Selden, 1995; IPCC, 2007). Moreover, the current CO_2 emissions trend mainly reflects the energy-related human activities, which were previously determined by economic growth, particularly in developing countries (IPCC, 2013).

Despite its contribution to global warming CO_2 is currently regarded as the most important greenhouse gas, as it can increase the net photosynthesis and reduce stomatal conductance and the rate of dark respiration. It therefore, increases the plant height and biomass production at a whole-plant level (Teskey, 1995; Gunderson *et al.*, 2002; Norby *et al.*, 2005). It also enters plants through stomata which respond to the

environmental stimuli and reduction of the intracellular CO_2 concentration (Ainsworth and Rogers, 2007).

The increase of the atmospheric CO_2 concentration and temperature drastically influence plants as these distinct elements disturbs their life cycles and cause them to mature early. This interaction causes early flowering, a shortened seed filling stage and limit the benefits of elevated CO_2. In addition, most plants under elevated CO_2 have a high rate of photosynthesis, increased growth, decreased water use and a decreased concentration of nitrogen and proteins (Hamerlynck *et al*., 2002; Kimball, 2004; Possell and Hewitt, 2009; Kumar *et al*., 2014). More so, net carbon assimilation responds much more to the increased CO_2 concentrations at low latitudes than at high latitudes. However, at low temperatures, elevated CO_2 concentration can actually decrease plant growth (Kimball, 1983).

Enriched CO_2 concentrations alleviate the negative effects of O_3 on the plant photosynthesis and growth (Krupa and Kickert, 1989). Elevated CO_2 concentration can increase photosynthesis and stomatal closure, which reduces the O_3 entry into the leave cavities. Thus, the interaction of elevated CO_2 and O_3 in the plants reduces the activity of Rubisco and the regeneration of the ribulose bisphosphate (RuBP), which reduces plant damage. Conversely, prolonged stomatal closure limit carbon fixation and the amount of assimilates, which are availed for grains and leaves (Mulchi *et al*., 1992; van Oosten *et al*., 1992; Rai and Agrawal, 2008; Reid and Fiscus, 2008; Rai *et al*., 2010). The effects of elevated CO_2 on the yield and grain quality will influence the supply and nutritional value of quinoa products, as grains tend to lose weight and have decreased protein concentrations (Kimball, 2004).

2.4 Ozone

Human activities are increasing the background of O_3 concentrations by an average of 0.3 parts per billion (ppb) per year (Wilkinson *et al*., 2011). It is expected to escalate globally, but may be severe in developing countries, due to high combustion of fossil fuels, deforestation and changes in the land use patterns (The Royal Society, 2008; Fuhrer, 2009). These changes pose a great challenge to the developing countries, as they require more energy production for economic growth. The production and high demand of energy in these countries result in the increased production of NO_x, VOCs

and O_3 formation (Ghude *et al*., 2008). However, anthropogenic emissions are expected to change in response to the economic, climatic and political pressures and implementation of policies (IPCC, 2013).

High surface O_3 concentrations are especially more prominent in the cities and industrial areas in the late afternoon and lower concentrations are found during the morning. In contrast, high ozone concentrations are experienced before sunrise in the marine and high latitude areas and lowest concentrations are found in the afternoon due to reduced levels of NO_x concentration (Oltmans and Levy II, 1994). Polluted areas can have up to 400 ppb of O_3 during peak time. However, unpolluted areas can have background O_3 that range from 20 ppb to 50 ppb (Seinfeld, 1989), but stratospheric input can cause occasional background O_3 levels that can exceed 60 ppb (Lefohn *et al*., 2001).

The flux of O_3 into the leaf apoplastic space is determined by the stomatal conductance. Various climatic and atmospheric conditions influence O_3 uptake via the stomata. Stomatal responses to the surrounding environment drastically influence the plant's sensitivity and tolerance to O_3 (Heath and Tylor, 1997; Rao and Davis, 2001; Foyer and Noctor, 2005). However, short bursts of acute O_3 levels over 100 ppb are associated with the reduction of the stomatal conductance (Vahisalu *et al*., 2010).

Plants respond to the damage caused by O_3 and its secondary by-products by reducing photosynthesis, biochemical and important physiological functions, which result in weaker, stunted plants, poor crop quality and low yield. O_3 damage to the plant tissues includes visible leaf injury and increased senescence due to ethylene production. Low concentrations of O_3 may not induce visible leaf injuries, but can decrease photosynthetic carbon gain by accelerating leaf yellowing (Pell *et al*., 1997). It can also directly affect reproductive parts by reducing bud formation and flowering, thereby causing pollen sterility, induce flower, ovule, grain injury and abortion (Mulholland *et al*., 1998; Black *et al*., 2000; Fiscus *et al*., 2005; Morgan *et al*., 2006; Black *et al*., 2007; Booker *et al*., 2009; Fuhrer, 2009). Thus, ozone effects on the plant metabolism are primarily induced by an increased production of reactive oxygen species (ROS) inside and outside of the plant cell, which is a common feature of biotic and edaphic stresses. The production of ROS can overwhelm the antioxidant quenching capacity of the

apoplast (Kangasjärvi *et al*., 2005). These stress conditions may activate signal transduction pathways that involve salicylic and jasmonic acid and ethylene. Changes in the plant metabolism can alter the plant's efficiency to capture light energy, energy transfer into carbon and carbon partitioning into biomass and yield (Leadley *et al*., 1990). However, the intensity and type of ozone damage depends heavily on both ozone concentration and exposure dynamics (Heath and Taylor, 1997).

2.5 Quinoa

Quinoa (*Chenopodium quinoa* Willd.) is an annual nutritious pseudocereal crop belonging to the C_3 group traditionally grown in the Andes region of South America. The seeds are the main part of the plant which is consumed by humans. Quinoa also contain high concentrations of saponin component in the pericarp that is anti-nutritious which is also dependent on the cultivar (Ward, 2000). However, saponins can be potentially used in the pharmaceutical industries as analgesic and urinary tract disinfectant and in pest control for good agricultural practices (Mujica, 1994; San Martin *et al*., 2007).

2.5.1 Origin and History

Quinoa has been cultivated in the Andean region of Bolivia, Peru, Ecuador and Colombia, dating back to 5000 years AD. The archeological evidence of the seeds found in the Peruvian tombs (7000 years old), show that quinoa has been known during the ancient Inca times (Tapia, 1997). It occupied a prominent place in the Inca Empire followed by maize. However, in 1532 AD when the Spaniards colonized the Andean region they forced the natives to consume other cereals by suppressing quinoa cultivation. Green revolution failure in this region was accompanied by a massive destruction of other introduced crops by drought. This has forced the comeback of quinoa and other native crops, because of their resistance to Andean harsh conditions (Cusack, 1984).

2.5.2 Distribution

Quinoa has been introduced to a wide range of environments where it grows from sea level to a high altitude ranging from 2000 to 4000 m and to a higher latitude of 40˚S and

2°N (Pulvento *et al*., 2010). The distribution starts from Andean region of Ecuador, Bolivia, Columbia and Peru, where it was domesticated successfully 3000 to 4000 years ago for human consumption and animal feed. Recently it has been introduced in Europe, North America, Asia and Africa where it has also produced good yields (Jacobsen *et al*., 2003).

The world's main producers of quinoa are Bolivia, Peru and the United States of America. However, quinoa has crossed continental boundaries to reach Europe, Asia and Africa (FAO, 2013). Quinoa in South Africa is not widely used, but it is gaining popularity quickly through health practitioners that recommend it for its high protein content and nutritional benefits (Abugoch, 2009).

2.5.3 Classification

Quinoa is an annual dicotyledonous pseudocereal C_3 plant belonging to the genus *Chenopodium* and the family *Chenopodiaceae*, but also placed under *Amaranthaceae*. The scientific name of quinoa is *Chenopodium quinoa* Willd (Wilson, 1990).

2.5.4 Morphology

Quinoa is an annual plant with an erect stem of 0.5 to 2 m tall, terminating in a panicle consisting of small flowers, each producing one seed ranging from 2.5 mg and 1 mm in diameter (Geerts *et al*., 2008). It bears alternate leaves with green, purple and red colour due to the presence of betacyanins. When water deficit conditions are present, its taproot can penetrate roughly 1.5 m below surface (Jacobsen and Stolen, 1993).

The wide spectrum of colors present in the vegetative organs and perigonium cause the variability in the color of plants and inflorescences of quinoa. The pericarp often ranges from white, yellow, orange and red with brown and black found in the wild species (Jacobsen and Stolen, 1993).

Quinoa also contains hermaphrodite and unisexual female flowers. The hermaphrodite flowers are positioned at the distal end and produce five perianth lobes, five anthers and a superior ovary which contains two or three stigma branches. However, some cultivars show male sterility in some or all female flowers (Hunziker, 1943).

The fruit is a disc like achene with numerous layers of pericarp, perigonium and episperm that may be conical and/or cylindrical with saponins concentrated in the pericarp. The size of the seed and color vary among cultivars where black seeds are dominant over red, yellow and white seeds (Mujica, 1994).

2.5.5 Climatic, soil, water and fertilizer requirements

The ideal conditions for sowing quinoa seeds is 1 to 2 cm depth in a fine homogeneous, well structured, moist seed bed at a temperature of 8˚C to 10˚C with 60% relative humidity. Quinoa is sensitive to low photoperiod and require a short day length (10 to 12 hrs) and cool temperatures (16˚C to 22˚C) for good growth (Bertero *et al*., 1999a; Jacobsen *et al*., 2003). It thrives well in the moist, well drained sandy-loamy to loamy-sandy soils containing organic matter with a pH of 6.0 to 7.5. Quinoa has a low water requirement as a drought tolerant plant that only require 50 to 70 mm of the rain during plant establishment, flowering and seed filling (Geerts *et al*., 2008). However, pre-sowing irrigation is essential in the arid regions with poor water quality, which also receive 50 to 70 mm annual rainfall when adopting deficit irrigation (Lanino, 2006; Geerts *et al*., 2008). Organic matter and low nitrogen and phosphorus fertilization can increase quinoa yields during drought spells. However, high levels of nitrogen and phosphorus fertilizers can reduce seed yields due to delayed maturity and intense lodging (Oelke *et al*., 1992; Bhargava *et al*., 2003a).

2.5.6 Economic and social importance

The dry seeds are the ultimate economic part in a quinoa plant. Quinoa seeds contain 14 to 18% soluble protein, a balanced composition of amino acids, wide range of minerals, vitamins, oil and antioxidants and the highest percentage of Omega 6 fatty acids vital for a balanced diet (Comai *et al*., 2007; FAO, 2013). To mention a few, quinoa has also been used to make products such as flour, soup, pasta and other processed products like biscuits, bread, beer, flakes and pancakes. Green leaves can also be consumed as a vegetable, while the whole plant can be used as green silage to feed cattle, pigs and poultry (Mujica, 1994). These leaves also contain a high content of quality proteins, vitamins and minerals, especially calcium, phosphorus and iron. Furthermore, leaves specifically contains 3.3% ash, 1.9% fibre, 0.4% nitrates, 289 mg/100 g sodium, 82 to 190 mg/kg carotenoids, 2.9 mg α-TE/100 g vitamin E, 1.2 to 2.3

mg/kg vitamin C and 27 to 30 mg/kg proteins (Koziol, 1992; Prakash *et al*., 1993; Repo-Carrasco *et al*., 2003). Quinoa has been considered as a potential crop for NASA controlled Ecological Life Support System to produce food, oxygen and water for crew on a long term missions, while removing carbon dioxide from their surrounding environment (Schlick and Bubenheim, 1996).

The world production of quinoa in 2011 was 80.200 metric tons (MT) on 86.203 hectares (FAO, 2013). However, there is no data showing South Africa's contribution to the world production of quinoa, let alone growing quinoa commercially.

Jacobsen *et al*. (2003) showed that the world demand for quinoa is very high, but the supply by quinoa producing countries is insufficient. Mujica *et al*. (2001) reported that quinoa cultivation in Kenya produced high seed yield and quality that is comparable to that obtained in Andes region. Therefore, the introduction of quinoa in South Africa and other African countries can benefit farmers, crop diversification in the agricultural sector, while cabbing malnutrition and improving food security (Jacobsen *et al*., 2003). Quinoa can be easily cultivated by subsistence farmers as it can survive on manure or fertilizer residues from previous crop (Aguilar and Jacobsen, 2003).

2.5.7 Abiotic, biotic and production constrains

Water stress reduces the osmotic potential of the soil solutes and is an escalating problem in the plant production. Plants respond and adapt to different levels of multiple abiotic stresses by accumulating osmolytes and proteins necessary for stress tolerance (Munns, 2002; Gregory, 2006; Shabala and Shabala, 2011).

Quinoa displays a high level of tolerance to various environmental stressors like soil salinity, drought, frost, diseases and pests (Jacobsen *et al*., 2003). It is also capable of tolerating soil pH ranging from 4.8 to 9.5 due to mycorrhizal associations that enable quinoa to maximize the use of rare nutrients (Mujica, 1994). However, Geerts *et al*. (2006b) reported that moderate and severe water stress can decrease the development of the above ground biomass, but the roots kept on developing. Water stress can also have a considerable effect during flowering, seed filling and maturity, which can cause considerable loss in the crop yield (Geerts *et al*., 2008).

Plants induced to waterlogging stress show growth reduction, low specific leaf area, decline in photosynthesis, reduced respiration, stomata closure and biomass production (Gonzalez *et al.*, 2009). Oelke *et al*. (1992) also reported that excessive irrigation to the well-established plants could result in tall plants with poor yield and cause stunting in the plants and dumping off disease during seedling stage.

The most important disease of quinoa is downy mildew that reduce yield by 33 to 58% in humid areas (Danielsen *et al*., 2001). This disease is characterized by chlorotic lesions on the ventral surfaces of the leaves and white mycelium on the dorsal surface (Valencia-Chamorro, 2003).

Other soil borne diseases that are less widespread like brown stalk rot (*Phoma exigua var. foveta*), *Rhizoctonia* damping off, *Fusarium* wilt, leaf spot (*Ascochyta hylospora*), seed rot and damping off (*Sclerotium rolfsii, Pythium zingiberum*) can also lead to high yield loss. These diseases are regarded as potential quinoa production constraints in the areas which are dominated by frequent low temperatures and high humidity (Danielsen *et al*., 2003)

There are several taxa of herbivorous gregarious insects which attack quinoa. Major and potential pest insects are insects, which belong to Coleoptera (Chrysomelidae and Curculionidae), Diptera (Agromyzidae), Homoptera (Aphididae), Hemiptera (Lygaeidae), Lepidoptera (Gelechiidae), Orthoptera (Gryllidae) and Thysanoptera (Thripidae) taxa (Rasmussen *et al.*, 2003). These polyphagous insect pests can reach populations levels of economic importance and reduce yield production by 8 to 40% to the plants with less saponins (Ortiz and Zanabria, 1979; Yábar *et al.*, 2002). However, actual yield loss by the insects is dependent on many factors, which can differ over and within season and location (Rasmussen *et al.*, 2003). The natural enemies that are important for the control of these pest insects include Hymenoptera (Braconidae), Diptera (Syrphidae), Coleoptera (Coccinellidae) and Araneae (Lycosidae and Salticidae) taxa (Yábar *et al.*, 2002; Rasmussen *et al.*, 2003).

Birds can also cause minor damage to quinoa during the inflorescence stage. However, saponins confer minor resistance to damage caused by the pests and birds (Risi and Galwey, 1984; Yábar *et al.*, 2002).

Considerable increase in temperature and photoperiod after anthesis can reduce the seed diameter (Bertero *et al*., 1999a). In addition, temperatures ranging from 32^0C to 35^0C cause pollen sterility (Hunziker, 1943). Quinoa growth is merely affected by temperature ranging from -1˚C to -5˚C at all stages, but the vegetative stage can tolerate frost temperatures of -14˚C to -16˚C. However, low temperatures induce oxidative stress, which is facilitated by ROS and can result in cold-induced photoinhibition during high light incidence. Night radiative frost induces visible damage on the plant aerial parts which are characterized of the foliar senescence (Cui *et al*., 2003; Rapacz *et al*., 2004; Bois *et al*., 2006; Winkel *et al*., 2009). Furthermore, low night temperatures coupled with high daily solar irradiance result in the long-term reduction of the photosynthetic efficiency and stunted plants. Generally, seed germination and imbibition, radicle elongation and seedling growth are delayed by the cold temperatures (Rosa *et al*., 2004). Nina Laura *et al*. (2004) also reported that a quinoa crop cycle exposed to an average night temperature of 2˚C can decrease the plant height and biomass by almost 50%.

2.6 Stomatal conductance (g_{H2O})

The stomatal conductance for water vapor (g_{H2O}) is one of the most significant indicators of plants exposed to water deficit stress (Centritto *et al*., 2003). It is controlled via the signals provided by the mesophyll cells, with hormonal signals playing an important role (Serna and Fenoll, 1997, 2000). Different plant types may induce stomata closure through activation of the root growth and increase in the hydraulic conductivity in all plant organs (Razzaghi *et al.,* 2011).

Stomata closure also responds to water stress in order to compensate for the transpirational water loss through the leaf surfaces with the rate at which water can be supplied by the roots. All stomata opening and closure actions are responsive to the ambient humidity. Plants also regulate stomata through hydropassive closure of the stomata, which is the closure of the stomata via the direct evaporation of water from the guard cells. In addition, hydroactive closure of the stomata is triggered by a reduction in the water potential in the leaf mesophyll cells. This process involves abscisic acid (ABA), which accumulates in the water stressed leaves and inhibit stomata opening (Hopkins and Hüner, 2006).

Plants exposure to water deficit stress may trigger the production of abscisic acid (ABA) in the roots. It acts as a hormonal signal that is transported to the shoot through the xylem system. The reduction in the turgor pressure induce the production of ABA in the cytoplasm of the leaf mesophyll cells which explains the presence of high concentrations of ABA induced by low leaf water potential in the shoots rather than in the root tissue (Liu *et al.*, 2005b; Hopkins and Hüner, 2006). However, Christmann *et al*. (2005) reported that higher concentration of ABA was found in the shoots than in the roots of the fully irrigated quinoa plants. Furthermore, Jacobsen *et al*. (2009) also reported that the chemical signalling was prompted by a high leaf water potential without hydraulic signals detected.

Mild water deficit in quinoa induce a decline in the root water potential, which leads to the accumulation of ABA in the xylem sap. However, the stomata of quinoa seems to be insensitive to ABA under moderate water stress (Jacobsen *et al*., 2007). Moreover, reduced root and soil water potential triggers the rapid stomata closure, low stomatal conductance and decreased leaf expansion rate. But quinoa can maintain the process of photosynthesis for up to 3 days when the stomata are fully closed (Vacher, 1998; Liu *et al.*, 2004; Liu *et al*., 2005a; Jacobsen *et al*., 2007).

Normal stomata openings enable plants to take up CO_2 for photosynthesis during moderate water stress. Moderate water stress induces high rates of transpiration and leaf growth which increase the productivity. The maintenance of the required water content under this condition is managed by the increase in the root systems capacity to support the shoot with water. This helps the stomata to remain open, therefore maintaining gaseous exchange, photosynthesis and biomass accumulation. However, transpiration and water uptake are partially controlled by the plants through the directive of the stomata opening (Kudoyarova *et al*., 2013). During the onset of the water stress stomatal conductance of quinoa decrease quickly and significantly, but with low changes in the leaf water potential. Contrary to that, stomatal conductance and photosynthesis of quinoa can remain relatively stable even when drought persists (Vacher, 1998). Hence, leaf expansion can be prevented at low water potential regardless of a complete maintenance of the turgor pressure due to osmotic adjustment (Kirnak *et al*., 2001).

During severe water stress stomatal conductance is controlled by low leaf water potential which also reduces the turgor pressure (Liu *et al.*, 2005a; Razzaghi *et al.,* 2011). The rapid reduction of a leaf water potential during water stress allows the leaf water potential to remain high which favours the extraction of the soil water. Furthermore, quinoa improves and maintains high leaf water use efficiency to compensate for the gradual reduction in the stomatal conductance and optimisation of the carbon gains while minimising water loss (Vacher, 1998).

Quinoa resists severe drought stress by developing thick-walled cells in the vegetative tissues. These thick-walled cells preserve turgor pressure and vesicular glands of calcium oxalate on the leaf surface which increases the albedo (reflected sunlight). This adaptation strategy guarantees a sustained low photosynthetic rate and partial leaf dropping at the bottom quarter of the plant without suffering complete senescence (Vacher, 1998; Jacobsen and Mujica, 1999; Jensen *et al*., 2000; Siener *et al*., 2006). However, osmotic active compounds and sugars provide the leaf water holding capacity as a way to balance the plant water potential (Jang *et al*., 2003).

When it is impossible for plants to maintain the water balance and the water potential is maximally reduced, ROS generation escalate sharply. These occur to induce the plant protection mechanism against oxidative stress and dehydration, thus premature massive leaf senescence and abscission of the older leaves becomes the first priority (Dizes, 1992; Hopkins and Hüner, 2006). During this process the chlorophyll content is degraded and lipids are converted to the soluble sugars through gluconeogenesis. This strategy enables plants to recover nutrients from the dead cells and tissues, thereby relocating them to the living plant parts or by storing them in the roots (Hopkins and Hüner, 2006; Koyro, 2006). In addition, more vesicles are formed on the stems and leaves of quinoa, but the volume varies with the severity of the water deficit (Dizes, 1992). Furthermore, reduction in the stomatal conductance also induces a reduction in the plant yield (Shabala, 2013).

Ambient O_3 concentrations can prevent full stomata closure of the sensitive plants that are experiencing additional abiotic stress such as drought. Consequently, poor stomata closure under drought conditions can lead to excessive water loss and high O_3 flux into the plant that can result in a poor plant performance. Drought-induced stomata closure

can limit the influx of O_3 into the plant and reduce O_3-induced yield losses. However, O_3 may reduce sensitivity of the stomata closure during soil drying. The latter can reduce the effectiveness of the stomatal closure in drought-induced plants to prevent the O_3 flux and maximise the regulation of water loss. (McLaughlin *et al*., 2007; Wilkinson and Davies, 2009, 2010).

Elevated CO_2 and severe water stress can induce stomatal closure. Severe drought stress can damage the photosynthetic apparatus (Xu *et al*., 2009). The induction of the stomata closure during severe drought stress can decrease the photosynthetic stimulation and WUE at elevated CO_2 concentrations (Zhao *et al*., 2004; Warren *et al*., 2011).

2.7 Photosynthesis and Chlorophyll *a* fluorescence

Water stress affects photosynthesis through the stomatal closure and reduction of the cellular water potential, which deprive chloroplast the atmospheric CO_2 and compromise the structural integrity of the photosynthetic machinery, respectively. CO_2 availability is the limiting factor of photosynthesis. However, plants use resources efficiently by maintaining intracellular CO_2 levels at transition zone without excess electron transport and carboxylation capacity (Hopkins and Hüner, 2006).

Factors that affect photosynthesis at different CO_2 concentrations and irradiance are facilitated by the rate of ATP utilization in the intact leaves which is determined by the magnitude of the steady state fluorescence yield (Baker and Rosenqvist, 2004). Light severely magnifies the direct effect of water stress on photosynthesis as water stress exposes the plants to excess light by inhibiting CO_2 assimilation. The excess light that is absorbed by the photosynthetic pigments is not processed by the photosynthetic electron transport chain. Plant failure to dissipate excess light safely can lead to photoinhibition. However, the photosynthetic apparatus can be protected from excess light by means of photorespiration and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) enzyme during water deficit conditions. The energy that is absorbed is channelled to the fixing of O_2 when CO_2 supply is limited due to the stomata closure (Hopkins and Hüner, 2006).

O_3 negatively affects the biochemical photosynthetic processes of the susceptible plants. In addition, O_3 disrupt the plants defence mechanism against abiotic stresses like drought stress. It alters the sensitivity of the stomata to abscisic acid (ABA) of drought-induced plants and induces up-regulation of ethylene (Wilkinson and Davies, 2009, 2010). Ethylene inhibits the shoot growth and promotes ripening, leaf senescence and abscission (Morgan and Drew, 1997).

Photosynthesis forms an integral part of the plant metabolism and the sensitivity of chlorophyll *a* fluorescence to the environmental stressors has been used as a non-invasive tool to study the behaviour of the Photosystem II (PSII) (Krause and Weis, 1991; Govindjee, 1995; Strasser *et al*., 1999, 2000). Physiological processes affecting PSII functions also affect chlorophyll *a* fluorescence, which enables early stress detection on the plant physiological status (Baker and Rosenqvist, 2004).

Through chlorophyll *a* fluorescence mechanism, excess absorbed energy is re-radiated as fluorescence light emitted by a chlorophyll *a* of the PSII antenna (Krause and Weis, 1991). Measuring the chlorophyll indicates the characteristic changes in the intensity of chlorophyll *a* fluorescence. The characteristic changes are associated with the absorption and transfer of the light energy in the electron transport chain in the chloroplast (Krause and Weis, 1991).

Analysing the fluorescence data display only 2 to 10% of the absorbed light energy. It confers the opportunity to estimate mesophyll diffusion conductance, relative CO_2 and O_2 specificity factor for RuBisCo enzyme and the proportion of photon flux density absorbed by the photosynthetic pigments channelled towards PSII. These photosynthetic parameters provide an insight into the photosynthetic regulation and provide valuable information regarding the changes in the photosynthetic performance of the plant (Laisk *et al.*, 2002; Makino *et al*., 2002; Yin *et al.*, 2011).

Stress induced plants use small amounts of radiant energy for photosynthesis and use different mechanisms to safely dissipate excess light to prevent photoinhibition and photooxidation. When stressed plants receive excess amounts of photon energy than required for CO_2 assimilation, some of that energy is dissipated as heat in the PSII (Flexas and Medrano, 2002).

However, environmental stressors and normal ontogenetic changes may temporarily alter PSII before visible effects of the irreversible morphological damage are apparent (Naumann *et al*., 2008). Furthermore, chlorophyll *a* fluorescence changes that are ontogeny related can be larger than those induced by the environmental stressors and genotypic difference (Krebs *et al*., 1996; Winkel *et al*., 2002).

The onset of water deficit induces an increase in the chlorophyll *a* fluorescence, but severe water stress decrease chlorophyll *a* fluorescence (Winkel *et al*., 2002). Moreover, chlorophyll *a* florescence parameter is a powerful tool to assess physiological changes of different developmental stages and water status of the plants at leaf level (Krause and Weis, 1991; Strasser *et al*., 2000).

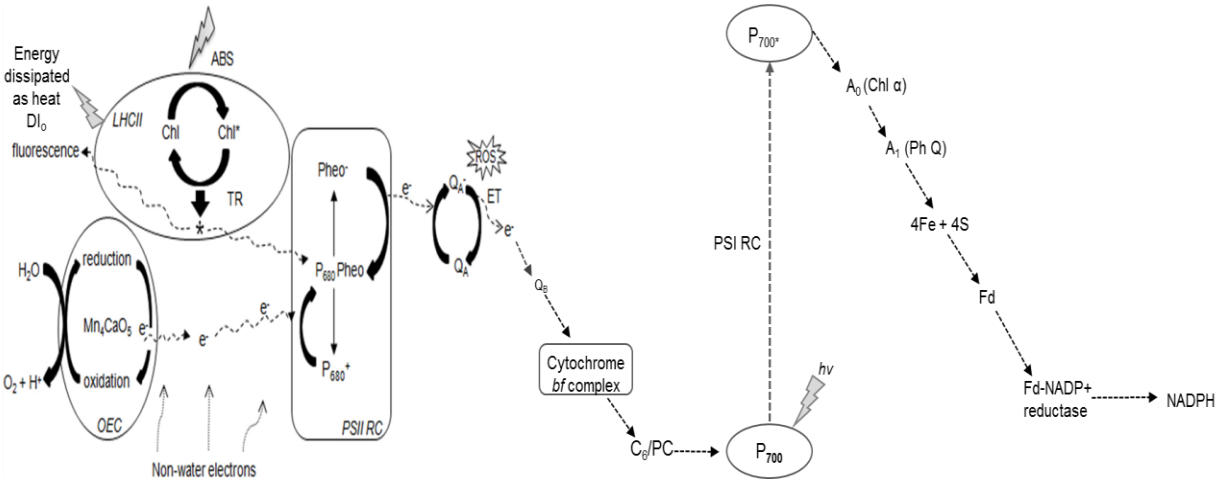


Figure 1. Hypothetical scheme of the photosynthetic electron transport and its relation to the emission of chlorophyll *a* fluorescence (Govindjee and Veit, 2010; Gururani *et al*., 2015).

Conversion of the light energy to chemical energy during photosynthesis occurs in three different basic consecutive steps, (i) ABS, absorption of photons by chlorophyll molecules in the light-harvesting complex (ii) TR, trapping of excitation energy by the reaction center (RC), and (iii) ET, electron transport through PSII reaction centers. The exposure of a plant to a short pulse of a strong light induces the absorption of the light energy by chlorophyll *a* which is used for photosynthesis. Hence, some light is emitted at a lower energy level as fluorescence. The chemical step occurs when an excited donor pigment molecule (P_{680}) donates an electron to pheophytin (Ph), producing oxidized Ph680 (Ph_{680+}) and reduced Ph (Ph^-) in PSII. The primary redox potential is

created within and around PSII. This occurs between a primary electron donor pigment Ph_{680^*} and an electron acceptor (Q_A) after the primary reactions of photochemistry which is determined by the integrity of the PSII (Figure 1). Despite the production of ROS molecules such as H_2O_2, environmental stressors disrupt the oxygen evolution at the oxygen evolution complex (OEC) of the PSII by reducing the flow of electrons to the reaction centers (RCs), thereby reducing the concentration of Q_A^- (Govindjee *et al*., 2010). Moreover, the reduced activity of the OEC is compensated by non-water electron donors for a short time, hence the fraction of the electrons donated by water is less in the water stressed plants (Figure 1). Intact manganese clusters (Mn_4CaO_5) at the OEC of the well-watered plants promote electron donation from water to the PSII reaction center and restricts the entry of non-water electrons (Gururani *et al*., 2015).

The JIP-test is used to interpret the original fluorescence measurements of the O-J-I-P transient into numerous phenological and biophysical expressions quantifying the PSII functions (Strasser *et al*., 2000; Strasser and Tsimilli-Michael, 2001). The O-J-I-P fluorescence transient is sensitive to environmental stressors (Krüger *et al*., 1997; Tsimilli-Michael *et al*., 1998, 1999). The analysis of the O-J-I-P fluorescence rise using the JIP-test (Strasser and Strasser, 1995; Srivastava *et al*., 1999; Strasser *et al*., 1999, 2000) provides a platform to measure the flux of energy passing through the photosystems and, assessing the photosynthetic performance of the plants and PSII function (Strasser *et al*., 2004; Tsimilli-Michael and Strasser, 2008).

Quantum yield of the primary photosystem (F_v/F_m) is one of the most employed parameters, as it provides evidence about the amount of the light absorbed by the chlorophyll pigment present in the PSII for photochemical processes (Genty *et al.*, 1989). This parameter only utilizes extreme values of the minimal variable fluorescence (F_o) and maximal variable fluorescence (F_m) of chlorophyll *a* fluorescence.

However, Force *et al*. (2003) elaborated on the benefit of using other JIP-test parameters to asses the PSII function. The advancement on chlorophyll *a* fluorescence techniques by Strasser *et al*. (2000) has led to the introduction of multi-parametric expression called the performance index (PI_{ABS}). The PI_{ABS} takes into account all main photochemical processes of the PSII reaction center complex, such as light energy

absorption, trapping of excitation energy, electron transport further than the primary plastoquinone (Q_A) and dissipation of excess excitation energy.

The total photosynthetic index (PI_{total}) and PI_{ABS} are considered to be the most sensitive parameters of the JIP-test and efficient tools to quantify stress in the plants (Tsimilli-Michael and Strasser, 2008). These parameters are suitable to investigate the plant's overall photosynthetic performance as they are sensitive to various environmental stressors (Appenroth *et al*., 2001; Hermans *et al*., 2003; Strauss *et al*., 2006; Christen *et al*., 2007; van Heerden *et al*., 2007).

2.8 Biomass

Biomass production and crop productivity is regulated primarily by the photosynthetic efficiency and the effective translocation of assimilates to the seeds which is measured by the harvest index. The rate of photosynthesis is determined by the concentration of the intracellular CO_2, which supplies CO_2 at the carboxylation site for the assimilation in the chloroplast. Plants use assimilated carbon during respiration to produce metabolic energy needed to increase and maintain biomass (Hopkins and Hüner, 2006; Bhargava *et al*., 2007).

Plant growth and productivity respond differently to drought severity and exposure period of the elevated CO_2 (Morgan *et al*., 2001; Luo *et al*., 2006; Xu *et al*., 2007a; Leakey *et al*., 2012). Elevated CO_2 concentration in the drought-induced plants acts as a mitigating factor by inducing a reduction in the stomatal conductance (Warren *et al*., 2011). Reduced stomatal conductance will reduce the amount of water loss, while increasing the osmotic adjustment and water use efficiency (WUE) of a leaf or whole-plant (Morgan *et al*., 2011; Nelson *et al*., 2004). Consequent increase in the WUE can deteriorate the plant water status and cause soil drying (Smith *et al*., 2000; Volk *et al*., 2000; Morgan *et al*., 2004; Wan *et al*., 2005; Zeppel *et al*., 2012). Plant growth stimulation by elevated CO_2 can be reduced under moderate drought stress and can possibly be inhibited under severe drought stress (Xu *et al*., 2007b Perry *et al*., 2013).

Water and O_3 stress induces reductions in the plant specific leaf area (Mulholland *et al*., 1998; McKee and Long, 2001; Wilkinson *et al*., 2011) which negatively affects the photosynthetic capacity. However, quinoa plants do not exhibit a significant reduction in

the leaf area during water stress (González *et al*., 2009; González *et al*., 2011). During water deficit, quinoa maintains a balance between the CO_2 assimilation and the transpirational water loss by decreasing the stomatal conductance. The consequent decrease in the stomatal conductance enables quinoa to reduce water loss, net CO_2 assimilation and transpiration rate, thus minimally reducing the plant dry weight (Munns, 2002; Shabala, 2013). The ability of the quinoa plant to regulate the stomata opening during the growing season determines the amount of CO_2 assimilate and biomass accumulation (Vacher, 1998; Shabala, 2013).

O_3 induces leaf injury, early leaf senescence and abscission and reduces the amount of healthy green leaf area that plants reserve for carbon fixation for biomass growth and grain filling (Mulholland *et al*., 1998; McKee and Long, 2001). The reduction of biomass growth can lead to the photosynthetic carbon fixation (Fiscus *et al*., 2005). These effects on a biomass growth are frequently increasing throughout the growing season (Singh and Agrawal, 2010).

2.9 Crop yield

Water stress and O_3 have detrimental effects on the plant growth and yield quality as they both induce reduction in the stomatal conductivity and leaf area. Plants also respond by synthesizing protective chemicals against O_3. But this defensive mechanism comes at a price as it can cause a reduction in a crop yield. It can also induce leaf injury, senescence, abscission and reduction in new growth, biomass and partitioning of available carbon to the seeds (McKee and Long, 2001; Betzerberger *et al*., 2010; Wilkinson *et al*., 2011).

Elevated CO_2 concentration in the drought-induced plants acts as a mitigating factor by inducing reduction in the stomatal conductance (Warren *et al*., 2011) and increasing the photosynthetic rate, growth, WUE and yield (Conroy *et al*., 1994; Kimball *et al*., 1999; Leakey *et al*., 2009). However, yield stimulation as a result of the elevated CO_2 can be reduced under moderate drought stress and can possibly be inhibited under severe drought stress (Xu *et al*., 2007b; Perry *et al*., 2013). Severe drought stress can reduce stimulation of photosynthesis which is caused by the elevated CO_2 (Houseman *et al*., 2003; Yu *et al*., 2012) as a result of damaged chloroplasts (Xu *et al*., 2009), changed chlorophyll components, reduced photosynthetic enzyme activity (Chaves *et al*., 2003)

and reduced efficiency of the PSII photochemistry (Hamerlynck *et al*., 2000; Xu and Zhou, 2006).

Water stressed plants prefer to decrease the osmotic potential and prevent a high reduction of the leaf water potential during water deficit conditions in order to maintain growth (Kudoyarova *et al*., 2013). Plants induced to moderate water stress produce high yield as they are able to maintain high rates of transpiration, leaf growth and gaseous exchange (Collins *et al*., 2008; Lawlor and Tezara, 2009). However, rapid growth of the vegetative mass can exhaust the water stored in the soil which is needed for the critical period of the reproductive organs initiation. More so, a high reduction of the leaf water potential and accumulation of ABA during initiation of the reproductive organs can cause massive pollen sterility and loss of grain quantity (Ji *et al*., 2011).

The ability of quinoa to temporarily double the photosynthetic rate ratio over transpiration during water stress when the leaf water potential is very low causes quinoa to reach comparable yield to the well-watered plants and higher water use efficiency (Geerts *et al*., 2008).

During severe water stress, plant and leaf growth are retarded, which influence the reduction of the water loss by leaves. The vein density of the leaves with reduced size tends to increase to allow plants to maintain water flow in other veins (Geerts *et al*., 2006b; Scoffoni *et al*., 2011). In addition, a reduction in the stomatal conductivity and leaf area disturbs gaseous exchange and photoassimilating surface, which drastically reduce yield (McDowell *et al*., 2008; Kudoyarova *et al*., 2013). Furthermore, quinoa yield reductions can be severe when plants experience drought during flowering and seed filling stages (Garcia *et al*., 2003). However, severe water stress that is only concentrated in the vegetative stages can stabilise the quinoa yield at a high level (Geerts *et al*., 2008).

2.10 Protein content

Quinoa seed protein content ranges from 14 to 18%, but it is dependent on the procedure used for seed purification (Koziol, 1992). The proteins mainly belong to albumin and globulin and contain a balanced composition of essential amino acids comparable to the composition of protein milk, casein (Repo-Carrasco *et al*., 2003).

The decrease in the protein content during water deficit conditions is associated with a decline in the photosynthetic gaseous exchange, chlorophyll *a* and chlorophyll *b* contents as well as the total chlorophyll content (Ranjbarfordoei *et al*., 2000). O_3 reduces the grain size, weight, number and nutritional quality (protein) of the O_3 sensitive plants (Mulholland *et al*., 1998; Fuhrer, 2009).

The reduction or inhibition of photosynthesis stimulation by the elevated CO_2 during severe water stress (Houseman *et al*., 2003; Yu *et al*., 2012; Perry *et al*., 2013) can reduce carbon fixation and the amount of assimilates availed for grains and leaves (Mulchi *et al*., 1992; van Oosten *et al*., 1992; Rai and Agrawal, 2008; Reid and Fiscus, 2008; Rai *et al*., 2010), due to prolonged stomata closure. Elevated CO_2 failure to compensate for the severe drought stress (Perry *et al*., 2013) could reduce grains weight and protein concentration (Kimball, 2004).

During severe water stress, the seed protein content is progressively reduced as the protein synthesizing apparatus of the plant tissue are changed, therefore, leading to a considerable decline in a plant's capacity to synthesize protein (Bhargava *et al*., 2007). Plant yield under this condition can also affect the protein content negatively and indirectly, but it is strongly dependent on the yielding accessions (Pleijel *et al*., 1999). However, quinoa produces a considerable high protein content during water deficit conditions owing to a high DNA and RNA content, which stimulates the synthesis and inhibit the protein decomposition (Ashraf and Foolad, 2007; Bhargava *et al*., 2007).

CHAPTER 3: MATERIALS AND METHODS

3.1 Experimental site and design

3.1.1 Water regimes (WR)

The experiment was conducted at the experimental station of the North-West University, Potchefstroom, North West province, South Africa (26°40′53″S, 27°5′57″E). Ten litre plastic pots with a 30 cm diameter were filled with a mixture of sand, soil and vermiculite with a ratio of 1:2:1. The 90 cm nylon wicks were rolled in a clockwise direction at different levels in the mixture, with one end protruding through a drainage hole(s) at the base of the pot to ensure uniform wetting. The 3 replicates of 1 to 4 water regimes were prepared with each regime representing the number of wicks the regime contains. The control had three wicks which represented 75% field capacity. One wick provided restricted water supply to induce severe drought stress (about 10% field capacity), two wicks induced moderate drought stress (about 20 to 30% field capacity), three wicks provided 75% field capacity and four wicks provided well-watered condition (about 90% field capacity) to avoid water lodging. Twenty eight grams of six-month slow-release fertilizer containing 17 nitrogen: 11 phosphorus: 10 potassium: 2 manganese oxide: TE (Osmocote® Pro) was added at various soil levels of each pot. Pots were placed over the buckets that served as water reservoirs, which were connected to a drip irrigation system that refill the water in the reservoir when necessary or once a week. The pots were placed in a fashion that they were not in contact with the water in the reservoir. Nylon wicks facilitated water uptake by capillary action to the soil medium.

3.1.2 Open Top Chambers (OTCs)

The experiment was conducted at the experimental station of the North-west University, Potchefstroom, North West province, South Africa (26°40′53″S, 27°5′57″E), using meticulous open-top chambers (OTCs). The experimental set-up consisted of twelve chambers was established at the experimental site, wherein the OTCs were in duplicate for each treatment (Heyneke *et al*., 2012). The control for drought-induced plants had one wick and the control for the well-watered plants had four wicks. One wick was used to induce drought stress (about 10% field capacity) and four wicks were used to

introduce the well-watered condition (about 90% field capacity). The same procedure above (3.1.1.) was used when filling pots with sand, soil and vermiculite and when applying fertilizer.

3.2 Soil-water status and pH measurements

The soil water content, conductivity and temperature were monitored every 60 minutes by using a ECH$_2$O Data Logger (Model DEm50, Decagon Devices, Pullman WA, USA) with soil moisture probes. The soil pH range suitable for quinoa was measured after mixing 1 g of soil with 100 ml of distilled water using a Jenway pH meter (Model 3520, Jenway Devices, Essex, England).

3.3 Plant cultivation

3.3.1 Water regimes trial

Quinoa (*Chenopodium quinoa* Willd.) seeds were hand sown in the polystyrene seed cell trays containing moistened vermiculite substrate in a greenhouse ventilated with ambient air during the pre-emergence phase at the North-West University. Plants were watered on alternate days for the first two weeks and after they were well established they were watered every day. These plants were manually transplanted into 30 cm diameter pots (two plants per pot) where watering was facilitated by wicks. Plants were placed in a randomised complete block design (RCBD) with 3 replicates for each regime.

3.3.2 OTCs trial

Quinoa seeds were sown in duplicate pots in the OTCs representing each treatment. Plants were manually watered on alternate days until they reach the 2nd leaf stage. The seedlings were thinned and only two plants were reserved. After plant thinning watering was facilitated by wicks in both well-watered and drought induced plants.

3.4 Fumigation treatments (O_3 and CO_2) in the OTCs

Plants were fumigated with elevated CO_2, O_3 and CO_2 + O_3 from 08:00 to 17:00 for six weeks in cylindrical OTCs with 1.5 meter diameter and 1.8 meter height. Carbon dioxide fumigation was initiated at 700 ppm. The selection of 700 ppm CO_2 level was based on

maintaining an atmospheric CO_2 concentration above current ambient levels of 404.70 ppm (Dlugokencky and Tans, 2016). At the same time, trying to understand how crops may need to be adapted to the changed and changing atmosphere (IPCC, 2013). The concentration range of 80 ppb O_3 and 120 ppb O_3 was selected to compare the data obtained from this trial with that of the studies done in the developed countries where O_3 levels of up to 200 ppb were used to study the physiological effects of O_3 at different concentrations (Pellegrini *et al*., 2011). Ozone generators containing UV lamps facilitated the break-down of oxygen and ensuring formation of O_3, while elevated CO_2 treatment was supplied by a regulated gas flow CO_2 cylinders. The treatments consisted of non-filtered chambers (NFCs) that received elevated CO_2 and O_3 and NFCs with 700 ppm CO_2 + 80 ppb O_3. The control OTCs received air that was filtered with charcoal (Purafil®) to remove gaseous pollutants like O_3, SO_2 and NO_2. The pots in the ambient air were provided to evaluate the effect of chambers on the plants, hence the treatments were in duplicates for each treatment.

3.5 Air quality monitoring

Air quality inside the OTCs was continuously monitored at regular intervals by using a Desktop CO_2 Logger (Model 7798 CO_2 /temp-relative-humidity monitor, A-Z Instrument Corporation, Inc., China) and O_3 monitor (Model 205 Ozone Monitor, 2B Technologies, Inc., USA) throughout the fumigation period from 08:00 to 17:00. A carbon dioxide monitor was also used to measure additional meteorological data like air temperature and humidity percentage. The airflow in each chamber was maintained at 10 $m.s^{-1}$ and it was monitored twice a day using the air flow meter (Model TA45 Airflow TM, Inc., UK).

3.6 Assessment of visible chamber and ozone symptoms on leaves

Plants were inspected daily in all OTCs, assessing chamber effect and the extent of ozone foliar symptoms. The number of senescent or dead leaves was recorded in all plant representatives for both well-watered and drought-induced plants. Measurements were done before and during fumigation to assess chamber effects and other environmental stress effects on the plants.

3.7 Sampling procedure

The leaves of the plants were numbered from the base of the plant to the top, the first leaf being the first to appear. Stomatal conductance and leaf temperature measurements were taken on the abaxial surface of the fully expanded upper canopy leaves without visible senescence signs by a steady state porometer (Model AP4, Delta-T Devices, Cambridge, UK) once a week between 06:00 to 09:00, 12:00 to 14:00 and 16:00 to 18:00 for a six week period. The fully expanded leaves were randomly selected on three plants in each water regime and fumigation treatment. Chlorophyll content was also measured on the same flag (canopy) leaves within the same time limit using chlorophyll content meter (Model CCM 300, Opti-science, USA).

3.8 Chlorophyll *a* fluorescence measurements and analysis of OJIP curves

The kinetics of the polyphasic prompt florescence rise, indicating Q_A reduction was measured *in vivo* using the Handy-PEA fluorimeter (Hansatech Instrument Ltd, King's Lynn, Norfolk, UK) on a dark adapted leaves. Plants were dark adapted for at least 1 hour prior to the start of the measurement. Measurements were taken at 4 different spots on the adaxial surface of 3 fully developed canopy leaves of 4 plants per treatment. Chlorophyll *a* fluorescence was recorded after illumination by a red actinic light of 3000 µmol photons $m^{-2}s^{-1}$ provided by 3 light-emitting diodes with 5 mm diameter focus spot and 12-bit resolution in 1s. Measured photo-induced transient readings were averaged using the Handy PEA software version 1.0 and analysed by PEA Plus data analyzer version 1.10 software (Hansatech Instrument Ltd, PEA Plus, King's Lynn, Norfolk, UK). The Handy-PEA fluorimeter data set points were set at 0.02 ms to 0.05 ms for initial fluorescence O step, intermediates steps J at 2 ms and I at 30 ms and peak P step at 300 ms. The curves were plotted on a logarithmic time-scale and exhibit a series of steps between the initial step O (F_o, when all the RCs of the PSII are open) and the maximum step P (F_m, when all RCs of the PSII are fully closed or reduced). The maximal fluorescence (F_m) and the minimal fluorescence (F_o) were used to calculate the F_v/F_m ratio, which is related to the quantum yield of the PSII photochemistry (Govindjee, 2004). The O-J (single turnover region) and J-I-P (multiple turnover regions) regions were also calculated. The JIP-test was used to calculate the prompt fluorescence transient induced by the first pulse of the red light according the

equations of the JIP-test, deriving the parameters such as the density of active Q_A reducing the PSII RCs per leaf cross-section (RC/CS_o) and the specific fluxes per active RC of the PSII (Strasser *et al*., 2004; Strasser *et al*., 2010; Yusuf *et al*., 2010).

The biophysical parameters and the performance index ($PI_{ABS,\ total}$) derived from the OJIP transient were calculated by using the average value of the original fluorescence data and the following parameters, which refer to time zero (start of the fluorescence induction) are (i) flux ratio of PSII; the maximum quantum yield of the primary photochemistry ($\varphi_{P0}= TR_o/ABS = F_v/F_m$); the efficiency with which a trapped exciton, having triggered the reduction of Q_A to Q_A^- can move an electron further than Q_A^- into the electron transport chain [$\psi_{E0} = ET_o/TR_o$], the quantum yield of the electron transport ($\varphi_{E0} = ET_0/ABS = \varphi_{Po} . \psi_0$) and the quantum yield of energy dissipation [$\varphi_{Do} = DI_o/ABS = 1- \varphi_{Po}$]; (ii) flux ratios of PSI: the efficiency of the electron transfer from reduced plastoquinone (Q_A^-) to the PSI end electron acceptors [$RE_o/ET_o = \delta_{Ro} = (1 - V_I)/(1 - V_J)$]; (iii) specific energy fluxes per reaction center (RC); absorption [ABS/RC= antenna size of PSII = $(M_o/V_J)\cdot F_M/(F_M - F_o)$]; electron transport [$ET_0/RC= (M_o/V_J\cdot(1 - V_J)$]; trapping ($TR_0/RC$); dissipation at the level of the antenna chlorophylls (DI_0/RC) and electron transport from PQH_2 to the reduction of PSI end electron acceptors [$RE_o/RC = (M_o/V_J)\cdot(1 - V_I)$]; (iv) phenomenological energy fluxes per excited cross section (CS): for absorption (ABS/CS), trapping (TR_0/CS), electron transport (ET_0 /CS) and dissipation (DI_0/CS); and (v) the fraction of active PSII-reaction centers per total absorption [$RC/ABS= (TR_o/RC)\cdot(TR_o/ABS)$] which is the reaction center II density within the antenna chlorophyll bed of PSII and the density of PSII RCs within the excited cross section [RC/CS= (ABS/CS)/(ABS/RC)].

Absorption of light energy (ABS), trapping (TR), conversion of excitation energy to the ET and the reduction of end electron acceptors are the four functional steps that regulate the initial stage of photosynthetic activity of a RC complex. These four independent steps contributing to photosynthesis, leads to the introduction of the multi-parametric expression named by Tsimilli-Michael and Stressor (2008), the so-called photosynthetic performance index ($PI_{ABS,\ total}$):

$$PI_{ABS} = [\gamma_{RC}/(1 - \gamma_{RC})]. [\varphi_{Po}/(1 - \varphi_{Po})].[\psi_{Eo}/(1 - \psi_{Eo})]$$

$$PI_{total} = (PI_{ABS}).(\delta_{Ro}/1 - \delta_{Ro})$$

The $\gamma_{RC}/(1 - \gamma_{RC})$ is the fraction of reaction center chlorophyll (Chl_{RC}) per total chlorophyll (Chl_{RC}+antenna). Two JIP-test parameters can be deconvoluted from this expression and estimated from the original fluorescence signals as $\gamma_{RC}/1 - \gamma_{RC} = RC/TR_0 . TR_0/ABS = [(F_{2ms} – F_{50\mu s})/4(F_{300\mu s} – F_{50\mu s})].F_V/F_0$. Factor 4 is used to express the initial fluorescence rise per 1 ms. The RC/ABS expression indicates the contribution to the $PI_{ABS,\ total}$ caused by the RC-density on a chlorophyll basis. The light reactions contributing to the primary photochemistry is estimated according to the JIP-test as $[\varphi_{P0}/(1 - \varphi_{P0})] = TR_0/DI_0 = k_P/k_N = F_V/F_0$. The electron transport contribution past Q_A is derived as $[\psi_{Eo}/(1 - \psi_{Eo})] = ET_0/(TR_0 – ET_0) = (F_M - F_{2ms})/(F_{2ms} - F_{50\mu s})$. The reduction of end reduction equivalent contribution is derived as $[\delta_{Ro}/(1 - \delta_{Ro})] = RE/ABS = (1 - F_{30ms})/(1 - F_{2ms})$.

The kinetic analyses of the prompt fluorescence transient kinetics were evaluated by calculating the difference in the relative variable fluorescence to present ΔV (expressed as V = f(t)) curves between the OK, OJ and KI steps, by normalising as follow: $V_{OK} = (F_t − F_0)/(F_K − F_0)$, as fluorescence data were normalized between O (0.02 ms) and K (0.3 ms) revealing the ΔV_L-band, $V_{OJ} = (F_t − F_0)/(F_J − F_0)$, as fluorescence data were normalized between O (0.02 ms) and J (2 ms) revealing the ΔV_K-band and $V_{KI} = (F_t - F_K)/(F_I - F_K)$, as fluorescence data were normalized between K (0.3 ms) and I (30 ms) revealing the ΔV_J-band. The difference between ΔV_{OK}, ΔV_{OJ} and ΔV_{KI} transients, relative to the control treatment were assessed and plotted as difference in kinetics $\Delta V_{OK} = V_{treatment} − V_{control}$, $\Delta V_{OJ} = V_{treatment} − V_{control}$ and $\Delta V_{KI} = V_{treatment} − V_{control}$ (Yusuf *et al*., 2010). The ΔV bands (ΔV_L, ΔV_K, ΔV_J) were revealed on the curves at specific time points corresponding to the limitation in the photosynthetic electron transport chain.

3.9 Growth increase measurement and calculations

The leave length and width of each plant was measured once a week. The leaf area (*A*) was calculated using a biometric relation between the leaf area, the length of the leaf blade (*L*) and maximal width of the leaf blade (*W*) where *A* = Light attenuation coefficient *K* of quinoa (0.59) x *L* x *W* (Schurr, 1997). The total leaf area of each representative was calculated as the growth leaf area multiplied by the total number of the leaves. Plant height measurements were also taken on the main stem from the soil level to the inflorescence tip panicle (once a week). Time from sowing date to visible

flower buds and grain filling was recorded for each plant. Flowering and grain filling date for each regime was estimated as the date when 70% of the plants had reached that stage. Visible flower buds were determined as the date when floral buds could be distinguished between leaf primordia in the main apex without dissecting. Plants were manually harvested and dust and residues were removed by exposing seeds to ventilation to determine the seed yield. Fresh weight and dry weight of each plant separated into leaves, shoots and seeds were determined after drying the plant material for 72 hrs at 65°C in an oven.

3.10 Statistical analysis

The data was analysed by using SigmaPlot version 12.0 (Systat Software, Inc., San Jose CA, USA) software. The normality test (Shapiro-Wilk) one-way analysis of variance (ANOVA) was applied in order to test the effects of drought stress at each water regime level. Comparison among means was determined through Fisher LSD method at $P \leq 0.05$. The data in the figures and tables were expressed as means ± standard deviation.

CHAPTER 4: RESULTS

4.1 The effects of water stress on quinoa

4.1.1 Soil-water status

The soil water content for water regime 1 ranged from 0.0195 to 0.0664 m^3/m^3 VWC (volumetric water content), water regime 2 ranged from 0.1024 to 0.2855 m^3/m^3 VWC, water regime 3 ranged from 0.3300 to 0.3989 m^3/m^3 VWC and water regime 4 ranged from 0.4229 to 0.6496 m^3/m^3 VWC over six weeks. All of these water regimes differ significantly ($P \leq 0.05$) from each other except for the control and water regime 3, which both had 3 wicks (Figure. 2A). Significant differences ($P \leq 0.05$) also occurred in the soil temperature and the soil conductivity of the pots (Table 1). Soil temperature of water regimes 1 and 2 were significantly ($P \leq 0.05$) higher compared to the control, as well as to water regimes 3 and 4 (Figure. 2B). The soil electrical conductivity of the control, water regimes 2 and 3 were significantly ($P \leq 0.05$) higher compared to water regime 1 and significantly ($P \leq 0.05$) lower compared to water regime 4 (Table 1).

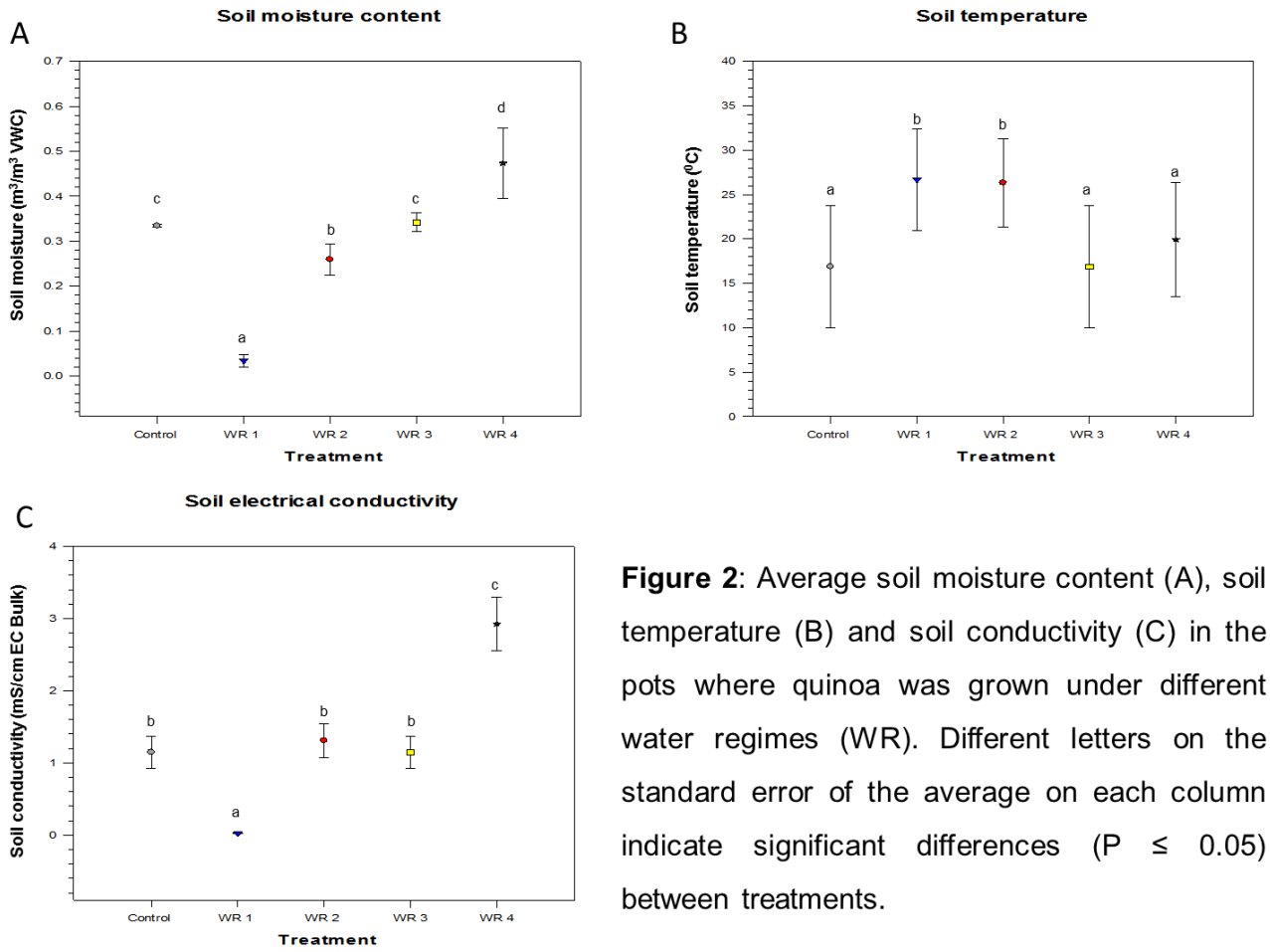


Figure 2: Average soil moisture content (A), soil temperature (B) and soil conductivity (C) in the pots where quinoa was grown under different water regimes (WR). Different letters on the standard error of the average on each column indicate significant differences (P ≤ 0.05) between treatments.

Table 1: Soil moisture, temperature and conductivity in the pots where quinoa was grown at different water regimes (WR). Data shown as mean ± standard deviation. Different letters indicate significant differences (*P* ≤ 0.05).

	Control	WR 1	WR 2	WR 3	WR 4
Soil moisture (m^3/m^3 VWC)	0.334±0.0025c	0.0343±0.0144a	0.259±0.0350b	0.342±0.0210c	0.474±0.0784d
Soil temperature (°C)	16.871±6.911a	26.692±5.722b	20.571±4.944b	16.871±6.911a	19.942±6.399a
Soil conductivity (mS/cm EC Bulk)	1.148±0.223b	0.0254±0.0078a	1.309±0.235b	1.148±0.223b	2.926±0.371c

4.1.2 Stomatal conductance (g_{H2O})

An increase in the stomatal conductance for water vapor (g_{H2O}) measured during the morning, afternoon and late afternoon was observed at six weeks after emergence. During the morning measurements the stomatal conductance ranged from 61 to 92 mmol $m^{-2}s^{-1}$ for water regime 1, water regime 2 ranged from 78 to 115 mmol $m^{-2}s^{-1}$, water regime 3 from 87 to 128 mmol $m^{-2}s^{-1}$ and water regime 4 from 118 to 240 mmol $m^{-2}s^{-1}$. There were no significant differences between water regimes 1 and 2, but the stomatal conductance of water regimes 3 and 4 were significantly ($P \leq 0.05$) higher compared to water regimes 1 and 2. These two water regimes also differed significantly from each other (Figure 3A and Table 2). Mid-day stomatal conductance ranged from 97 to 152 mmol $m^{-2}s^{-1}$ for water regime 1, water regime 2 from 118 to 218 mmol $m^{-2}s^{-1}$, water regime 3 from 198 to 282 mmol $m^{-2}s^{-1}$ and water regime 4 from 242 to 465 mmol $m^{-2}s^{-1}$. There were significant differences between water regimes 1 and 2, but the stomatal conductance of water regimes 3 and 4 were significantly ($P \leq 0.05$) higher, which also differed significantly from each other (Figure 3B and Table 2). Late afternoon stomatal conductance ranged from 71 to 120 mmol $m^{-2}s^{-1}$ for water regime 1, water regime 2 ranged from 71 to 188 mmol $m^{-2}s^{-1}$, water regime 3 from 141 to 280 mmol $m^{-2}s^{-1}$ and water regime 4 from 223 to 300 mmol $m^{-2}s^{-1}$. There were no significant differences between water regimes 1 and 2, but the stomatal conductance of water regimes 3 and 4 were significantly ($P \leq 0.05$) higher compared to water regimes 1 and 2. These two water regimes also differed significantly from each other (Figure 3C and Table 2). As expected, the stomatal conductance decreased when there was a decrease in the soil water content and increased with an increase in the soil water content.

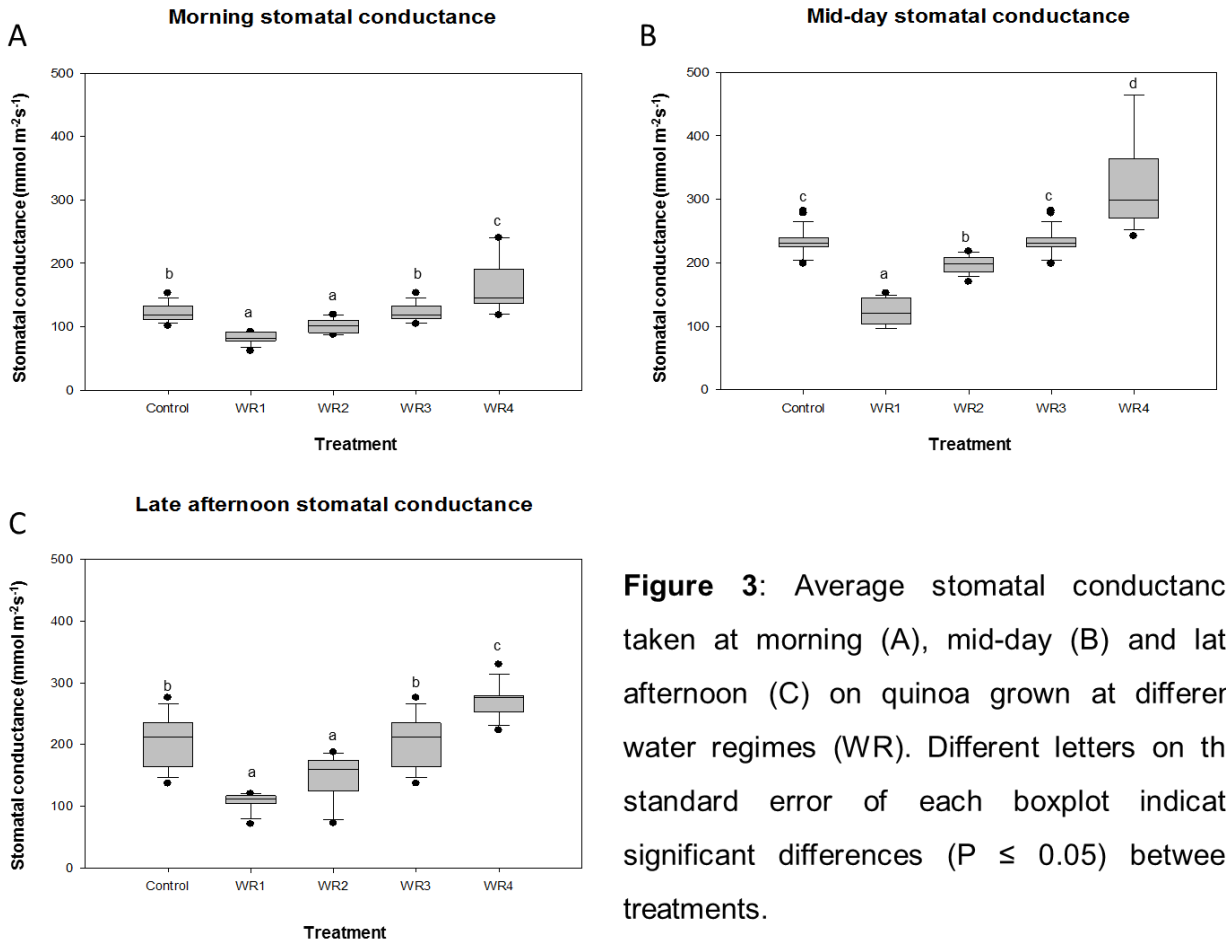


Figure 3: Average stomatal conductance taken at morning (A), mid-day (B) and late afternoon (C) on quinoa grown at different water regimes (WR). Different letters on the standard error of each boxplot indicate significant differences (P ≤ 0.05) between treatments.

Table 2: Average morning, mid-day and late afternoon stomatal conductance on quinoa leaves grown at different water regimes (WR). Data shown as mean ± standard deviation. Different letters indicate significant differences ($P \leq 0.05$).

Stomatal conductance (mmol $m^{-2}s^{-1}$)	Control	WR 1	WR 2	WR 3	WR 4
Morning	122.731±15.501b	82.182±9.877a	101.455±11.562a	123.720±14.960b	163.182±43.822c
Mid-day	232.958±21.745c	122.500±21.039a	194.167±19.178b	235.958±21.745c	324.083±76.920d
Late afternoon	204.500±46.366 b	106.500±15.350a	146.077±39.878a	205.500±46.356b	273.200±29.918c

4.1.3 Chlorophyll *a* fluorescence

4.1.3.1 OJIP transient

Chlorophyll *a* fluorescence transients of the dark-adapted quinoa leaves are shown on a logarithmic scale from 0.01 ms to 1000 ms for all treatments (Figure 4A-C). The OJIP shape was prominent in all of the treatments, with a varying maximum variable fluorescence (F_m - F_o = F_v), therefore indicating that all plants were photosynthetically active (Tsimilli-Michael and Strasser, 2008). A decline in the soil water content resulted in significant changes in the shape of the OJIP transient (Figure 2A). These changes became more evident as the weeks progressed (Figure 4C), which indicate that the OJIP transient is water regime-dependent. A decrease in the OJIP transient were especially noticeable between 2 ms and 300 ms (multiple turn-over phase). An increase in the chlorophyll *a* fluorescence transient is the direct measure of the PSII photosynthetic behaviour and provides valuable information about the physiological differences between the water regimes effects.

There were no significant differences between water regimes 1 and 2 at 0.05 ms. However, the fluorescence intensity of the control and water regimes 3 and 4 were significantly ($P \leq 0.05$) lower and these treatments did not differ significantly from each other (Table 3).

Fluorescence intensity at 2 ms between water regimes 1 and 2 only differ significantly during the second week, but the control, water regimes 3 and 4 fluorescence intensity were significantly ($P \leq 0.05$) higher and these treatments did not differ significantly from each other (Table 3).

There were significant differences between water regimes 1 and 2 at 30 ms during week 2. Though, the fluorescence intensity of the control and water regimes 3 and 4 were significantly ($P \leq 0.05$) higher and these treatments did not differ significantly from each other between week 1 and 2, hence water regime 4 differed significantly in week 3 (Table 3).

The fluorescence intensity at 300 ms between water regimes 1 and 2 only differed significantly during the second week. However, the control and water regimes 3 and 4

fluorescence intensity were significantly ($P \leq 0.05$) higher and water regime 4 differed significantly from the control and water regime 3 in week 3 (Figure 4A-C and Table 3).

The 10% field capacity (WR 1) resulted in changes in the single turn-over events of the PSII function (Figure 4A-C), which is the transition from O (0.02 ms) to J (2 ms). At the same time, the effects were also evident in the multiple turnover range, which is a transition between J (2 ms) to P (1000 ms). Hence, the effect of 20% field capacity (WR 2) only manifested in the multiple turn-over events of the PSII function (Figure 4A-C), which is the transition between J (2 ms) to P (1000 ms). Quinone A (Q_A) is reduced once at the transition from O to J, which represents the single turn-over events of the transient. This shows the photochemical reactions leading to the reduction of electron acceptor (Strasser *et al*., 1999).

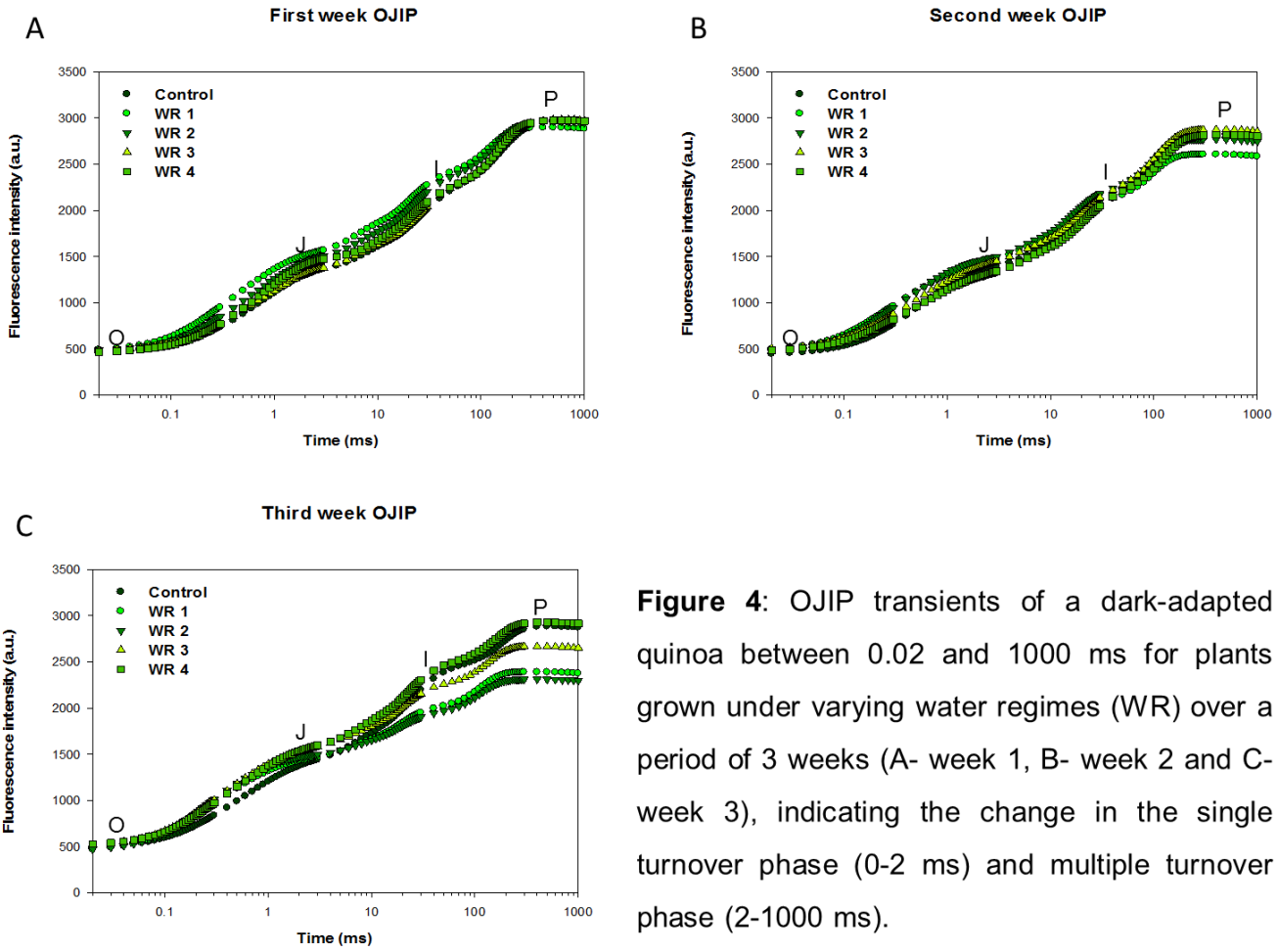


Figure 4: OJIP transients of a dark-adapted quinoa between 0.02 and 1000 ms for plants grown under varying water regimes (WR) over a period of 3 weeks (A- week 1, B- week 2 and C- week 3), indicating the change in the single turnover phase (0-2 ms) and multiple turnover phase (2-1000 ms).

Table 3: The fluorescence intensity of the OJIP steps on quinoa leaves grown at different water regimes (WR). Data shown as mean ± standard deviation. The measurements were taken on alternate weeks; different letters indicate significant differences ($P \leq 0.05$).

Week	OJIP step	Control	WR 1	WR 2	WR 3	WR 4
Week 1	O (0.05 ms)	500.13±39.68a	536.60±34.54c	522.53±22.64b	512.10±29.90a	491.92±21.46a
	J (2 ms)	1370.40±155.73c	1202.03±158.43a	1290.24±143.85b	1375.35±159.64c	1420.93±100.60c
	I (30 ms)	2003.61±180.62a	2272.02±151.96c	2203.69±83.86b	2041.65±105.75a	2089.98±132.51a
	P (300 ms)	2947.78±140.23b	2891.69±117.39a	2909.41±106.92a	2943.82±117.39b	2946.06±140.71b
Week 2	O (0.05 ms)	507.67±47.91a	546.16±39.77b	537.95±33.23b	507.67±47.91a	520.63±40.22a
	J (2 ms)	1479.29±226.98b	1398.12±150.13a	1391.95±146.72a	1479.29±226.98b	1534.93±220.90b
	I (30 ms)	2118.67±134.66b	2055.71±125.93a	2178.46±211.62c	2118.21±134.32b	2114.69±196.81b
	P (300 ms)	2834.89±114.76b	2995.75±97.31c	2770.23±202.84a	2842.42±112.74b	2824.62±102.26b
Week 3	O (0.05 ms)	558.31±51.85b	524.13±39.17a	531.38±45.56a	558.31±51.85b	571.61±52.11b
	J (2 ms)	1454.39±150.87a	1543.27±122.37b	1541.07±141.66b	1460.97±154.59a	1488.11±144.29a
	I (30 ms)	2176.60±207.78b	1947.73±130.63a	1968.13±155.71a	2176.60±207.78b	2305.78±211.85c
	P (300 ms)	2758.74±252.58b	2387.93±163.94a	2388.15±209.24a	2758.74±252.58b	2916.32±197.49c

4.1.3.2 Difference in relative variable fluorescence

4.1.3.2.1 ΔV_{OP}

The difference in the relative variable fluorescence ($\Delta V = V_{treatment} - V_{control}$) was obtained by normalising the transients between F_o and F_m, yielding the ΔV_{OP} plots, which provided the in-depth analyses of the fluorescence transient (Figure 5A-C). The ΔV_{OP} plots were obtained by normalising the relative variable fluorescence transients between O (0.02 ms) and P (300 ms). The difference in the fluorescence (ΔV) transient is provided by subtracting the normalised (V_t) transients (normalised between F_o and F_m)

of the control plants from the corresponding transient of the stressed plants (Strasser *et al*., 2007).

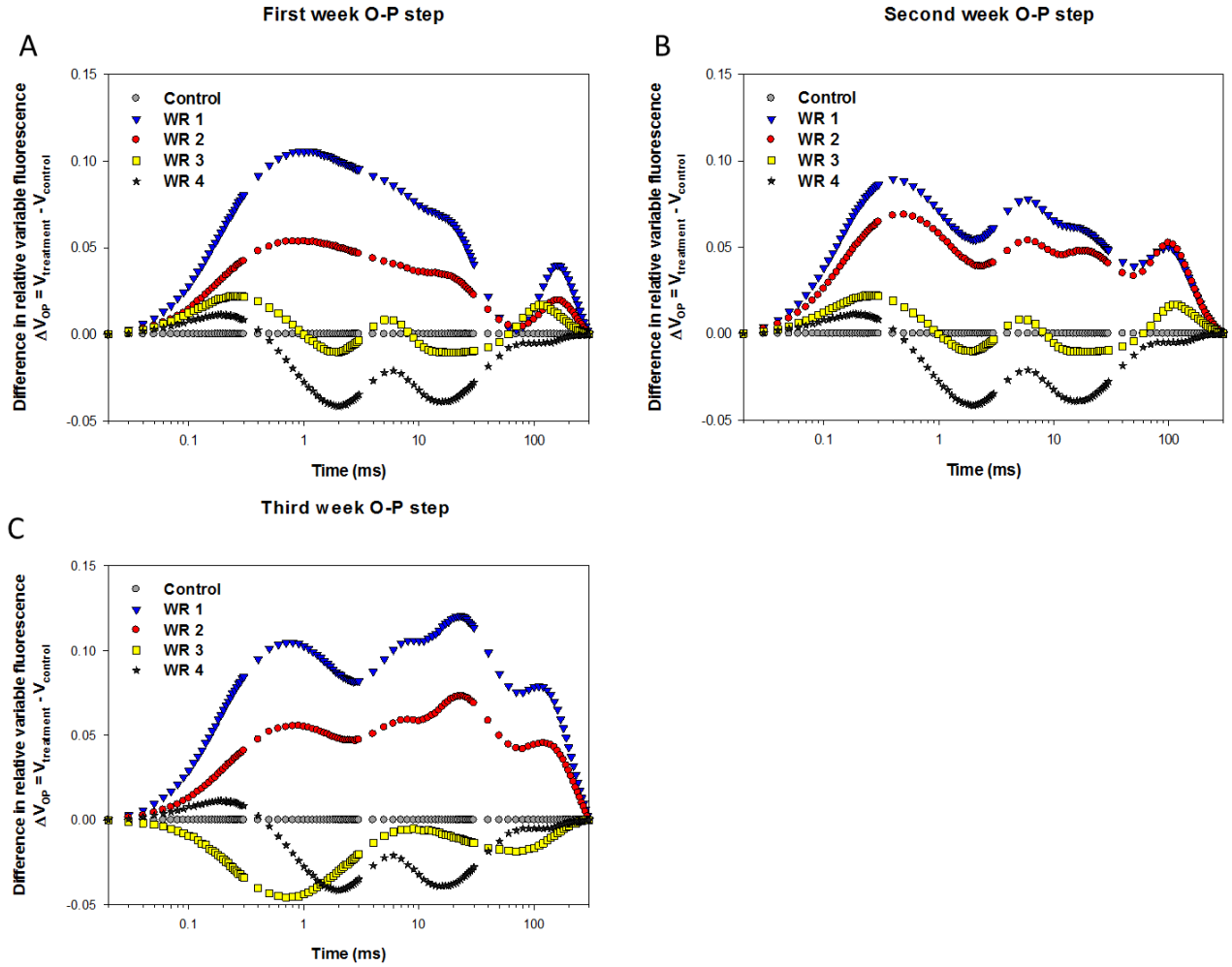


Figure 5: Changes in the difference of the relative variable chlorophyll *a* fluorescence transients (A- week 1, B- week 2 and C- week 3) of a dark-adapted quinoa leaves normalised between F_O and F_P [$V_{OP} = (F_t - F_o)/(F_p – F_o)$, $\Delta V_{OP} = V_{treatment} – V_{control}$] recorded in plants grown under varying water regimes.

4.1.3.2.2 ΔV_L-Band

Normalising the fluorescence data between O (0.02 ms) and K (0.3 ms) will reveal the presence of the ΔV_L-band, which is an indicator of the degree of energetic groupings (connectivity) of the PSII units. Quinoa subjected to WR 1 and 2 revealed a positive ΔV_L-bands (Figure 6A-C). In addition, there were no significant differences in ΔV_L-bands between water regimes 1 and 2 from week 1 to 3 (Table 4).

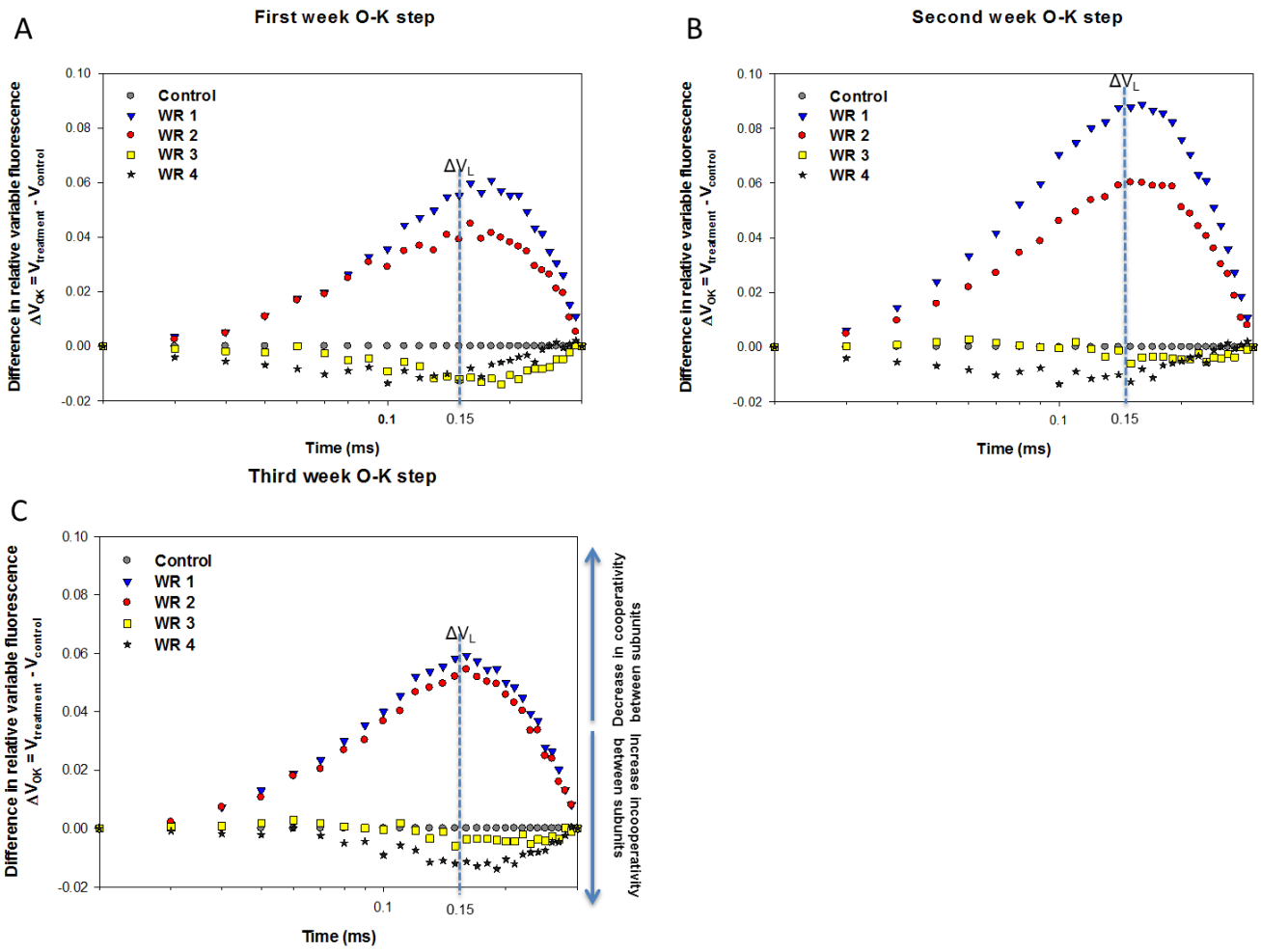


Figure 6: Changes in the difference of the relative variable chlorophyll *a* fluorescence transients (A- week 1, B- week 2 and C- week 3) of a dark-adapted quinoa leaves normalised between F_O and F_K [$V_{OK} = (F_t - F_o)/(F_K – F_o)$, $\Delta V_{OK} = V_{treatment} – V_{control}$] recorded in plants grown under different water regimes, indicating the drought-induced decrease in energetic connectivity of the PSII units.

4.1.3.2.3 ΔV_K-Band

Normalising the fluorescence data between O (0.02 ms) and J (2 ms) will reveal the presence of the ΔV_K-band. The ΔV_K-band is an indication of a dysfunctional oxygen evolving complex (OEC) or the functional PSII antenna size was increased. The water deficit treatments (water regimes 1 and 2) resulted in the formation of a positive ΔV_K-band (Figure 7A-C), which could be an indication that the OEC was inactivated or the functional PSII antenna size was increased in the drought-stressed plants. As a result, there were significant differences between water regimes 1 and 2 from week 1 to 2 only,

but the ΔV_K-bands of water regimes 3 and 4 were significantly ($P \leq 0.05$) higher which also differed significantly from each other (Table 4).

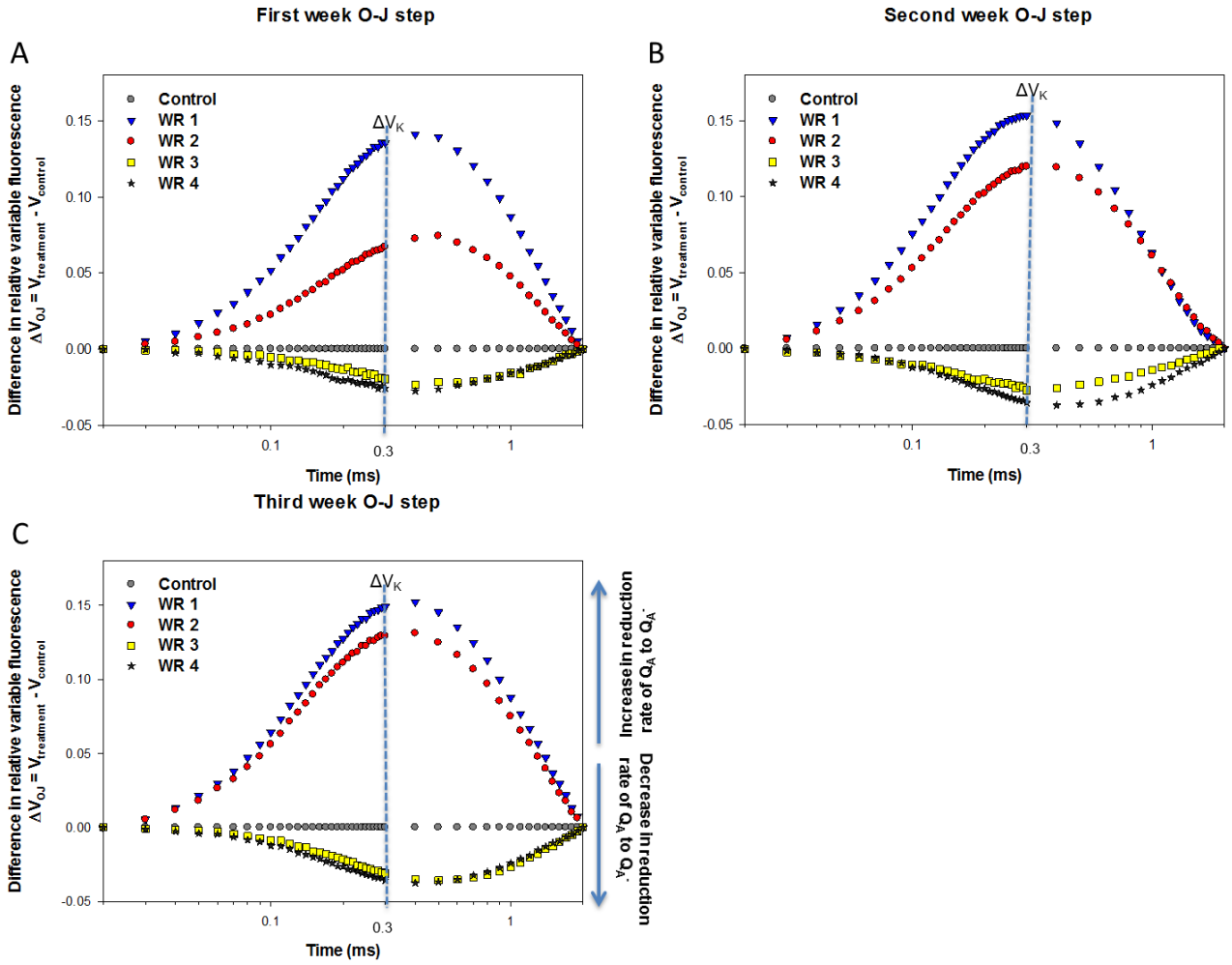


Figure 7: Changes in the difference of the relative variable chlorophyll *a* fluorescence transients (A- week 1, B- week 2 and C- week 3) of a dark-adapted quinoa leaves normalised between F_O and F_J [$V_{OJ} = (F_t - F_o)/(F_J - F_o)$, $\Delta V_{OJ} = V_{treatment} - V_{control}$] recorded in plants grown under different water regimes, showing intactness of the OEC and the effect of different water regimes on the functional antenna size of PSII.

4.1.3.2.4 ΔV_J-Band

Normalising the fluorescence data between K (0.3 ms) and I (30 ms) revealed the ΔV_J-band. The ΔV_J-bands were more prominent in the drought-induced plants compared to the well-watered plants (Figure 8A-C) as was also reported by Kalaji *et al*. (2014).

Though a slight positive ΔV_J-band appeared in the well-watered (water regimes 3 and 4) treatments at 2 ms, these bands did not differ significantly from each other. However, a negative ΔV_J-band appeared within the two water deficit treatments (water regimes 1 and 2). These treatments only differed significantly in the first week, but did not differ significantly from each other between week 2 to 3 (Table 4). The presence of a negative ΔV_J-band indicates a decrease in the fraction of the primary oxidised plastoquinone acceptor (Q_A^-). This decrease can either be due to a restriction in the flow of electrons to Q_A^- or an increase in the demand of electrons by photosystem I (PSI).

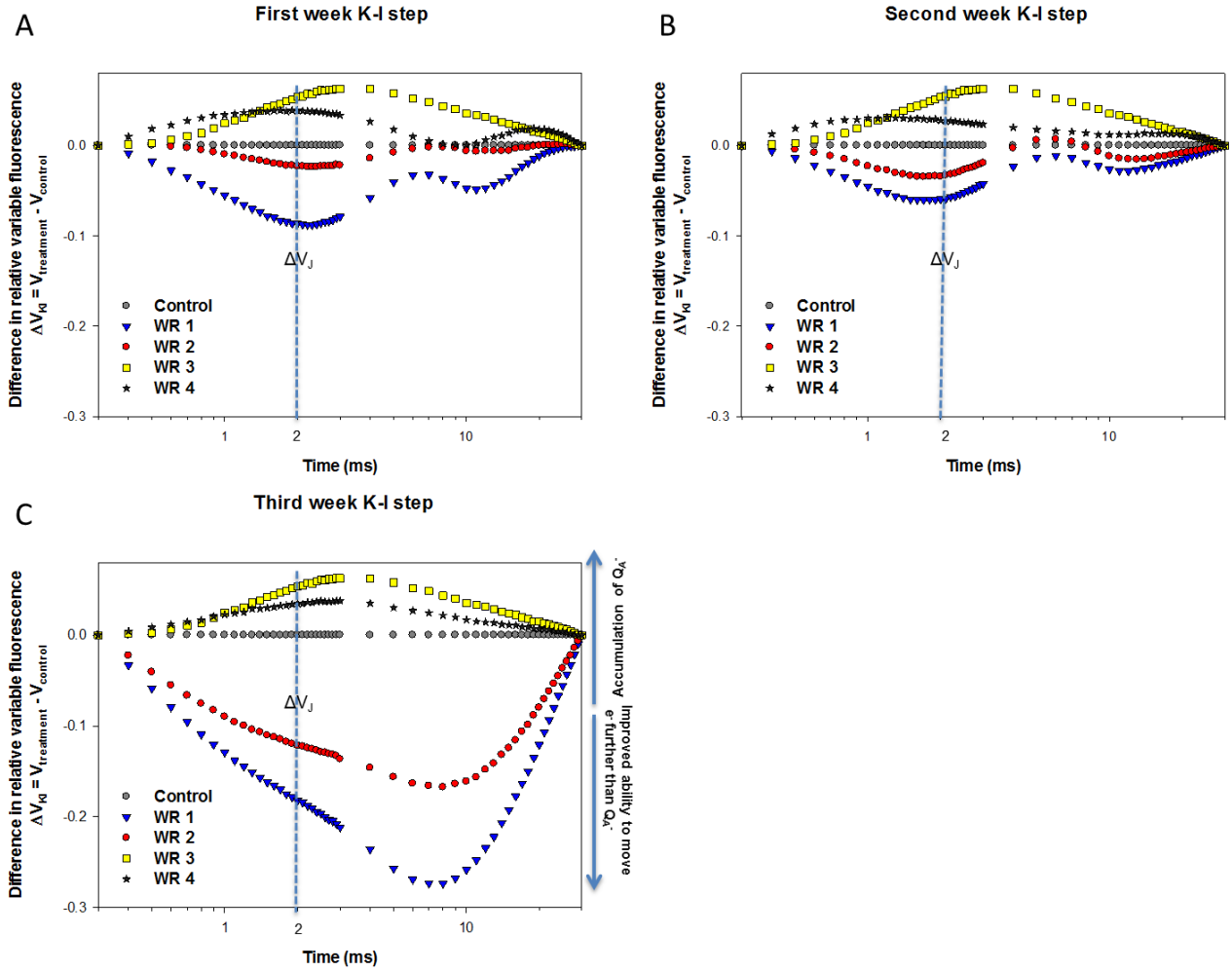


Figure 8: Changes in the difference of the relative variable chlorophyll *a* fluorescence transients (A- week 1, B- week 2 and C- week 3) of a dark-adapted quinoa leaves normalised between F_K (0.3 ms) and F_I (30 ms), [$V_{KI} = (F_t - F_o)/(F_I – F_K)$, $\Delta V_{KI} = V_{treatment} – V_{control}$] recorded in plants grown under varying water regimes reflecting an increase or decrease in Q_A^- concentration.

Table 4: Fluorescence intensity at 0.15; 0.3 and 2 ms of the water regimes (WR). Data shown as mean ± standard deviation. The measurements were taken on alternate weeks; different letters indicate significant differences ($P \leq 0.05$) (n = 80).

Week	Band	Control	WR 1	WR 2	WR 3	WR 4
Week 1	L (0.15 ms)	2635.02±129.92a	2773.15±112.90b	2747.43±119.31b	2647.61±131.61a	2669.19±163.84a
	K (0.3 ms)	2023.23±147.29a	2272.02±151.96c	2203.69±83.86b	2029.24±145.73a	2000.00±106.16a
	J (2 ms)	1370.40±155.73c	1202.03±158.43a	1290.24±143.85b	1375.35±159.64c	1420.93±100.60c
Week 2	L (0.15 ms)	2703.99±120.38b	2646.85±192.91a	2639.56±184.84a	2702.56±117.45b	2705.26±138.64b
	K (0.3 ms)	2833.71±114.44c	2605.06±145.04a	2770.23±202.84b	2833.71±114.44c	2832.31±116.27c
	J (2 ms)	1479.29±226.98b	1398.12±150.13a	1391.95±146.72a	1479.29±226.98b	1534.93±220.90b
Week 3	L (0.15 ms)	2577.02±224.95b	2319.76±191.42a	2312.23±158.70a	2577.02±224.95b	2724.30±182.49c
	K (0.3 ms)	2758.74±252.58b	2399.71±204.52a	2387.93±163.94a	2758.74±252.58b	2916.32±197.49c
	J (2 ms)	1454.39±150.87a	1543.27±122.37b	1541.07±141.66b	1460.97±154.59a	1488.11±144.29a

4.1.3.2.5 ΔV_I-Band

The evaluation of the JP phase [double normalisation at F_J (2 ms) and F_P (300 ms)] reveals the ΔV_I-band. The ΔV_I-band permits the assessment of the sequence of events of the electron transport from the plastoquinol (PQH_2) to the final electron acceptor of the PSI. The fluorescence increase in WR 1 and 2 and decrease in WR 3 and 4 (Figure 9A-C) mirrors the pool size of the final electron acceptors of the PSI (Yusuf *et al*., 2010). Thus, WR 1 and 2 indicated that quinoa's response was dependent on the severity of water stress as reflected by the regulation of the pool size of the final electron acceptors on the acceptor side of PSI (Figure 9A-C).

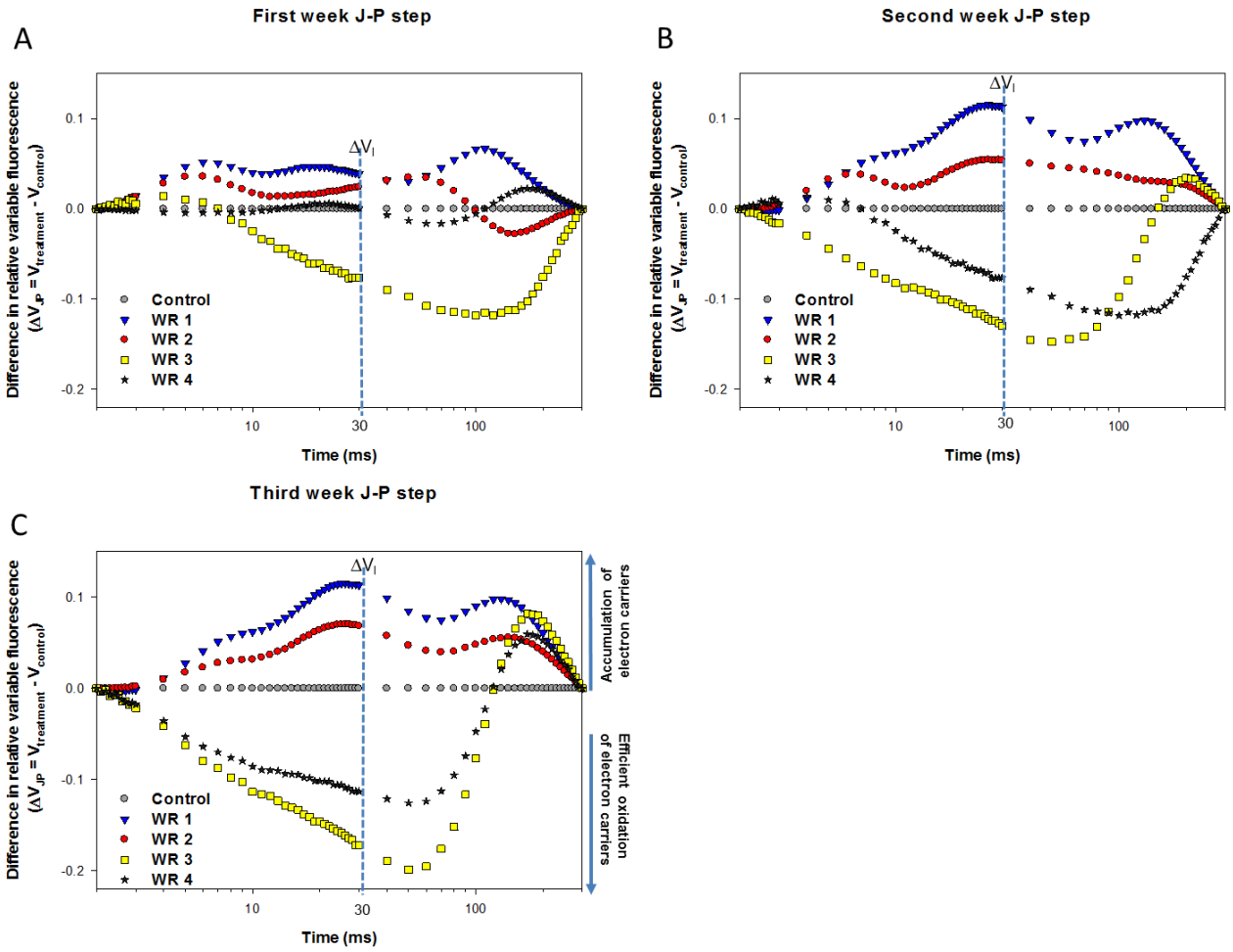


Figure 9: Changes in the difference of the relative variable chlorophyll *a* fluorescence transients (A- week 1, B- week 2 and C- week 3) of a dark-adapted quinoa leaves normalised between F_J and F_P [$V_{JP} = (F_t - F_o)/(F_P – F_J)$, $\Delta V_{JP} = V_{treatment} – V_{control}$] recorded in plants grown under different water regimes indicating the accumulation and efficient utilisation of plastoquinone.

4.1.3.3 Specific energy fluxes

The relative values of the JIP-test parameters are shown in a radar plot, where all of the values were normalised against the control (Figure 10A-C). The specific energy fluxes of the chlorophyll *a* fluorescence transient were calculated. These specific energy fluxes include the maximum quantum efficiency (φ_{P0}), performance index (PI_{ABS}) and the total photosynthetic performance index (PI_{total}).

4.1.3.3.1 Maximum quantum efficiency (φ_{P0} or F_v/F_m)

The same trend of a stable quantum efficiency was observed after the normalisation of the JIP-test parameters of the well-watered and drought-induced plants. However the amplitude of the fluorescence intensity in quinoa's response was different (Figure 10A-C). Quinoa F_v/F_m values were lower under drought conditions compared to the well-watered conditions. There was no significant variation in the F_v/F_m ratio of the well-watered and drought-induced plants when compared to other JIP parameters with significant variations between treatments. However, there were fractional changes in the F_v/F_m values for both treatments. There was a positive correlation between F_v/F_m of the well-watered and drought-induced plants, with higher F_v/F_m values observed in both treatments.

Generally, both drought-induced and well-watered plants maintained high F_v/F_m values (~0.839 to 0.853), respectively. When the plants started to flower the F_v/F_m slightly decreased in both treatments. The F_v/F_m was 0.853 in drought-induced plants during the vegetative stage and 0.842 at the flowering stage. Minor variations were observed in the well-watered plants, which had a F_v/F_m value of 0.853 during the vegetative stage and 0.848 at the flowering stage (Figure 10B-C). The effects of the well-watered and drought-induced conditions on the PSII parameters were similar to the trends observed for the stomatal conductance (Figure 3A-C).

4.1.3.3.2 Specific fluxes per reaction center (RC)

The specific flux or activity of the active PSII reaction center (RC) of absorption (ABS/RC); trapping (TR_o/RC); electron transport (ET_o/RC); dissipation (DI_o/RC) and (RE_o/RC) reduction flux of the final PSI electron acceptors per RC were highly influenced by the water regimes and the interaction between the growth stages and the treatments (Figure 10A-C).

The ABS/RC parameter mirrors the total absorption of the PSII antenna chlorophylls per active RC. The increase in this parameter could mean that a fraction of the RCs is inactivated or the apparent antenna size is increased. The JIP analyses of the quinoa plants in water regimes 1, 2, 3 and 4 suggests that high values of ABS/RC indicated significant increase in the absorption of flux photon per active RCs. Studies suggest that

the latter could only mean an increase in the antenna size and not a structural increase of the antenna size of a biochemical complex (Kalaji *et al*., 2014; Gururani *et al*., 2015). The increase of ABS/RC (decrease of the active RCs) was complemented by a decrease in the trapping of the excitation energy (TR/RC) which resulted in the decrease in reduction of Q_A to Q_A^-.

Generally, quinoa exhibited a high ET_o/RC in all of the treatments, therefore indicating a thermal activation of the dark reactions. However, ET_o/RC and RE_o/RC were reduced in water regimes 1 and 2 as the drought stress intensified. Moreover, the progressive increase in ABS/RC and DI_o/RC during drought stress indicated that more energy was dissipated as heat.

4.1.3.3.3 Phenomenological fluxes per excited cross section (CS)

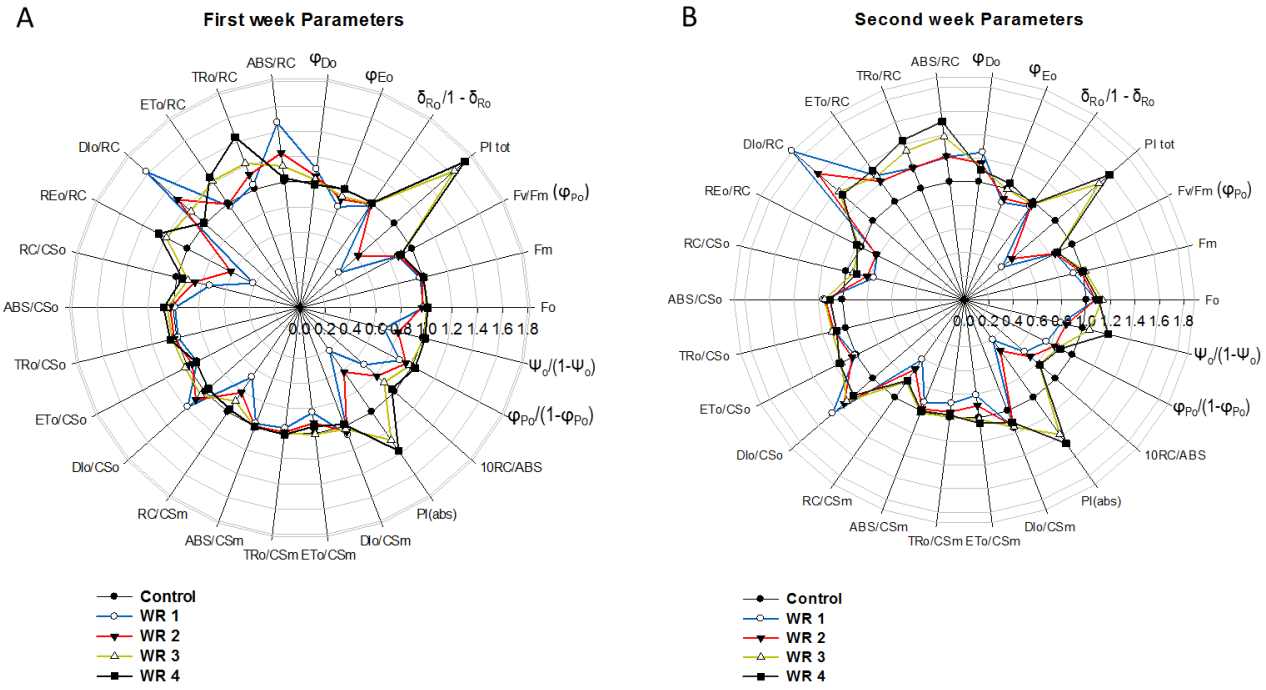
The values for phenomenological fluxes per excited cross section (CS) for ABS/CS, TR_o/CS were reduced in water regimes 1 and 2 and increased in water regimes 3 and 4 (Figure 10A-C). However, ET_o/CS values were higher during the vegetative stage, but later decreased during the flowering stage. Results indicated that the drought stress induced the down-regulation of the PSII function, as indicated by the inactivation of RC/CS_o and decrease in TR_o/CS, ET_o/CS and RE_o/RC. Moreover, drought-stressed plants had lower values for the specific flux per reaction center and phenomenological flux per excited cross section compared to the well-watered plants (Figure 10A-C).

4.1.3.3.4 Performance indexes (PI_{ABS} and PI_{total}) and the partial parameters

The PI_{ABS} and PI_{total}, as a measure of the plant performance indicated quinoa's significance response to drought stress. These parameters are used to quantify the PSII behaviour and measure plant performance up to the reduction of the PSI end-electron acceptors, respectively. The PI_{ABS} slightly decreased at the 10% and 20% field capacity, relative to the control (Figure 10A-C and Figure 11A). However, the PI_{ABS} parameter started to decrease as the plants were aging for both treatments, but at 75% and 90% field capacity showed a slight decrease. Various driving components of PI_{ABS}, density of operative photosystems (reaction center per chlorophyll, RC/ABS), efficiency of energy trapping ($\psi_o/(1 - \psi_o)$ and conversion of excitation energy to the electron transport ($\varphi_{Po}/(1 - \varphi_{Po})$ showed similar trends for the well-watered and drought-induced quinoa. The

values for the RC/ABS and (φ_{Po}/(1 - φ_{Po}) parameters were reduced in drought-stressed plants. The parameter (ψ_0) represents the efficiency with which a trapped exciton transfers the electron to the photosystem electron transport chain further than Q_A^-. The increase in the value for (ψ_{Eo}) induced gain in the electron capacity, which was indicated by an increase in the value of (ψ_o/(1 - ψ_o) only in the well-watered plants (Figure 10A-C).

Performance index (PI_{total}) measures the plant performance up to the reduction of the PSI end-electron acceptors. This parameter represents partial potential for energy conservation and was strictly connected to the plant overall growth and survival under stress conditions. Moreover, the PI_{total} was significantly reduced in WR 1 and 2, but was also reduced in WR 3 and 4 as the plants were aging (Figure 10C and Figure 11B).



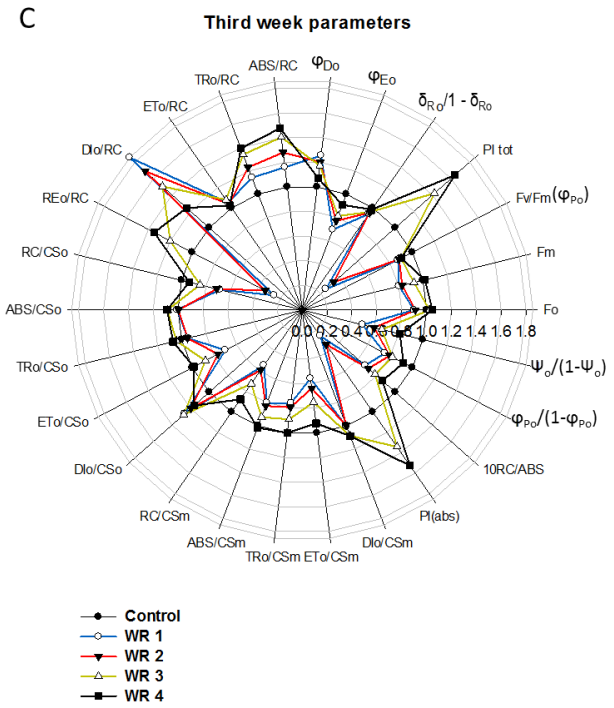


Figure 10: Radar plots (A- week 1, B- week 2 and C- week 3) depicting possible changes in energy fluxes, ratios, structure and function of the photosynthetic apparatus of quinoa subjected to different water regimes (WR). The deviation of the behaviour pattern from the control demonstrates the fractional impact of water treatment to the fluorescence parameters.

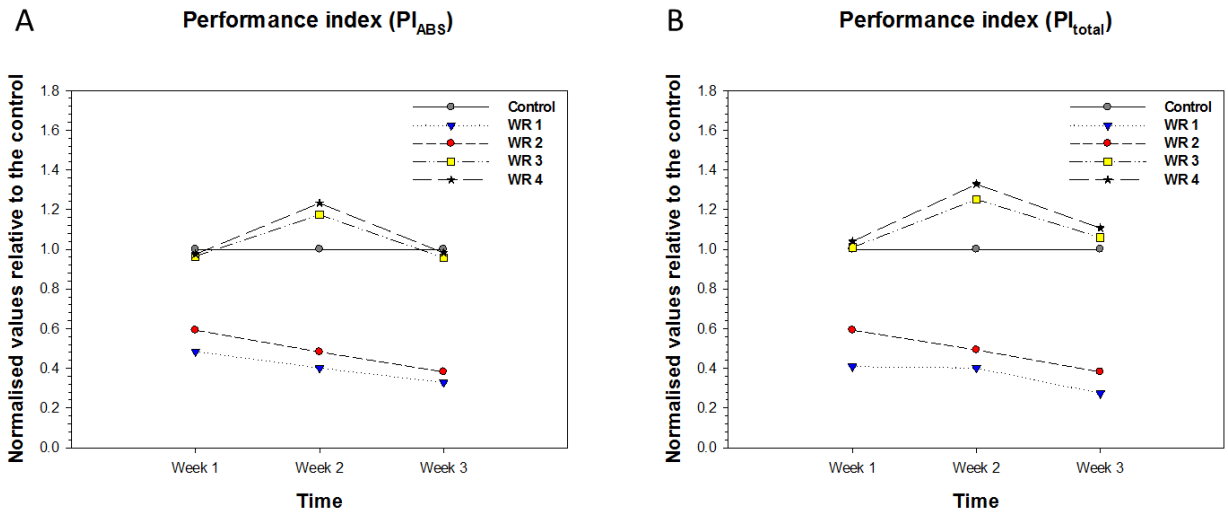


Figure 11: Normalised average photosynthetic performance index [PI_{ABS} (A) and PI_{total} (B)] as measured in plants subjected to different water regimes.

4.1.4 Days to flowering

Both drought-induced and the well-watered plants were exposed to the same environmental conditions. However, there were relative moderate variations in the time to flowering, being 31 days in spring to 44 days in winter after sowing of the well-watered plants and 36 days in spring to 45 days in winter after sowing of the drought-induced plants (Figure 12). The delay in flowering for 4 days of the drought-induced plants was observed on the plants exposed to pre-anthesis severe water stress. During spring the well-watered plants flowered in 31 days from sowing date and 36 days in drought-induced plants (Figure12).

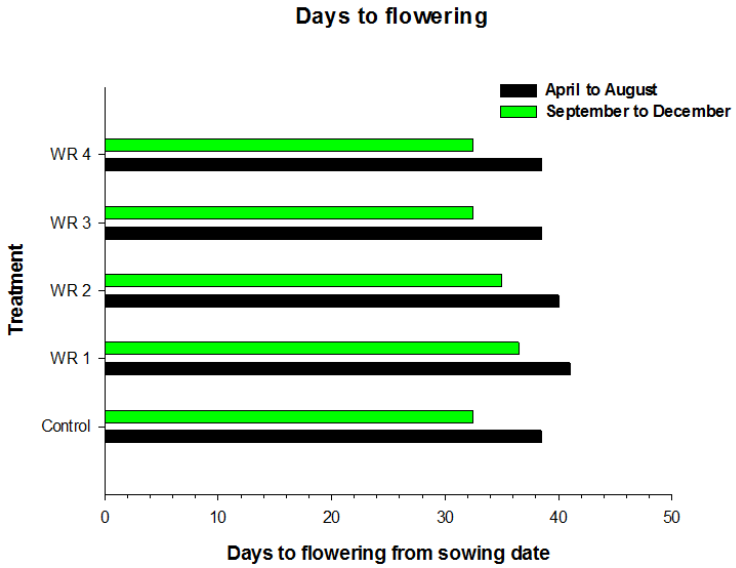


Figure 12: Flowering days of quinoa plants grown at different water regimes (WR) during autumn to winter and spring to summer season.

4.1.5 Plant growth, specific leaf area and biomass

Plant height was reduced by 52% ($P \leq 0.05$) at 10% field capacity (WR 1) (Figure 13 and Table 5). The leaf area was reduced by 56% ($P \leq 0.05$) and 20% ($P \leq 0.05$) at 10% (WR 1) and 20% (WR 2) field capacity, respectively, relative to the control plants (Figure 14A and Table 6). The wet and dry weight was 75% ($P \leq 0.05$) higher in the well-watered plants compared to the drought-induced plants (Figure 14B-C). The lowest values were observed in the plants that were induced to the 10% field capacity (WR 1). This decrease in biomass is attributed to the shoot biomass.

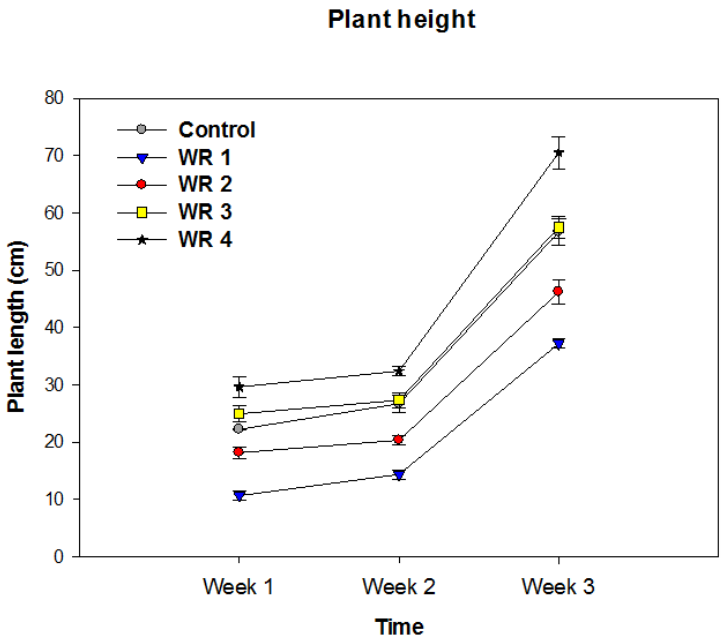


Figure 13: Average plant height of quinoa plants at different water regimes (WR) during the period of experiment. Different letters on the standard error of the average on each column indicate significant differences (P ≤ 0.05) between treatments.

Table 5: Average plant height (cm) of quinoa plants at different water regimes (WR). Data shown as mean ± standard deviation. The measurements were taken every week; different letters indicate significant differences (*P* ≤ 0.05).

	Control	**WR 1**	**WR 2**	**WR 3**	**WR 4**
Week 1	19.23±0.74c	10.67±0.81a	18.17±1.01 b	24.933±1.46c	29.63±1.79d
Week 2	23.70±1.48c	14.37±0.81a	20.33±0.76b	27.30±1.38c	32.40±0.89d
Week 3	56.70±2.29c	37.233±0.74a	46.20±2.05b	57.53±1.91c	70.53±2.81d

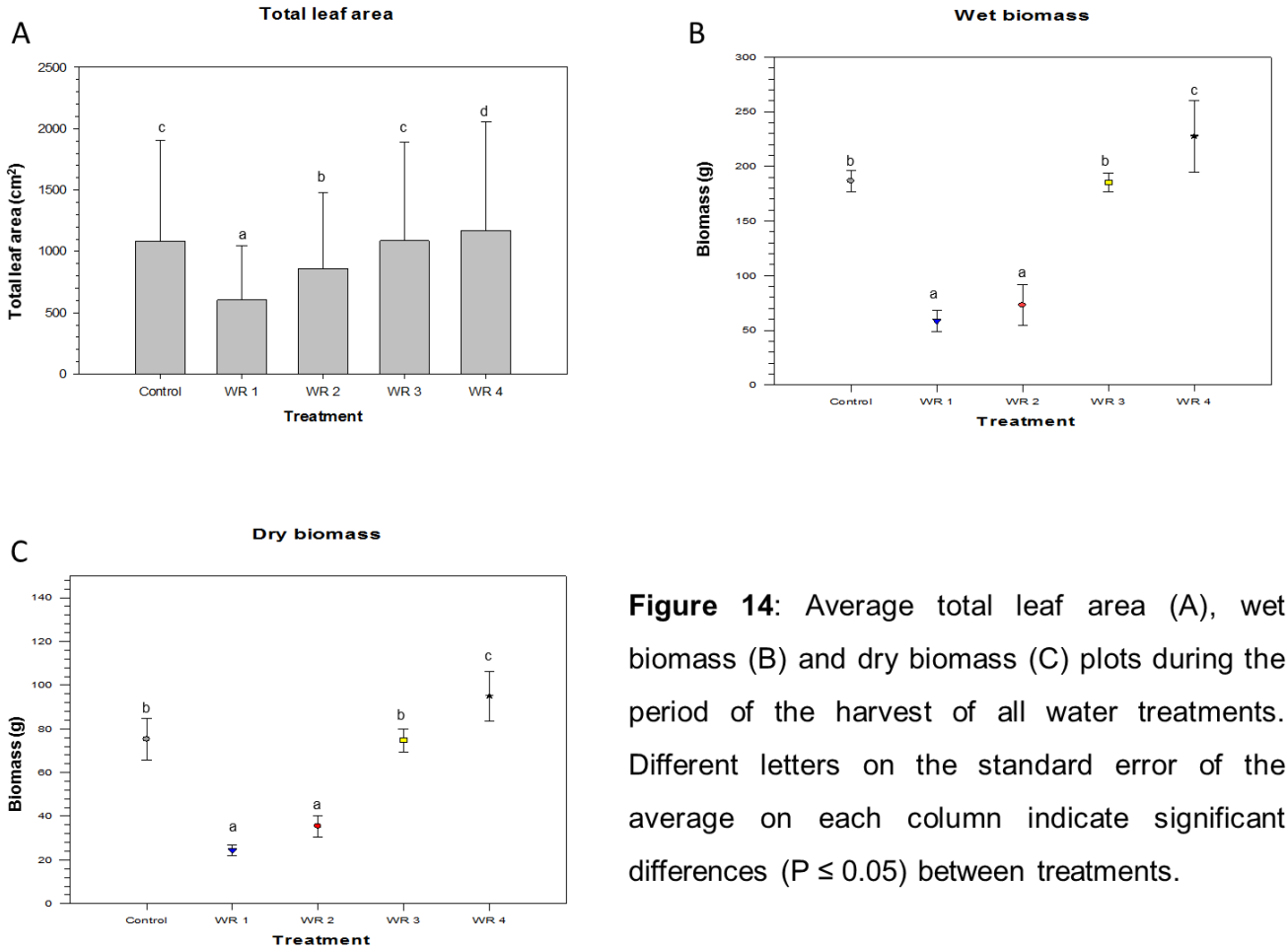


Figure 14: Average total leaf area (A), wet biomass (B) and dry biomass (C) plots during the period of the harvest of all water treatments. Different letters on the standard error of the average on each column indicate significant differences ($P \leq 0.05$) between treatments.

4.1.6 Seed yield

There were no significant differences in the weight of the seeds per plant between the 10% (WR 1) and 20% (WR 2) field capacity treatment. However, there was 83% ($P \leq 0.05$) increase in the weight of seeds per plant at 75% (WR 3) and 90% (WR 4) field capacity and in the control and when compared to a decrease by 46% ($P \leq 0.05$) and 36% ($P \leq 0.05$) at 10% (WR 1) and 20% (WR 2) field capacity, respectively (Figure 15 and Table 6).

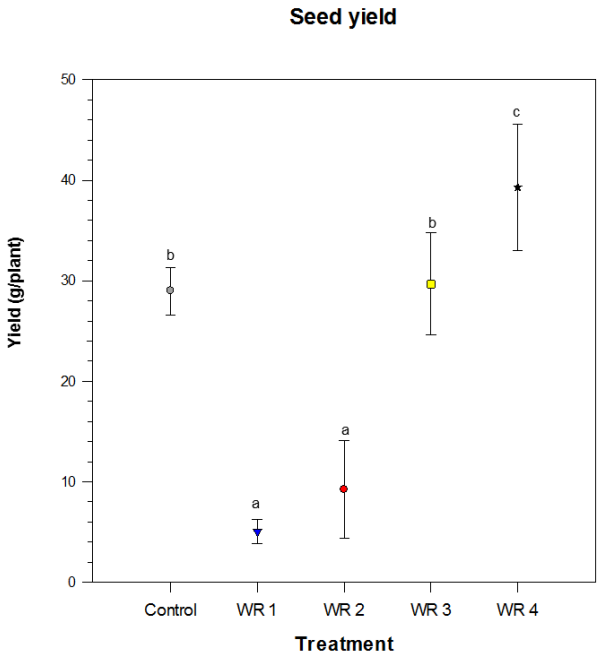


Figure 15: Average quinoa seed yield during the period of the harvest in all water treatments. Different letters on the standard error of the average on each column indicate significant differences (P ≤ 0.05) between treatments.

Table 6: Quinoa total leaf area, wet and dry biomass and seed yield grown at different water regimes (WR). Data was shown as mean ± standard deviation. Different letters indicate significant differences (*P* ≤ 0.05).

	Control	**WR 1**	**WR 2**	**WR 3**	**WR 4**
Total leaf area (cm^2)	1082.00±821.53c	605.67±440.42a	860.67±622.00b	1086.67±807.15c	1171.33±885.59d
Wet biomass (g)	186.59±9.95b	58.41±9.50a	72.92±18.72a	185.47±8.67b	227.51±32.89c
Dry biomass (g)	75.21±9.53b	24.41±2.46a	34.03±6.24a	66.41±10.04b	95.04±11.38c
Seed yield (g/plant)	25.32±4.88b	5.05±1.21a	9.20±4.86a	29.67±5.17b	39.31±6.28c

4.2 The effects of O_3 on quinoa and its interaction with elevated CO_2 levels and drought

4.2.1 Soil-water status

The average soil water content of the pots, which were placed in the (OTCs) was 0.15 m^3/m^3 VWC (volumetric water content) for the drought stressed plants and 0.44 m^3/m^3 VWC for the well-watered plants. There were significant differences between the soil water content (Figure 16A), conductivity (Figure 16C) and temperature (Figure 16B) between the well-watered plants and the water deficit plants (Table 7). It is evident that the drought-induced conditions initiated a decrease in the soil electrical conductivity, which can prevent an efficient water absorption by the plant. The average soil moisture content of 0.15 m^3/m^3 VWC in the controlled environment (OTCs) induces significant reduction in the soil electrical conductivity (Figure 16A).

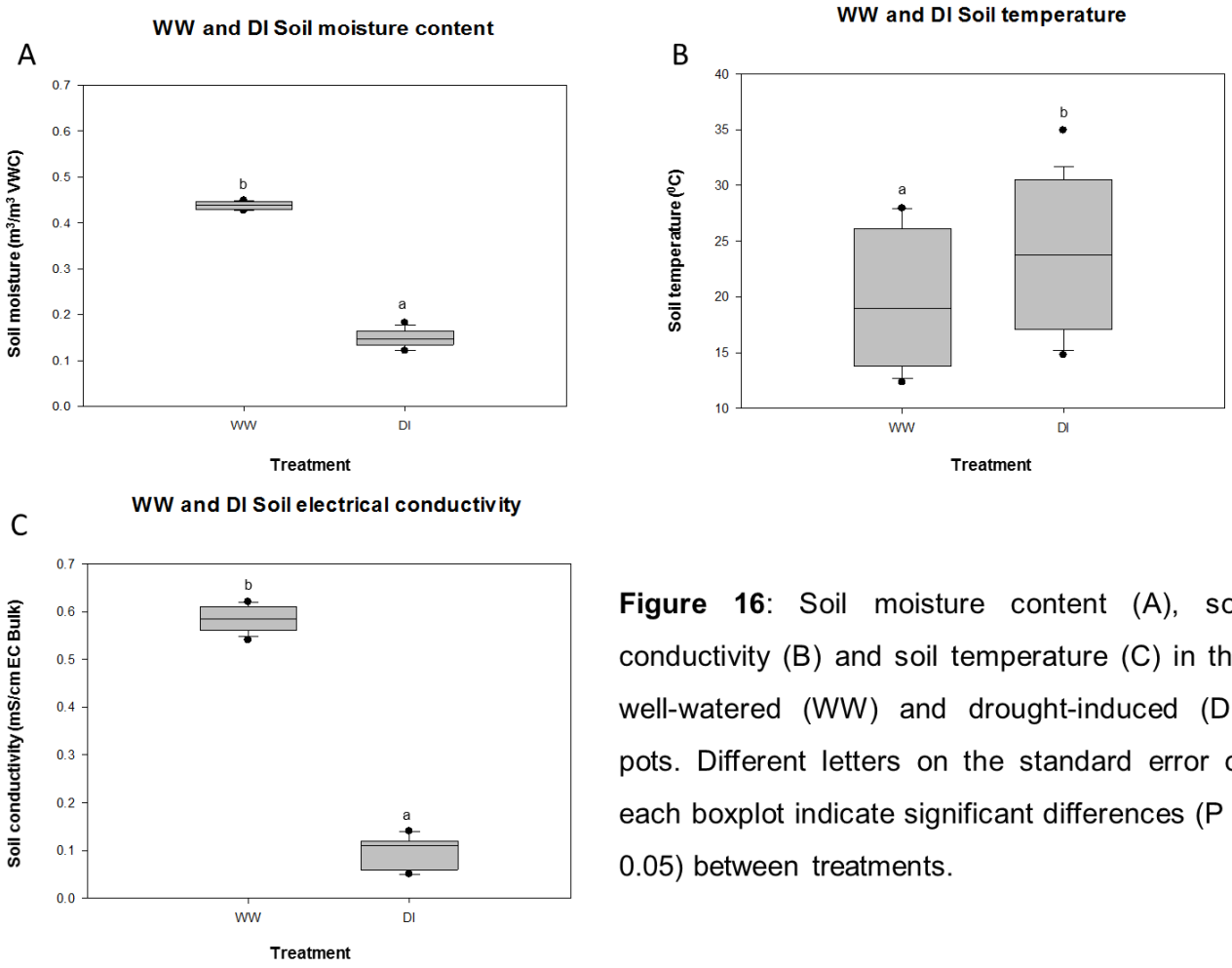


Figure 16: Soil moisture content (A), soil conductivity (B) and soil temperature (C) in the well-watered (WW) and drought-induced (DI) pots. Different letters on the standard error of each boxplot indicate significant differences ($P \leq 0.05$) between treatments.

Table 7: Soil moisture, temperature and conductivity in the well-watered (WW) and drought-induced (DI) pots. Data shown as mean ± standard deviation. The measurements were taken from the beginning of the experiment to the end; different letters indicate significant differences ($P \leq 0.05$).

	Well-watered	**Drought-induced**
Soil moisture content (m^3/m^3 VWC)	0.438±0.008b	0.149±0.019a
Soil electrical conductivity (mS/cm EC Bulk)	0.584±0.027b	0.0975±0.033a
Soil temperature (°C)	19.700±5.936a	23.891±7.016b

4.2.2 Visible injury

Plants showed severe roundish brown necrosis in the interveinal adaxial of both young and old expanded leaves. Notably, more O_3 damage was observed on the leaves located towards the bottom of the plant. These are also the older leaves and has been exposed for a longer period to O_3. The damaged leaf area was about 5% to 10% and 20% to 30% on the canopy leaves and the bottom leaves of the plants fumigated with 80 ppb O_3, respectively (Figure 17D-E). There was about 20% to 30% damage on the canopy leaves and 60% to 70% on the bottom leaves of the plants fumigated with 120 ppb O_3 (Figure 17F-G). Plants fumigated with elevated CO_2 + O_3 had about 5% damage on both canopy and bottom leaves (Figure 17C). Plants fumigated with elevated CO_2 showed no signs of the damage and remained green for a long period, relative to the control plants (Figure 17A-B).

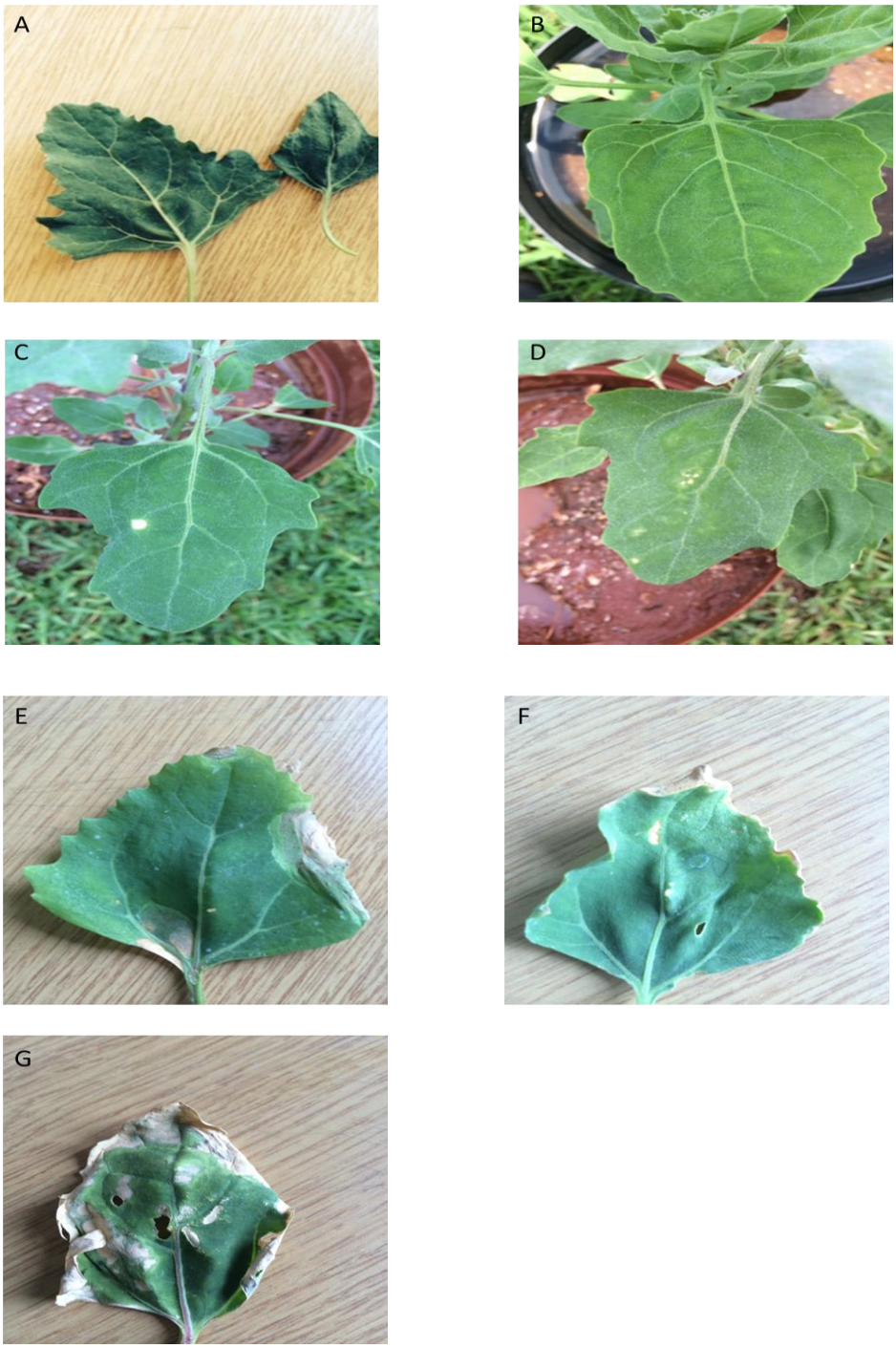


Figure 17: The comparative effects of quinoa leaves in (A) control, (B) plants fumigated with elevated CO_2 and (C) a combination of 700 ppm CO_2 + 80 ppb O_3, (D) 80 ppb O_3 canopy leaves and (E) 80 ppb O_3 bottom leaves and (F) 120 ppb O_3 canopy leaves and (G) 120 ppb O_3 bottom leaves in the open top chambers.

4.2.3 Stomatal conductance (g_{H2O})

4.2.3.1 Well- watered conditions

Stomatal conductance was measured on the canopy leaves before and after the onset of elevated CO_2, O_3 and CO_2 + O_3 fumigation. The stomatal conductance significantly ($P \leq 0.05$) decreased in the well-watered plants fumigated with elevated CO_2 just between 14 and 35 days after the onset of fumigation when compared to other treatments and the control plants. A significant ($P \leq 0.05$) increase in the stomatal conductance was observed where the plants were fumigated with 80 ppb O_3 and 120 ppb O_3, relative to the control plants (Table 8).

After 21 days of fumigation, the well-watered plants had significant ($P \leq 0.05$) differences between all treatments. However, the stomatal conductance of the CO_2 fumigated plants were significantly ($P \leq 0.05$) lower when compared to the other fumigation treatments (Figure 18C).

After 28 days of fumigating the well-watered plants with elevated CO_2, 80 ppb O_3 and 120 ppb O_3 they had no significant differences in the stomatal conductance. The stomatal conductance of the control plants and plants fumigated with CO_2 + O_3 were significantly ($P \leq 0.05$) higher (Figure 18E) when compared to the other treatments.

After 35 days of fumigation, there were no significant differences between the well-watered plants fumigated with 80 ppb O_3 and the plants fumigated with CO_2 + O_3. Though these treatments had stomatal conductance that were significantly ($P \leq 0.05$) higher than the CO_2 treated plants. At the same time, their stomatal conductance were also significantly ($P \leq 0.05$) lower than plants fumigated with 120 ppb O_3 (Figure 18G). There were a decrease of 33% ($P \leq 0.05$) in the stomatal conductance of the well-watered plants fumigated with elevated CO_2. In addition, the stomatal conductance increased by 6% ($P \leq 0.05$) and 31% ($P \leq 0.05$) of the well-watered plants fumigated with 80 ppb O_3 and 120 ppb O_3, respectively (Table 8).

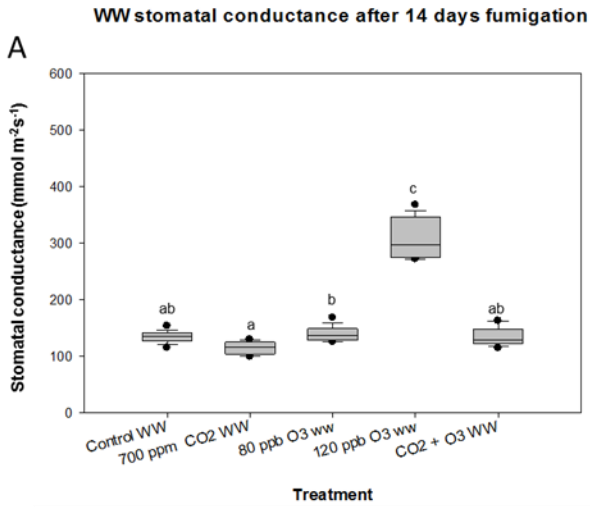
4.2.3.2 Drought-induced conditions

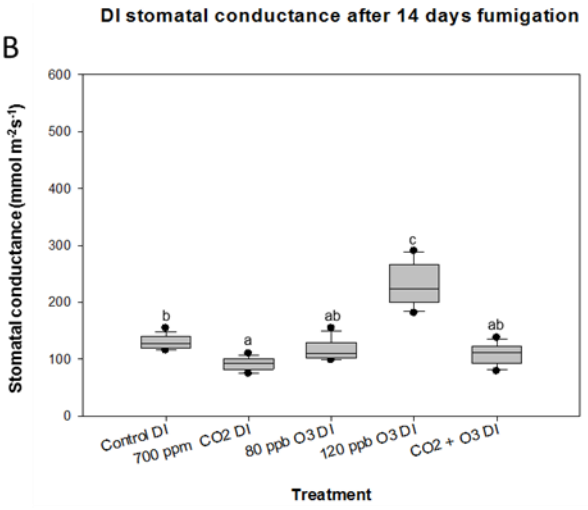
The stomatal conductance significantly ($P \leq 0.05$) decreased in the drought-induced plants fumigated with elevated CO_2 just between 14 and 35 days after the onset of

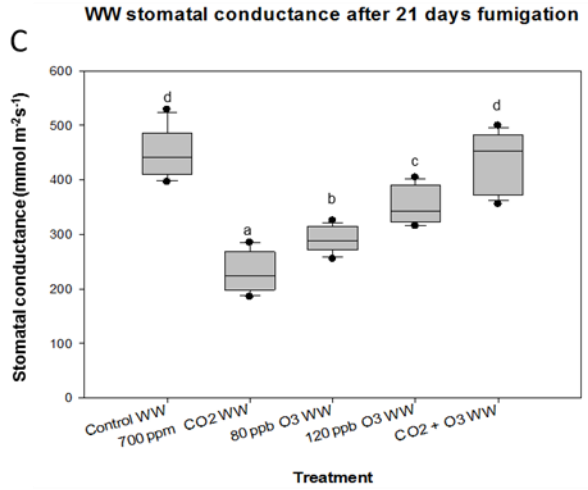
fumigation when compared to the other treatments and the control plants. After 14 days of fumigation there were no significant differences in the stomatal conductance between the plants fumigated with 700 ppm CO_2, 80 ppb O_3 and 700 ppm CO_2 + 80 ppb O_3 of the drought-induced plants. The stomatal conductance of the plants fumigated with 120 ppb O_3 was significantly ($P \leq 0.05$) higher when compared to all treatments (Figure 18B). There were no significant differences in the stomatal conductance of the drought-induced plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3, but they were significantly ($P \leq 0.05$) higher when compared to the CO_2 fumigated plants 21 days after fumigation (Figure 18D).

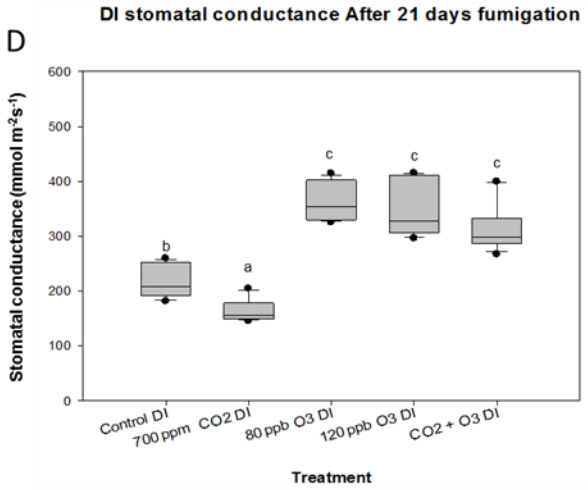
After 28 days of fumigation the stomatal conductance of the drought-stressed plants fumigated with elevated CO_2 were significantly ($P \leq 0.05$) lower compared to the plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3. The 80 ppb O_3 and CO_2 + O_3 fumigated plants did not differ significantly from each other. However, significant differences were observed between 80 ppb O_3 and 120 ppb O_3 fumigated plants (Figure 18F).

As expected, after 35 days the stomatal conductance decreased by 45% ($P \leq 0.05$) of the drought-stressed control plants (Table 8). There were no significant differences between the drought stressed plants fumigated with 80 ppb O_3 and the plants fumigated with CO_2 + O_3. Though these treatments had stomatal conductance that were significantly ($P \leq 0.05$) higher than the CO_2 fumigated plants, but lower than plants fumigated with 120 ppb O_3 (Figure 18H). In addition, there were a decrease of 77% ($P \leq 0.05$) in the stomatal conductance of the plants fumigated with elevated CO_2 and subjected to drought stress simultaneously (Table 8). This is an indication that the elevated CO_2 and soil water content induces stomata closure. The stomatal conductance of the drought-stressed plants fumigated with 80 ppb O_3 and 120 ppb O_3 increased by 33% ($P \leq 0.05$) and 56% ($P \leq 0.05$), respectively (Table 8).


A
WW stomatal conductance after 14 days fumigation
Stomatal conductance (mmol m-2s-1)
600
500
400
300
200
100
0
ab
a
b
c
ab
Control WW
700 ppm CO2 WW
80 ppb O3 ww
120 ppb O3 ww
CO2 + O3 WW
Treatment


B
DI stomatal conductance after 14 days fumigation
Stomatal conductance (mmol m-2s-1)
600
500
400
300
200
100
0
b
a
ab
c
ab
Control DI
700 ppm CO2 DI
80 ppb O3 DI
120 ppb O3 DI
CO2 + O3 DI
Treatment


C
WW stomatal conductance after 21 days fumigation
Stomatal conductance (mmol m-2s-1)
600
500
400
300
200
100
0
d
a
b
c
d
Control WW
700 ppm CO2 WW
80 ppb O3 WW
120 ppb O3 WW
CO2 + O3 WW
Treatment


D
DI stomatal conductance After 21 days fumigation
Stomatal conductance (mmol m-2s-1)
600
500
400
300
200
100
0
b
a
c
c
c
Control DI
700 ppm CO2 DI
80 ppb O3 DI
120 ppb O3 DI
CO2 + O3 DI
Treatment

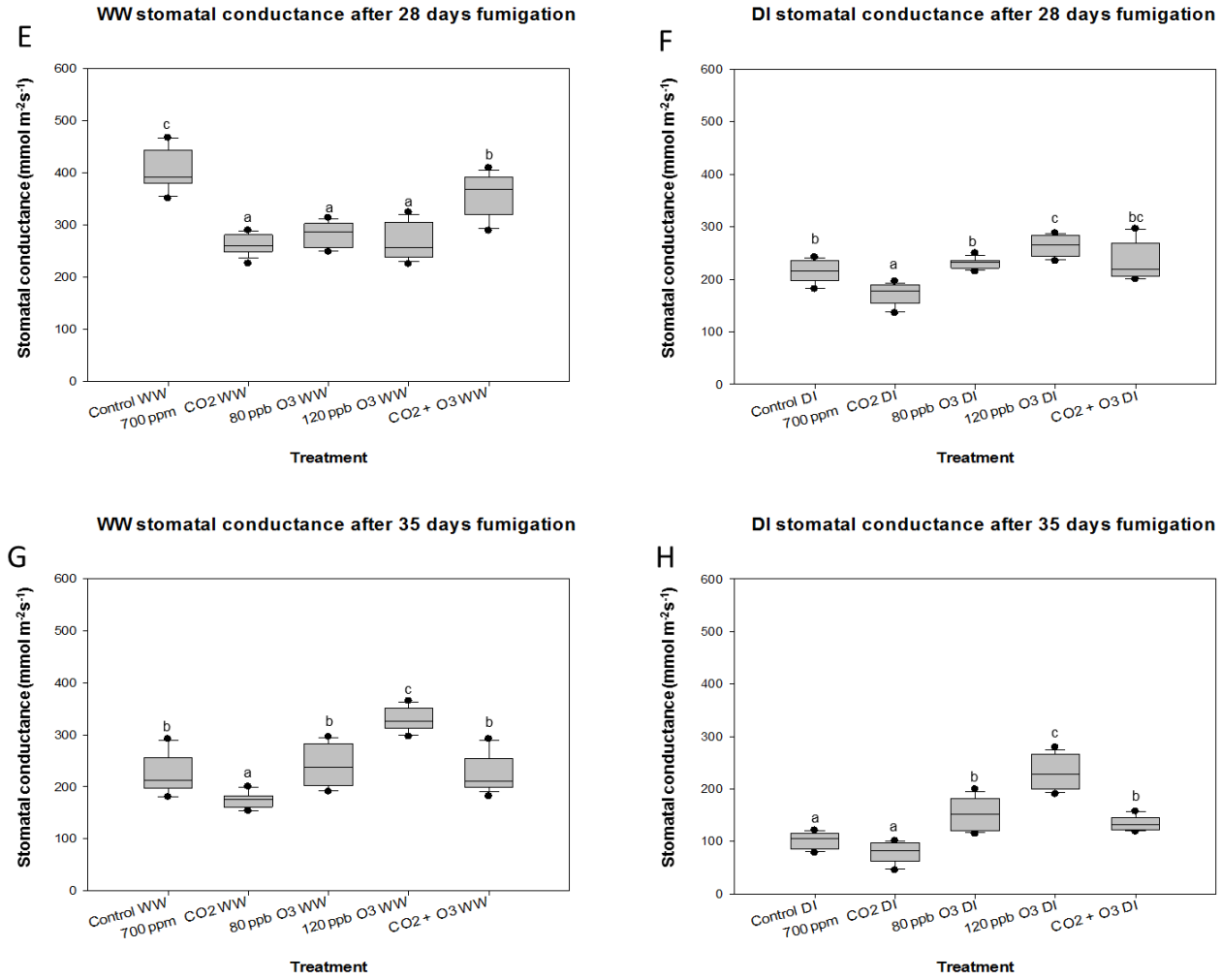


Figure 18: Stomatal conductance (mmol $m^{-2}s^{-1}$) of the well-watered (WW) and drought-induced (DI) quinoa plants fumigated with 700 ppm CO_2, 80 ppb O_3, 120 ppb O_3 and a combination of 700 ppm CO_2 and 80 ppb O_3 after 14 days of fumigation (A and B), 21 days of fumigation (C and D), 28 of fumigation (E and F) and 35 days of fumigation (G and H). Different letters on the standard error of each boxplot indicate significant differences ($P \leq 0.05$) between treatments.

Table 8: Stomatal conductance (mmol $m^{-2}s^{-1}$) of the well-watered (WW) and drought-induced (DI) quinoa plants during fumigation. Data shown as mean ± standard deviation. Different letters indicate significant differences ($P \leq 0.05$).

Treatment	Fumigation period	Control	700 ppm CO_2	80 ppb O_3	120 ppb O_3	CO_2+O_3
Well-watered	After 14 days	134.083±10.570 ab	115.250±11.403 a	140.417±13.581 b	309.583±36.004 c	135.083±16.528 ab
	After 21 days	450.000±46.363 d	232.500±38.206 a	290.667±24.537 b	354.000±35.675 c	436.750±54.816 d
	After 28 days	406.167±40.781 c	262.417±20.483 a	281.083±24.704 a	269.583±36.552 a	357.583±42.113 b
	After 35 days	226.417±40.031 b	174.333±15.587 a	241.000±42.032 b	329.167±23.296 c	225.500±39.530 b
Drought-induced	After 14 days	129.917±12.831 b	91.583±11.774 a	116.750±19.165 ab	231.417±38.714 c	108.583±19.737 ab
	After 21 days	217.500±30.345 b	164.833±20.915 a	364.833±35.118 c	352.083±50.628 c	314.583±44.639 c
	After 28 days	215.250±22.120 b	171.917±21.339 a	230.250±10.721 b	262.750±20.280 c	235.917±37.860 bc
	After 35 days	102.417±15.957 a	78.417±20.593 a	152.167±31.362 b	233.083±33.082 c	134.333±13.580 b

4.2.4 Chlorophyll *a* fluorescence

4.2.4.1 OJIP transient

The photosynthetic performance of the well-watered and drought-induced quinoa treated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 was determined by using chlorophyll *a* fluorescence (JIP-test). Chlorophyll *a* fluorescence transients of a dark-adapted quinoa leaves were plotted on a logarithmic time scale from 0.01 ms to 1000 ms for all treatments (Figure 19A-H). The OJIP shape was prominent in all treatments with comparable maximum variable fluorescence (F_m - F_o = F_v). This

indicates that all treatments of both well-watered and drought-induced plants were photosynthetically active.

Reduced soil water content and fumigation with 80 ppb O_3 and 120 ppb O_3 resulted in a change in the shape of the OJIP transient (Figure 16A, Figure 19B, D, F and H and Table 10). This change became more evident as the weeks progressed, which indicated that the OJIP transient is influenced by the plant water status, CO_2 levels and O_3 fumigation period and concentration. Changes of the OJIP transient was especially noticiable during the multiple turn-over phase (2 ms to1000 ms).

The O_3 levels at 120 ppb influenced both the single and multiple turn-over events of the PSII function. The effect of elevated CO_2, 80 ppb O_3 and CO_2 + O_3 were more evident in the multiple turn-over events of the PSII function (Figure 19A-H and Table 9 and 10).

4.2.4.1.1 Effect of the well-watered conditions on the OJIP transient

There were no significant differences between the well-watered plants (Table 9) fumigated with 700 ppm CO_2, 80 ppb O_3, 120 ppb O_3 and 700 ppm CO_2 + 80 ppb O_3 at 0.05 ms. After 21 and 35 days of fumigation significant differences were found between well-watered plants fumigated with elevated CO_2 and CO_2 + O_3 (Table 9).

At 2 ms the fluorescence signal of the well-watered plants had significant differences between 80 ppb O_3 and CO_2 + O_3 from 14 to 28 days after fumigation. The fluorescence intensity were significantly ($P \leq 0.05$) lower after 14 to 35 days of fumigation when compared to the plants fumigated with 120 ppb O_3 under well-watered conditions (Table 9).

The significant differences were observed only in the well-watered plants fumigated with 80 ppb O_3 and CO_2 + O_3 at 30 ms after 21 and 35 days of fumigation. Furthermore, the fluorescence intensity after 35 days of fumigating the well-watered plants with 80 ppb O_3 and 120 ppb O_3 was significantly ($P \leq 0.05$) higher when compared to the other treatments (Table 9). These two treatments also differ significantly from each other.

At 300 ms, there were no significant difference in the fluorescence intensity between the 80 ppb O_3 and the 120 ppb O_3 fumigated plants after 28 days of fumigation under well-watered conditions (Table 9). There were no significant differences between the well-

watered plants fumigated with 120 ppb O_3 and CO_2 + O_3 from 21 to 35 days after fumigation. The fluorescence intensity of the well-watered plants fumigated with elevated CO_2 were significantly ($P \leq 0.05$) lower from 14 to 21 days and after 35 days of fumigation (Table 9). As expected, there were no significant differences in the fluorescence intensity of the well-watered plants fumigated with elevated CO_2 and CO_2 + O_3, which is an indication that elevated CO_2 was able to ameliorate the negative effects of 80 ppb O_3 (Table 9) under well-watered conditions.

4.2.4.1.2 Effect of drought stress on the OJIP transient

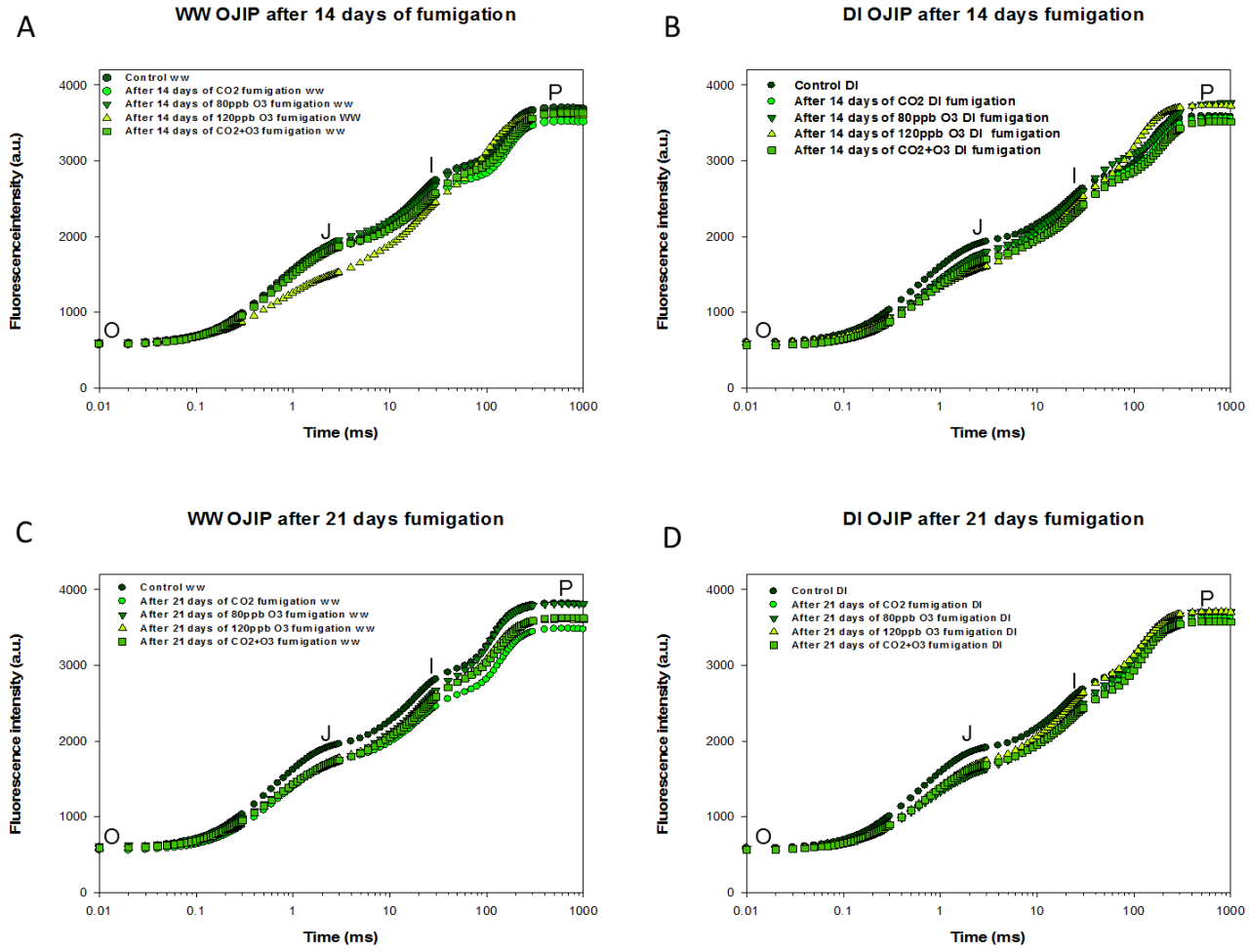
At 0.05 ms, there were no significant differences between drought-induced plants fumigated with elevated CO_2 and CO_2 + O_3 and between the plants fumigated with 80 ppb O_3, 120 ppb O_3 after 14 days of fumigation (Table 10). Furthermore, the plants fumigated with CO_2 and CO_2 + O_3 differ significantly from the plants fumigated with 80 ppb O_3 and 120 ppb O_3.

The were significant differences between drought-induced plants fumigated with 80 ppb O_3 and CO_2 + O_3 at 2 ms from 14 to 28 days after fumigation. The fluorescence intensity were significantly ($P \leq 0.05$) higher after 14 to 35 days of fumigation when compared to the plants fumigated with 120 ppb O_3 subjected to drought stress (Table 10).

There were significant differences between drought-induced plants fumigated with 700 ppm CO_2, 80 ppb O_3, 120 ppb O_3 and 700 ppm CO_2 + 80 ppb O_3 at 30 ms after 35 days of fumigation (Table 10). In addition, the fluorescence intensity after 35 days of fumigating the drought-induced plants with 80 ppb O_3 and 120 ppb O_3 was significantly ($P \leq 0.05$) lower when compared to other treatments. These two treatments also differ significantly from each other.

There were significant differences in the fluorescence intensity at 300 ms between 700 ppm CO_2, 80 ppb O_3, 120 ppb O_3 and 700 ppm CO_2 + 80 ppb O_3 of the drought-stressed plants after 14 days of fumigation (Table 10). After 21 days of fumigation, there were no significant difference in the fluorescence intensity between 80 ppb O_3 and 120 ppb O_3 fumigated plants subjected to drought stress. However, the fluorescence intensity of the drought-induced plants fumigated with elevated CO_2 was significantly (P

≤ 0.05) higher from 14 to 35 days after fumigation compared to the other treatments (Table 10). At the same time, there were no significant differences in the fluorescence intensity of the drought-stressed plants fumigated with elevated CO_2 and CO_2 + O_3, which is an indication that the interaction of drought stress and elevated CO_2 was able to ameliorate the negative effects of 80 ppb O_3 (Table 10).



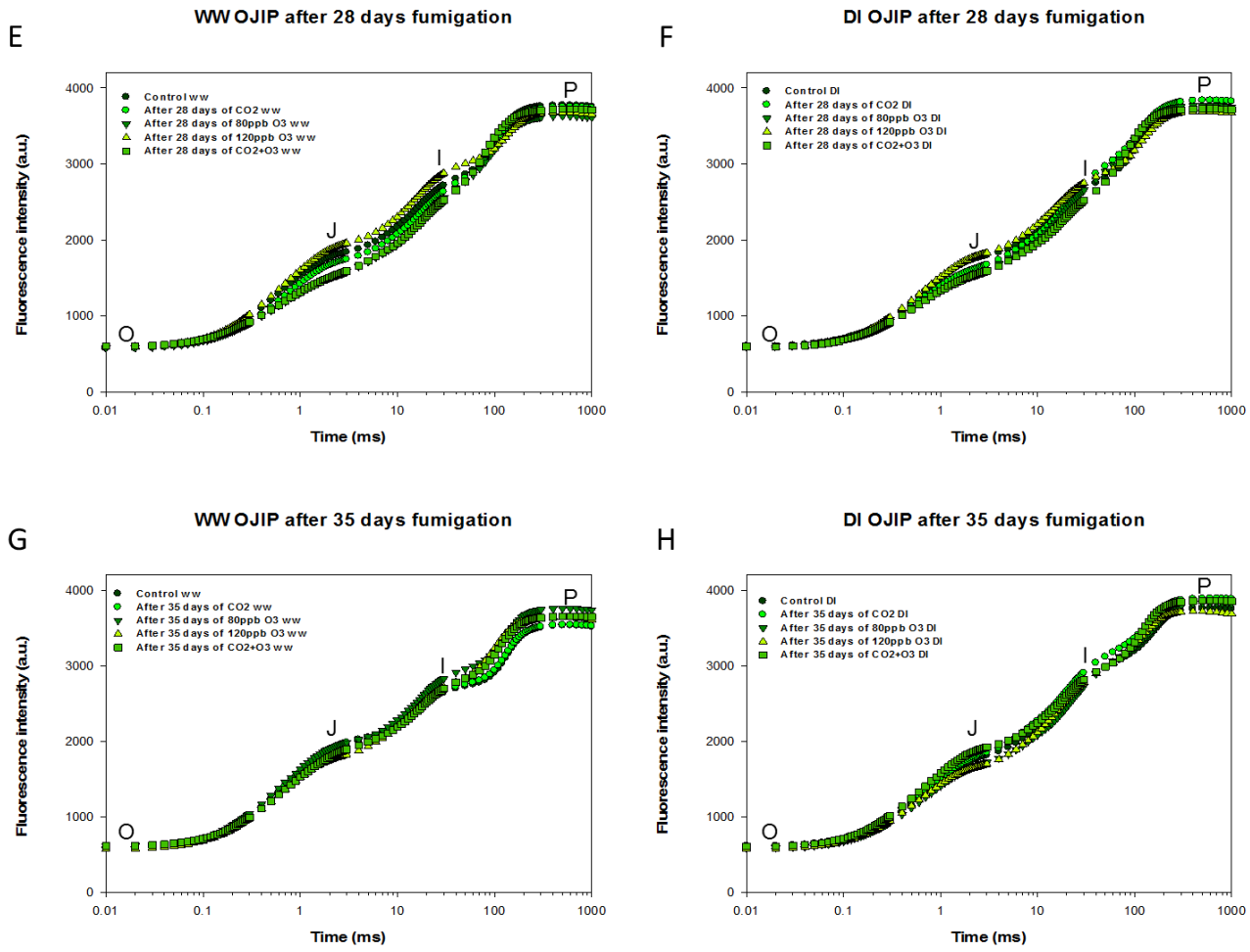


Figure 19: Polyphasic fluorescence OJIP of the chlorophyll *a* fluorescence exhibited by a dark-adapted leaves of quinoa plants fumigated with 700 ppm CO_2, 80 and 120 ppb O_3 and a combination of 700 ppm CO_2 and 80 ppb O_3. Plots A, B, C, D, E, F, G and H show the average response of the well-watered (WW) and drought-induced (DI) quinoa to elevated levels of CO_2 and O_3 and combination of both when normalised at F_o (0.02 ms) to F_p (1000 ms) phase; $V_{OP} = (F_t-F_o)/(F_p-F_o)$ from 14 to 35 days after fumigation, indicating the change in the single turnover phase (0-2 ms) and multiple turnover phase (2-1000 ms).

Table 9: The fluorescence intensity of the OJIP steps on well-watered (WW) quinoa leaves after 14, 21, 28 and 35 days of fumigation. Data shown as mean ± standard deviation. The measurements were taken on alternate weeks; different letters indicate significant differences ($P \leq 0.05$).

Fumigation period	OJIP step	Control WW	700 ppm CO_2 WW	80 ppb O_3 WW	120 ppb O_3 WW	CO_2+O_3 WW
After 14 days	0.05 ms	617.28±24.98a	631.39±33.45ab	640.50±42.23b	623.15±19.55a	617.297±26.17a
	2 ms	1825.44±152.08d	1774.12±123.30b	1832.66±182.44c	1528.00±114.77a	1764.84±194.64c
	30 ms	2746.19±131.58d	2551.81±123.62ab	2677.34±158.49c	2526.950±109.60a	2580.32±137.39b
	300 ms	3668.75±127.83c	3478.02±155.79a	3577.97±175.02b	3700.93±81.69c	3559.65±108.77b
After 21 days	0.05 ms	636.55±37.99c	579.63±28.16a	653.51±44.31c	619.24±27.88bc	620.96±75.35b
	2 ms	1882.61±225.37c	1651.03±108.08a	1655.10±203.89b	1675.00±110.88a	1659.58±217.75b
	30 ms	2814.36±221.48d	2454.90±143.64a	2667.36±225.03bc	2619.08±91.32b	2584.69±256.77c
	300 ms	3794.25±195.12c	3437.40±153.19a	3787.256±190.14c	3575.41±114.89b	3584.58±250.51b
After 28 days	0.05 ms	625.98±36.85c	599.64±23.17a	611.33±51.99ab	627.43±25.04b	632.00±36.12c
	2 ms	1765.12±168.70c	1665.77±142.60b	1501.71±136.89a	1870.22±140.30d	1503.90±119.88a
	30 ms	2715.12±190.28c	2633.05±177.74b	2554.33±207.29a	2877.35±154.47d	2524.61±142.18a
	300 ms	3754.76±145.84bc	3726.87±136.10c	3615.24±175.67a	3658.62±109.07ab	3701.76±126.92b
After 35 days	0.05 ms	627.85±37.10b	627.74±30.75ab	651.88±39.88c	613.00±33.85a	650.35±25.83c
	2 ms	1862.51±188.82d	1882.26±152.64c	1900.71±111.59cd	1751.81±109.51a	1791.98±164.56b
	30 ms	2643.38±165.99b	2658.23±121.82a	2831.98±108.67c	2709.22±148.83ab	2699.96±128.49b
	300 ms	3504.85±190.61a	3516.56±162.72a	3742.21±127.37c	3637.17±147.76b	3634.43±139.78b

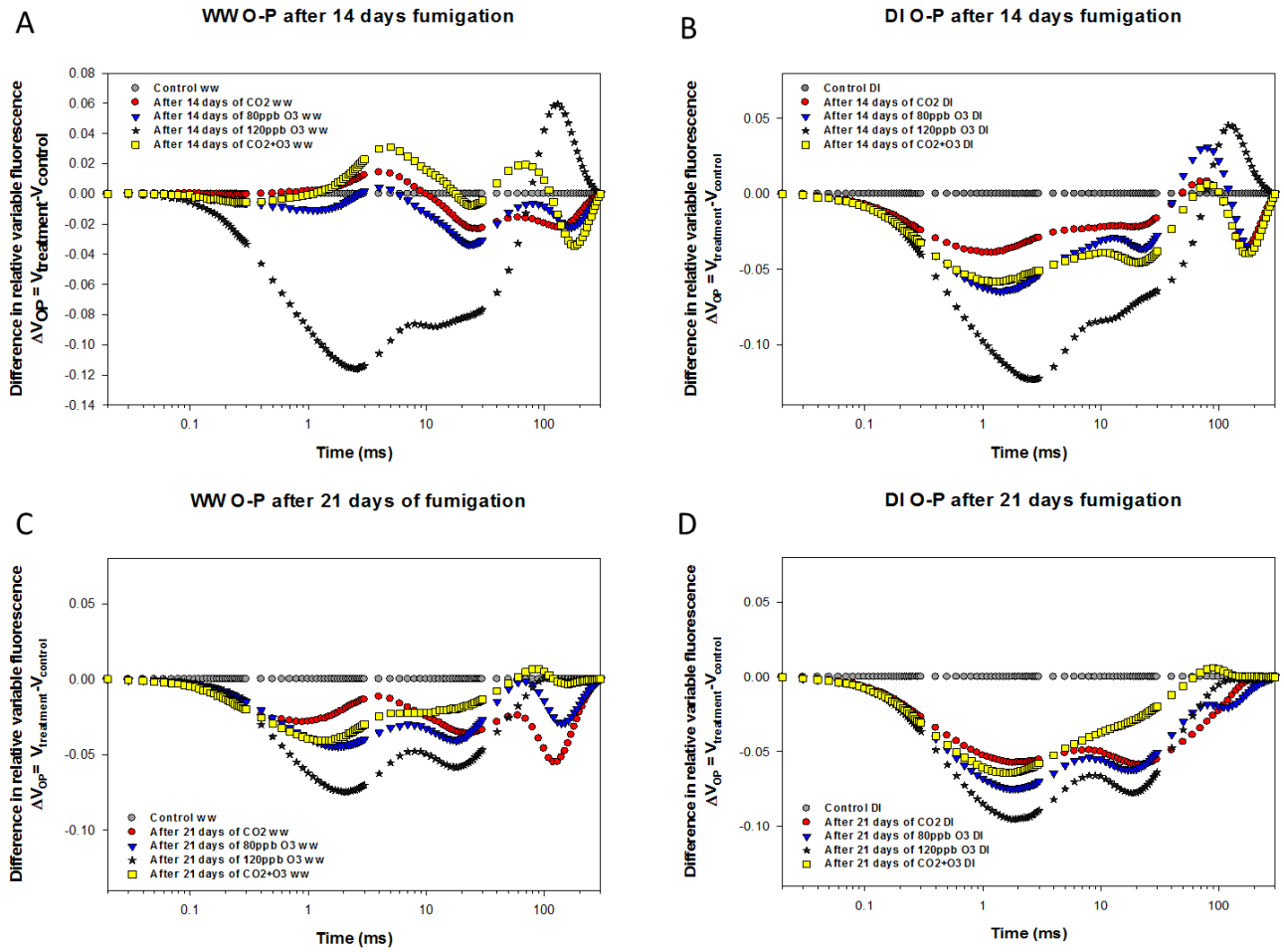
Table 10: The fluorescence intensity of the OJIP steps on drought-induced (DI) quinoa leaves after 14, 21, 28 and 35 days of fumigation. Data shown as mean ± standard deviation. The measurements were taken on alternate weeks; different letters indicate significant differences ($P \leq 0.05$).

Fumigation period	OJIP step	Control DI	700 ppm CO_2 DI	80 ppb O_3 DI	120 ppb O_3 DI	CO_2+O_3 DI
After 14 days	0.05 ms	642.75±39.29 c	589.63±28.29 a	621.77±20.06 b	623.15±19.55 b	586.92±30.56 a
	2 ms	1849.68±172.00 d	1690.53±154.94 c	1686.67±162.10 c	1528.00±114.77 a	1606.24±124.51 b
	30 ms	2633.27±154.98 c	2519.30±120.82 b	2602.23±112.65 c	2526.95±109.60 b	2418.53±87.71 a
	300 ms	3558.02±165.86 c	3482.20±116.43 b	3641.53±86.72 d	3700.93±81.69 e	3425.97±103.07 a
After 21 days	0.05 ms	622.49±31.36 c	594.73±27.08 a	609.69±23.88 b	602.73±42.21 b	591.10±20.69 a
	2 ms	1839.46±186.29 c	1578.11±182.07 a	1551.82±117.44 b	1648.48±136.57 b	1611.50±108.31 b
	30 ms	2681.17±168.03 d	2481.73±175.66 b	2498.41±108.75 ab	2638.30±146.32 c	2436.00±91.19 a
	300 ms	3643.61±90.22 c	3586.11±113.78 b	3667.39±78.07 c	3675.98±120.20 c	3537.65±91.51 a
After 28 days	0.05 ms	606.76±32.08 a	635.65±40.07 b	643.08±37.67 b	619.93±27.43 ab	623.38±45.98 b
	2 ms	1725.26±143.32 c	1592.61±130.21 b	1513.95±116.39 a	1751.58±97.11 c	1514.62±139.96 a
	30 ms	2650.24±139.79 b	2724.54±120.26 c	2655.21±113.25 b	2747.00±158.84 c	2518.81±212.32 a
	300 ms	3678.35±130.91 a	3812.76±114.58 c	3752.97±105.25 b	3670.71±124.12 a	3709.81±176.07 ab
After 35 days	0.05 ms	606.34±32.39 a	648.88±37.94 b	621.261±31.09 a	612.67±26.83 a	644.09±30.89 b
	2 ms	1730.82±129.72 b	1755.43±150.47 b	1642.98±112.08 a	1629.16±108.36 a	1842.22±156.22 c
	30 ms	2796.34±230.31 bc	2905.13±185.60 d	2728.54±166.02 a	2780.13±159.39 b	2819.13±151.16 c
	300 ms	3731.87±162.24 ab	3867.50±145.99 c	3789.26±159.93 b	3709.87±133.28 a	3843.60±151.57 bc

4.2.4.2 Difference in relative variable fluorescence

4.2.4.2.1 ΔV_{OP}

The ΔV_{OP} plots were constructed by normalising the relative variable fluorescence transients between O (0.02 ms) and P (300 ms). This plots revealed the hidden bands of the fluorescence kinetics (ΔL, ΔK, ΔJ and ΔI-bands), which provides more insight about the information the OJIP transient contain (Figure 20A-H).



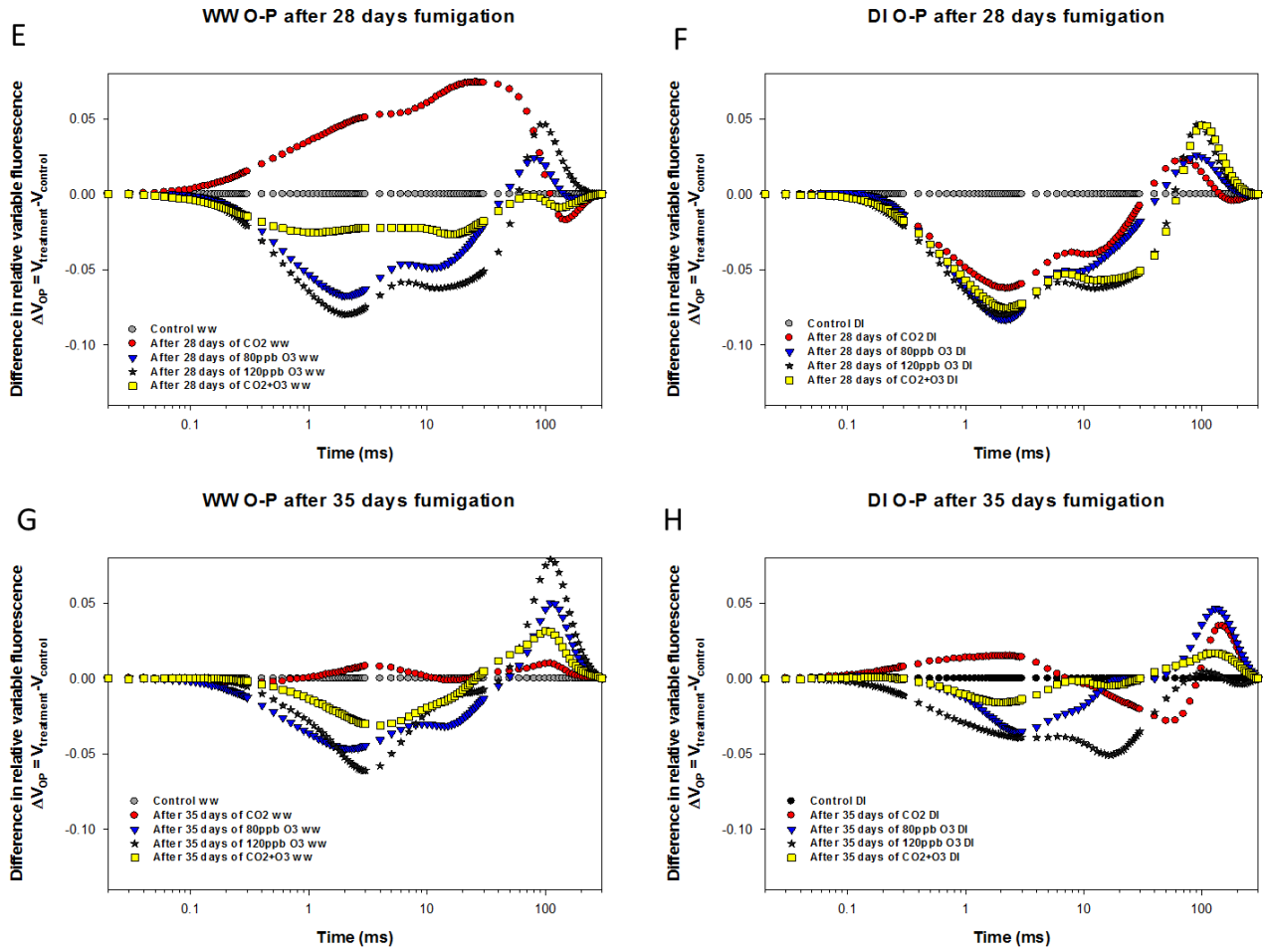
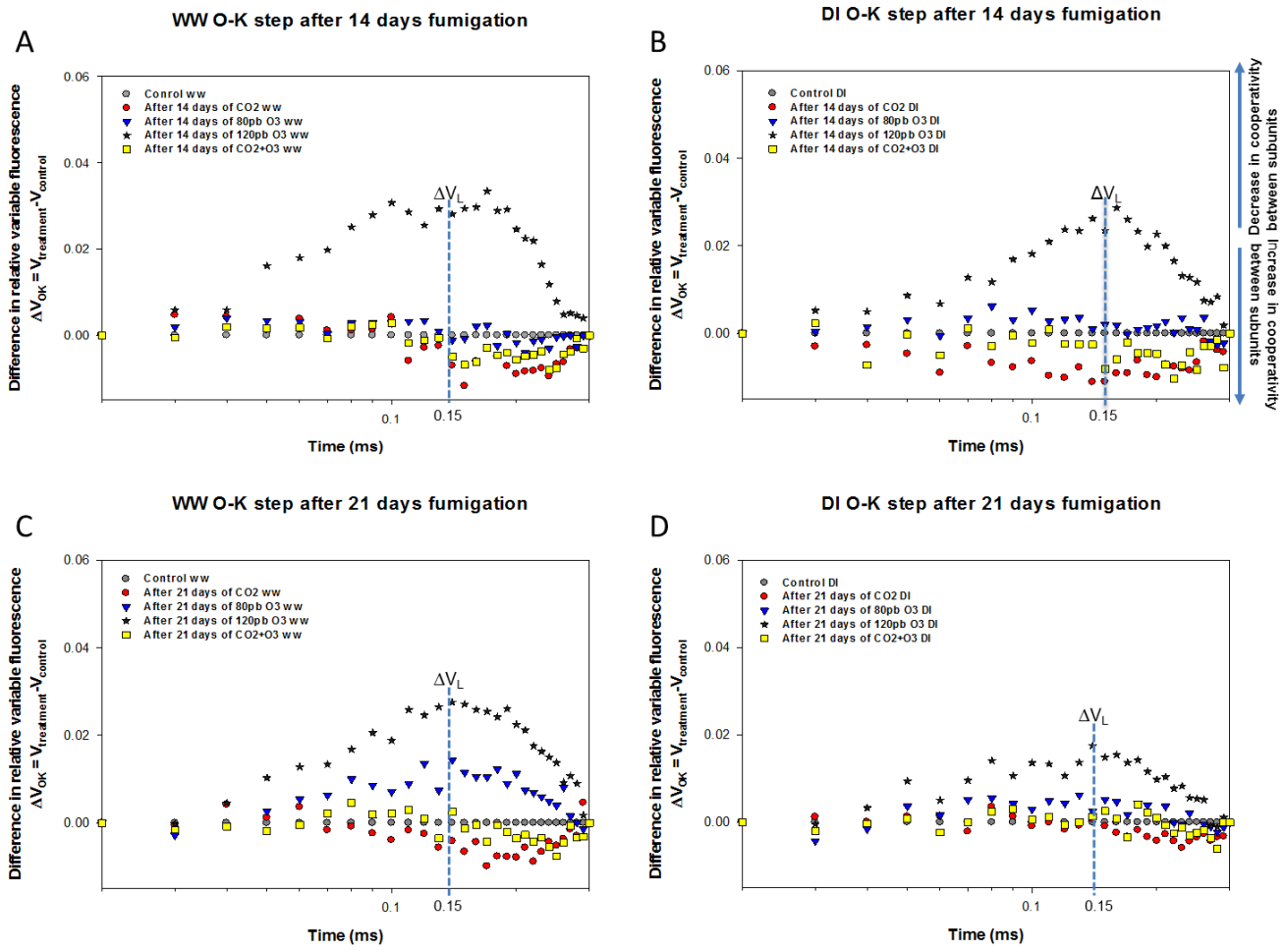


Figure 20: Changes in the difference of the relative variable chlorophyll *a* fluorescence transients (A-H) of a dark-adapted quinoa leaves normalised between F_O and F_P [$V_{OP} = (F_t - F_o)/(F_p – F_o)$, $\Delta V_{OP} = V_{treatment} – V_{control}$] recorded in plants fumigated with 700 ppm CO_2, 80 ppb O_3, 120 ppb O_3 and 700 ppm CO_2 + 80 ppb O_3 grown under well-watered and drought-induced conditions.

4.2.4.2.2 ΔV_L-Band

The ΔV_L-band is revealed by normalising data between O (0.02 ms) and K (0.3 ms), which is an indicator of the energetic grouping (connectivity) of the PSII units. After 14 days of fumigation with elevated CO_2, 80 ppb O_3 and CO_2 + O_3, well-watered quinoa revealed a negative ΔV_L-bands indicating a higher cooperativity of excitation energy between the PSII units, relative to the control (Figure 21A). There were significant differences between all of these treatments (Table11). However, drought-induced quinoa fumigated with 80 ppb O_3 and 120 ppb O_3 revealed a positive ΔV_L-band (Figure 21A). The well-watered quinoa exhibited a positive ΔV_L-band only on plants fumigated

with 120 ppb O_3 (Figure 21B), which is an indication of decreased energetic groupings of the PSII units. In addition, all of these treatments significantly differ from each other, but ΔV_L-band of the plants fumigated with 120 ppb O_3 was significantly ($P \leq 0.05$) higher (Table11). As a result, the presence of the negative ΔV_L-bands indicate effective consumption of the excitation energy and high stability of the system between 14 to 21 days after fumigation in both well-watered and drought-stressed plants fumigated with elevated CO_2, 80 ppb O_3 and CO_2 + O_3 (Figure 21A-D). The positive ΔV_L-bands were present on the drought-induced plants fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3. Similar results were also observed in the well-watered plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 from 28 to 35 days after fumigation (Figure 21E-H). This low energy cooperativity also reveals a sigmoidal rise between 0 ms and 0.3 ms that matches a significant decrease in ΔV_{OK} at 0.15 ms, which resulted in a reduced ΔV_L-band. However, the well-watered plants fumigated with elevated CO_2 exhibited a negative ΔV_L-band throughout the experiment indicating that the elevated CO_2 fumigated plants did not lose energetic connectivity of PSII units (Figure 21A-H).



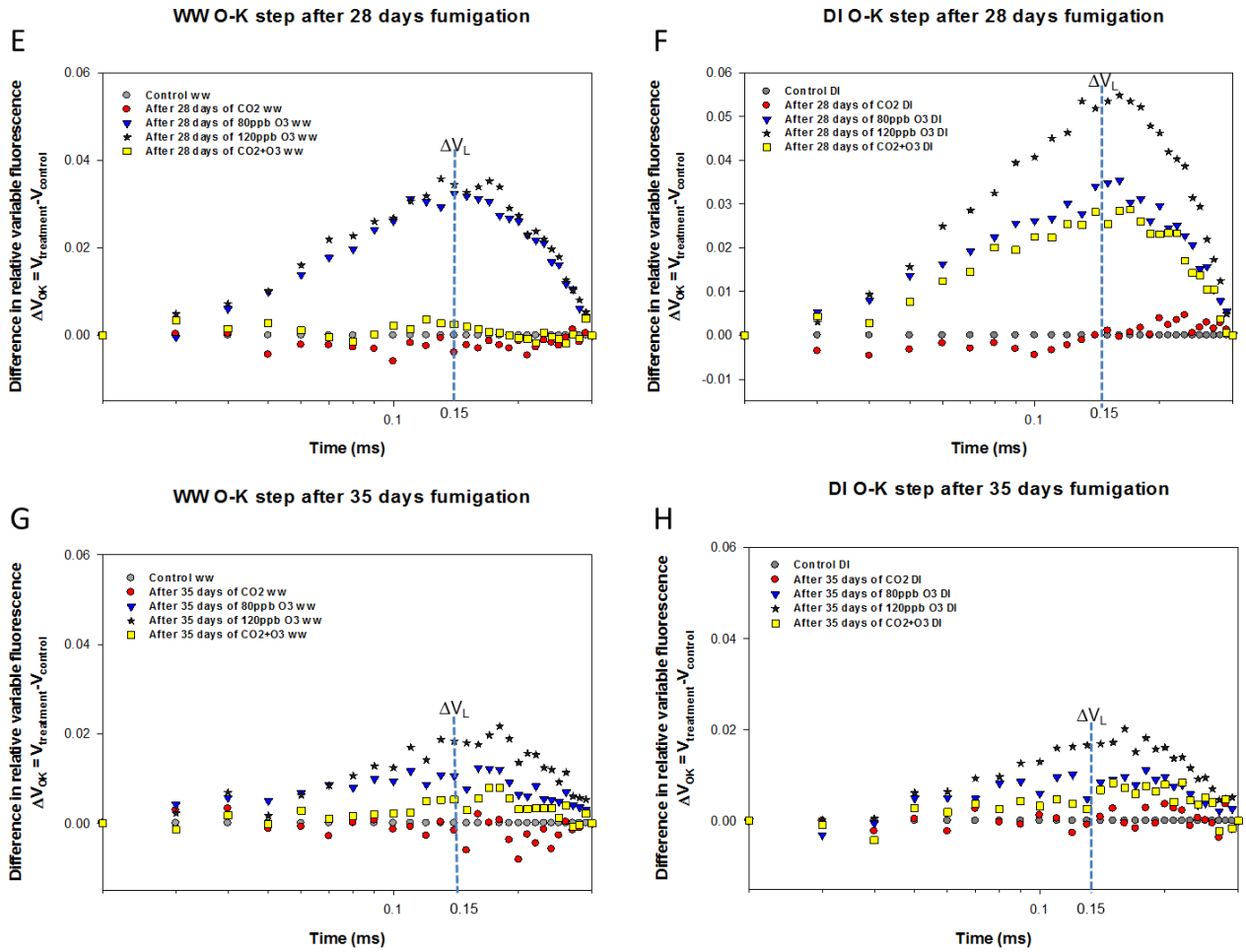


Figure 21: Changes in the difference of the relative variable chlorophyll *a* fluorescence transients (A-H) of a dark-adapted quinoa leaves normalised between F_o (0.02 ms) and F_k (0.3 ms), [$V_{OK} = (F_t - F_o)/(F_k – F_o)$, $\Delta V_{OK} = V_{treatment} – V_{control}$], indicating the energetic connectivity of the PSII units and difference between the well-watered (WW) and drought-induced (DI) quinoa fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3.

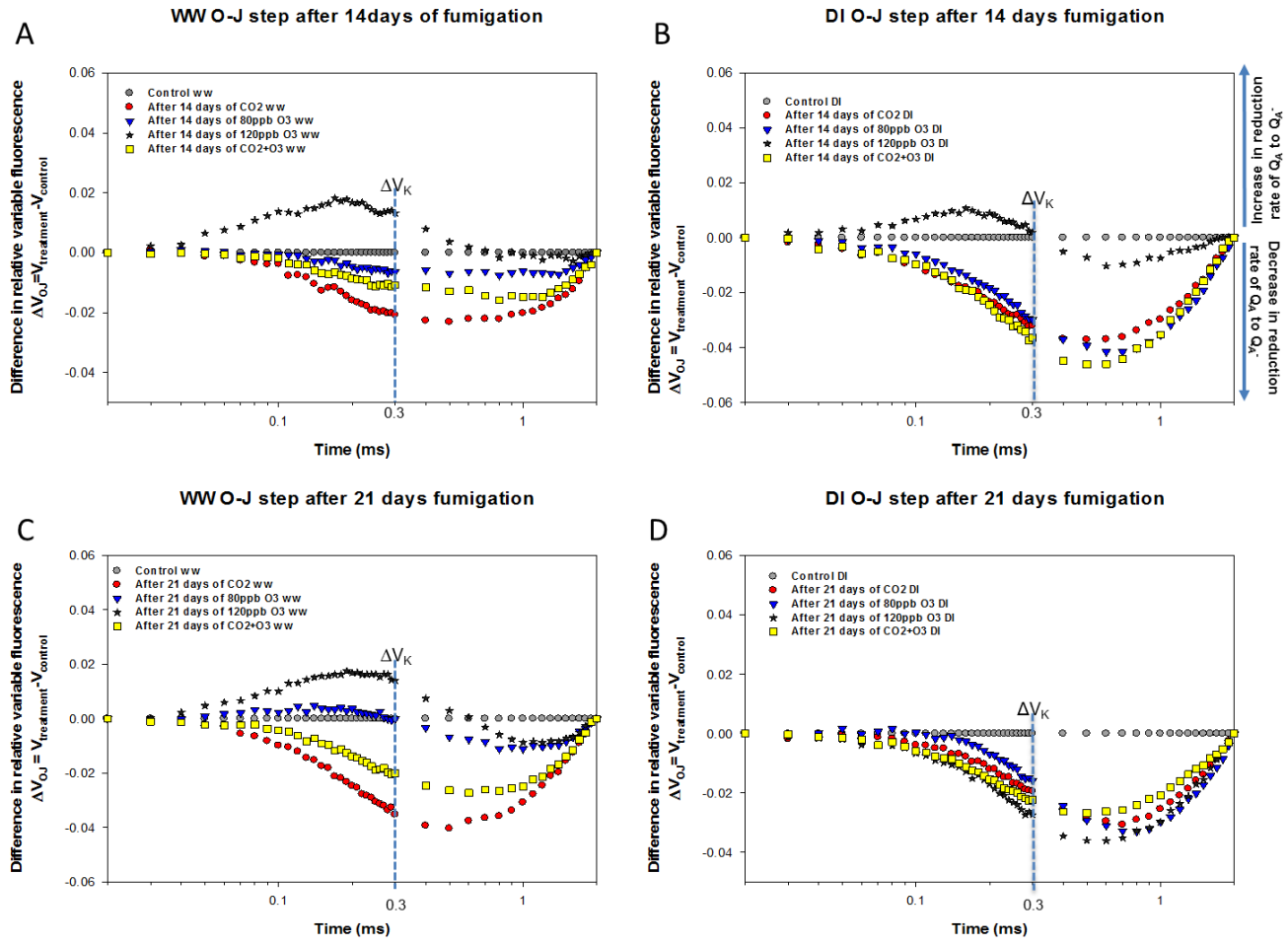
Table 11: Fluorescence intensity at 0.15 ms of the well-watered (WW) and drought-induced (DI) quinoa leaves after 14, 21, 28 and 35 days of fumigation. Data shown as mean ± standard deviation. Different letters indicate significant differences ($P \leq 0.05$) (n = 112).

Fumigation period		Treatment	Control	700 ppm CO_2	80 ppb O_3	120 ppb O_3	CO_2+O_3
After days	**14**	WW	3309.94±152.81c	3154.05±190.38b	3080.56±153.73a	3488.23±89.35d	3152.68±112.69b
		DI	3243.84±173.73c	3077.08±119.33b	3242.00±73.96c	3488.23±89.35d	3023.90±109.49a
After days	**21**	WW	3579.64±273.67c	3105.90±166.21a	3297.89±332.31b	3570.54±215.89c	3366.08±120.20b
		DI	3383.15±115.32b	3262.88±114.37a	3280.27±155.76a	3398.62±99.45b	3412.65±142.42b
After days	**28**	WW	3582.98±190.74b	3439.49±105.38a	3585.73±122.46b	3447.31±197.55a	3530.36±178.99b
		DI	3479.52±183.19a	3457.73±139.89a	3603.04±133.58b	3584.21±131.35b	3588.33±190.21b
After days	**35**	WW	3227.40±223.04a	3252.90±156.00a	3448.82±120.90b	3505.83±163.01b	3495.74±165.33b
		DI	3413.58±179.34a	3631.11±154.06b	3468.65±188.10a	3530.91±153.04a	3591.80±185.35ab

4.2.4.2.3 ΔV_K-Band

Normalising the fluorescence data between O (0.02 ms) and J (2 ms) reveals the ΔV_K-band. The well-watered plants fumigated with elevated CO_2, 80 ppb O_3 and CO_2 + O_3 exhibited a negative ΔV_K-bands after 14 days of fumigation indicating a decreased reduction rate of Q_A, which is the primary electron acceptor of the PSII. Hence, well-watered plants fumigated with 120 ppb O_3 exhibited a positive ΔV_K-band after 14 days of fumigation indicating an increase in the reduction rate of Q_A (Q_A to Q_A^-) (Figure 22A). The latter could mean that the oxygen evolving complex (OEC) was leaking and offering access to the non-water electron donors. More so, a positive ΔV_K-band could be an indication that the OEC was inactivated or the functional PSII antenna size was increased as reported by Yusuf *et al*. (2010). The well-watered plants fumigated with elevated CO_2 maintained a negative ΔV_K-band throughout the experiment (Figure 22A,

C, E and G), while plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 exhibited a positive ΔV_K-band from 28 to 35 days after fumigation (Figure 22E-H). There were no significant differences between these treatments after 28 days of fumigation. However, these treatments differed significantly after 35 days of fumigation except in the plants fumigated with 80 ppb O_3 and CO_2 + O_3 (Table 12). Drought-stressed plants had a negative ΔV_K-band from 14 to 21 days after fumigation, indicating a delayed response to water stress and fumigation treatments (Figure 22B and D). The exhibition of a positive ΔV_K-band from 28 to 35 days after fumigation for all treatments indicated that the OEC was inactivated. Furthermore, all of these treatments significantly differ from each other under drought-induced conditions. At the same time, the ΔV_K-band of the plants fumigated with 120 ppb O_3 was significantly ($P \leq 0.05$) higher compared to other treatments (Table 12), which is an indication of O_3 damage.



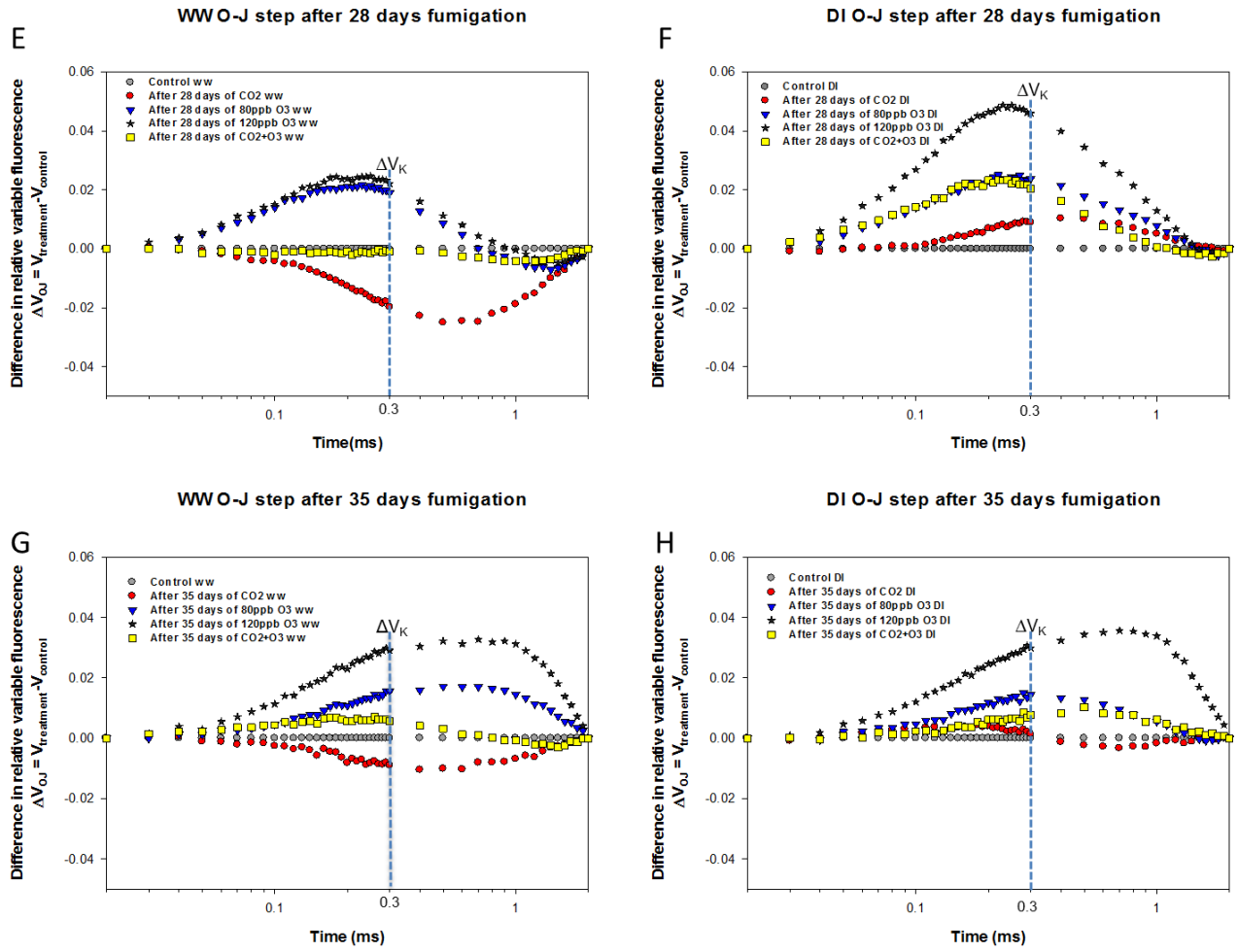


Figure 22: Changes in the difference of the relative variable chlorophyll *a* fluorescence transients (A-H) of a dark-adapted quinoa leaves normalised between F_o (0.02 ms) and F_J (2 ms), [$V_{OJ} = (F_t - F_o)/(F_J - F_o)$, $\Delta V_{OJ} = V_{treatment} - V_{control}$] showing intactness of the OEC and the effect of elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 on the functional antenna size of PSII of the well-watered (WW) and drought-induced (DI) quinoa.

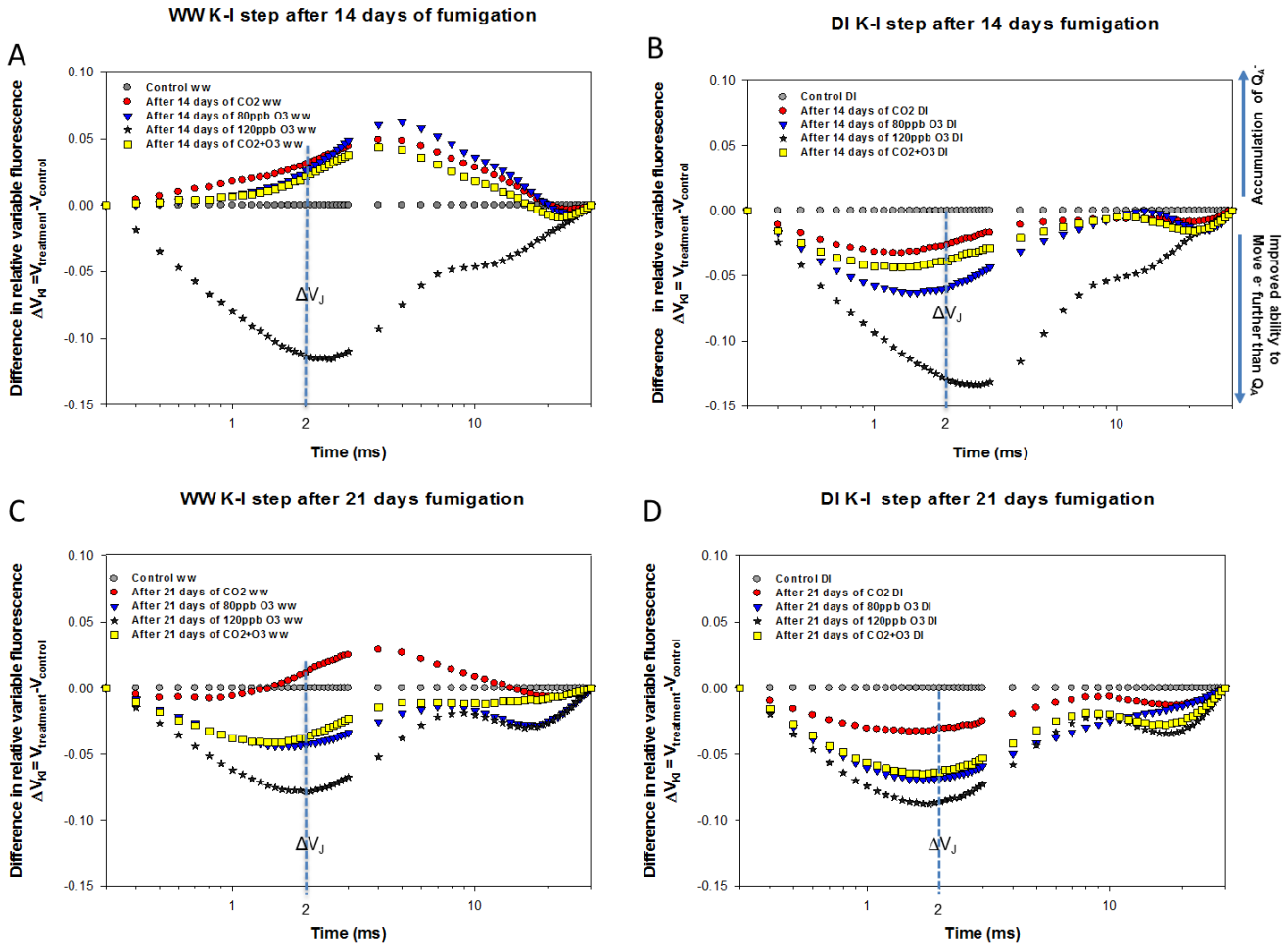
Table 12: Fluorescence intensity at 0.3 ms of the well-watered (WW) and drought-induced (DI) quinoa leaves after 14, 21, 28 and 35 days of fumigation. Data shown as mean ± standard deviation. Different letters indicate significant differences ($P \leq 0.05$) (n = 112).

Fumigation period		Treatment	Control	700 ppm CO_2	80 ppb O_3	120 ppb O_3	CO_2+O_3
After days	**14**	WW	3668.75±127.83c	3577.97±175.02b	3478.02±155.79a	3700.93±81.69c	3559.65±108.77b
		DI	3558.02±165.86c	3482.20±116.43b	3641.53±86.72d	3700.93±81.69e	3425.97±103.07a
After days	**21**	WW	3794.25±195.12c	3437.40±153.19a	3584.58±20.51b	3787.26±190.14c	3575.41±114.89b
		DI	3643.61±90.22c	3586.11±113.78b	3667.39±78.07c	3675.98±120.20c	3537.60±91.51a
After days	**28**	WW	3754.76±145.84b	3726.87±136.00b	3658.62±109.07a	3615.24±175.67a	3701.76±126.92ab
		DI	3678.35±130.91a	3812.76±114.58c	3752.90±105.25b	3670.71±124.12a	3709.81±176.07a
After days	**35**	WW	3504.85±190.61a	3516.56±162.72a	3637.17±147.76b	3742.21±127.37c	3634.43±139.78b
		DI	3709.87±133.28a	3789.20±159.93b	3843.60±151.57bc	3867.50±145.99c	3731.87±162.24ab

4.2.4.2.4 ΔV_J-Band

Normalising the fluorescence data between K (0.3 ms) and I (30 ms) reveals the ΔV_J-band. The decrease in oxygen evolution and Q_A^- concentration is reflected by a negative ΔV_J-band. Negative ΔV_J-band could mean that the build-up of reduced electron carriers like plastoquinone and plastocyanin was less (Schansker *et al*., 2003; Ranjan *et al*., 2014). The ΔV_J-bands of the drought-induced plants fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 had a higher amplitude than the well-watered plants (Figure 23B, D, F and H). The presence of a negative ΔV_J-bands in these treatments indicates a significant ($P \leq 0.05$) decrease in the fraction of the primary oxidised quinone acceptor (Q_A^-). This also indicated that drought stress disrupted the OEC of the drought-induced plant fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 by decreasing the flow of electrons to the RCs of PSII, thus decreasing

the concentration of Q_A^-. Similar results were observed on the well-watered plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 (Figure 23A, C, E and G). Consequently, CO_2 fumigated well-watered plants exhibited a slight positive ΔV_J-band suggesting an increase in the OEC integrity and Q_A^- concentration, but these bands do not differ significantly from the plants fumigated with 80 ppb O_3 after 35 days of fumigation (Table 13). However, damaged OEC activity can be compensated by non-water electron donors for drought-induced plant (Kalaji *et al*., 2014; Gururani *et al*., 2015).



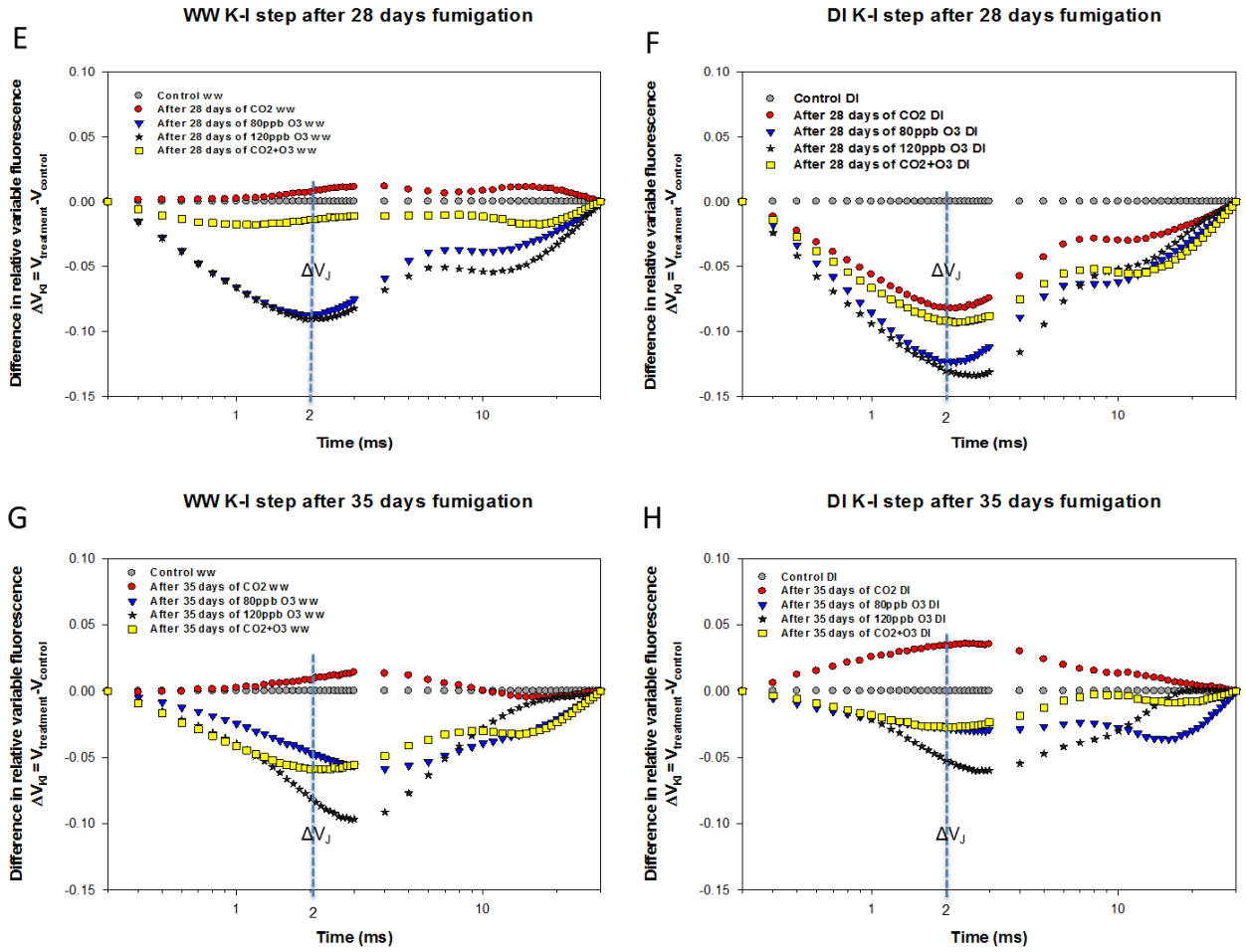


Figure 23: Changes in the difference of the relative variable chlorophyll *a* fluorescence transients (A-H) of a dark-adapted quinoa leaves normalised between F_K (0.3 ms) and F_I (30 ms), [$V_{KI} = (F_t - F_o)/(F_I – F_K)$, $\Delta V_{KI} = V_{treatment} – V_{control}$], reflecting an increase or decrease in oxygen evolution and Q_A^- concentration of the well-watered (WW) and drought-induced (DI) quinoa fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3.

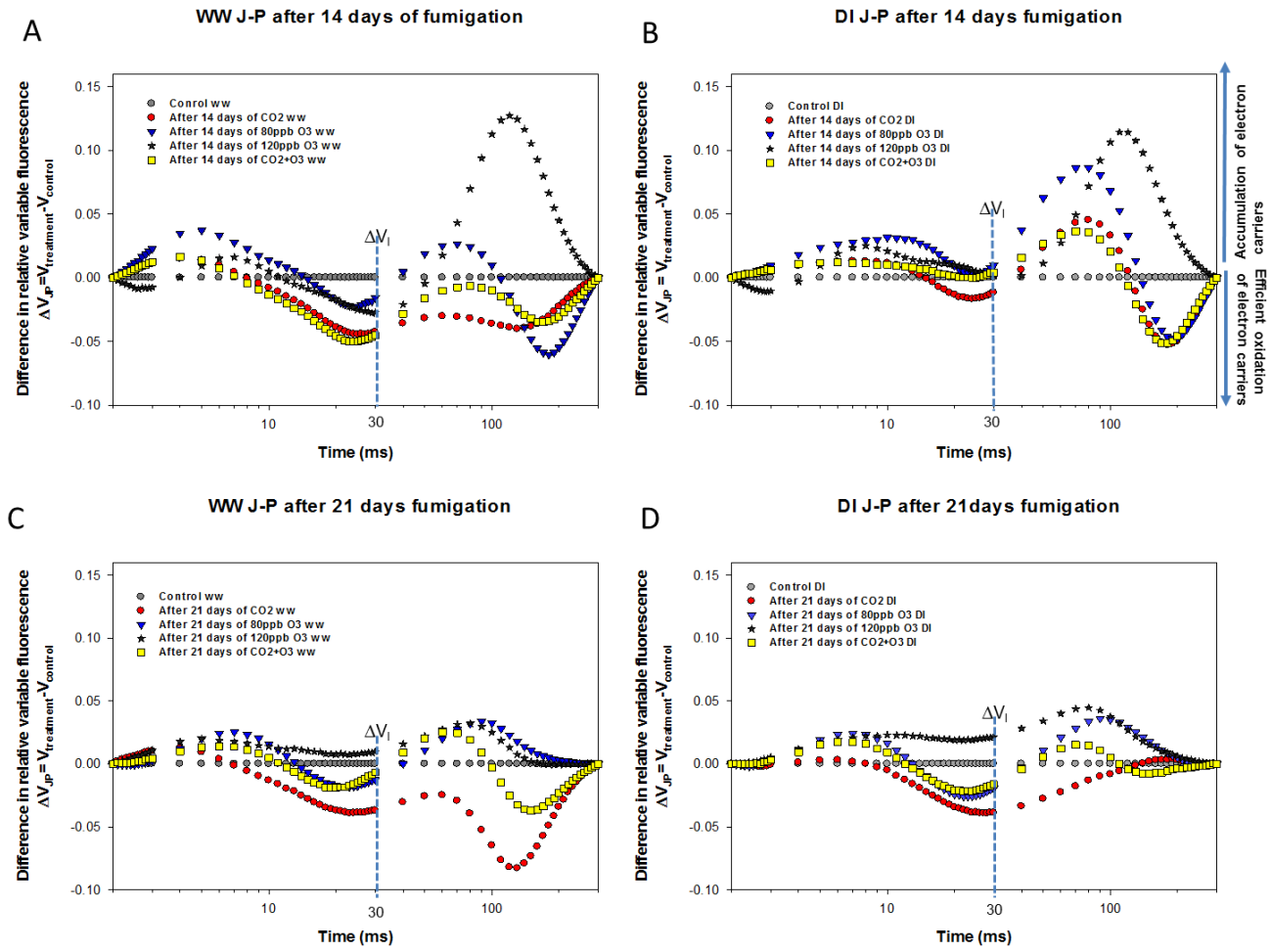
Table 13: Fluorescence intensity at 2 ms of the well-watered (WW) and drought-induced (DI) quinoa leaves after 14, 21, 28 and 35 days of fumigation. Data shown as mean ± standard deviation. Different letters indicate significant differences ($P \leq 0.05$) (n = 112).

Fumigation period		Treatment	Control	700 ppm CO_2	80 ppb O_3	120 ppb O_3	CO_2+O_3
After days	**14**	WW	1825.44±152.08d	1774.12±123.30b	1832.66±182.44c	1528.00±114.77a	1764.84±194.64c
		DI	1849.68±172.00d	1690.53±154.94c	1686.67±162.10c	1528.00±114.77a	1606.24±124.51b
After days	**21**	WW	1882.61±225.37c	1651.03±108.08a	1655.10±203.89b	1675.00±110.88a	1659.58±217.75b
		DI	1839.46±186.29c	1611.50±108.31b	1648.48±136.57b	1551.82±117.44b	1578.11±182.07a
After days	**28**	WW	1765.12±168.70c	1665.77±142.60b	1501.71±136.89a	1870.22±140.30d	1503.90±119.88a
		DI	1725.26±143.32c	1592.61±130.21b	1513.95±116.39a	1751.58±97.11c	1514.62±139.96a
After days	**35**	WW	1862.51±188.82d	1882.26±152.64c	1900.71±111.59cd	1751.81±109.51a	1791.98±164.56b
		DI	1730.82±129.72b	1842.22±156.22c	1642.98±112.08a	1629.16±108.36a	1755.43±150.47b

4.2.4.2.5 ΔV_I-Band

Normalising the fluorescence data between F_J (2 ms) and F_P (300 ms) reveals the ΔV_I-band, which permits the assessment of the sequence of events of the electron transport from the plastoquinol (PQH_2) to the final electron acceptor of the PSI. The pool size of the final electron acceptors of the PSI were revealed by the increase in the variable fluorescence of the plants fumigated with 80 ppb O_3 and 120 ppb O_3 after 28 days of fumigation of the well-watered plants. However, the variable fluorescence decreased in the well-watered plants fumigated with elevated CO_2 and CO_2 + O_3 (Figure 24E and G). As expected, the variable fluorescence increased in the drought-stressed plants for all treatments (Figure 24B, D, F and H). Thus, an increase in the variable fluorescence indicated that quinoa response was dependent on the severity of water stress and the

concentration of elevated CO_2 and O_3 as revealed by the regulation of pool size of the final electron acceptors on the acceptor side of PSI (Figure 24E-F).



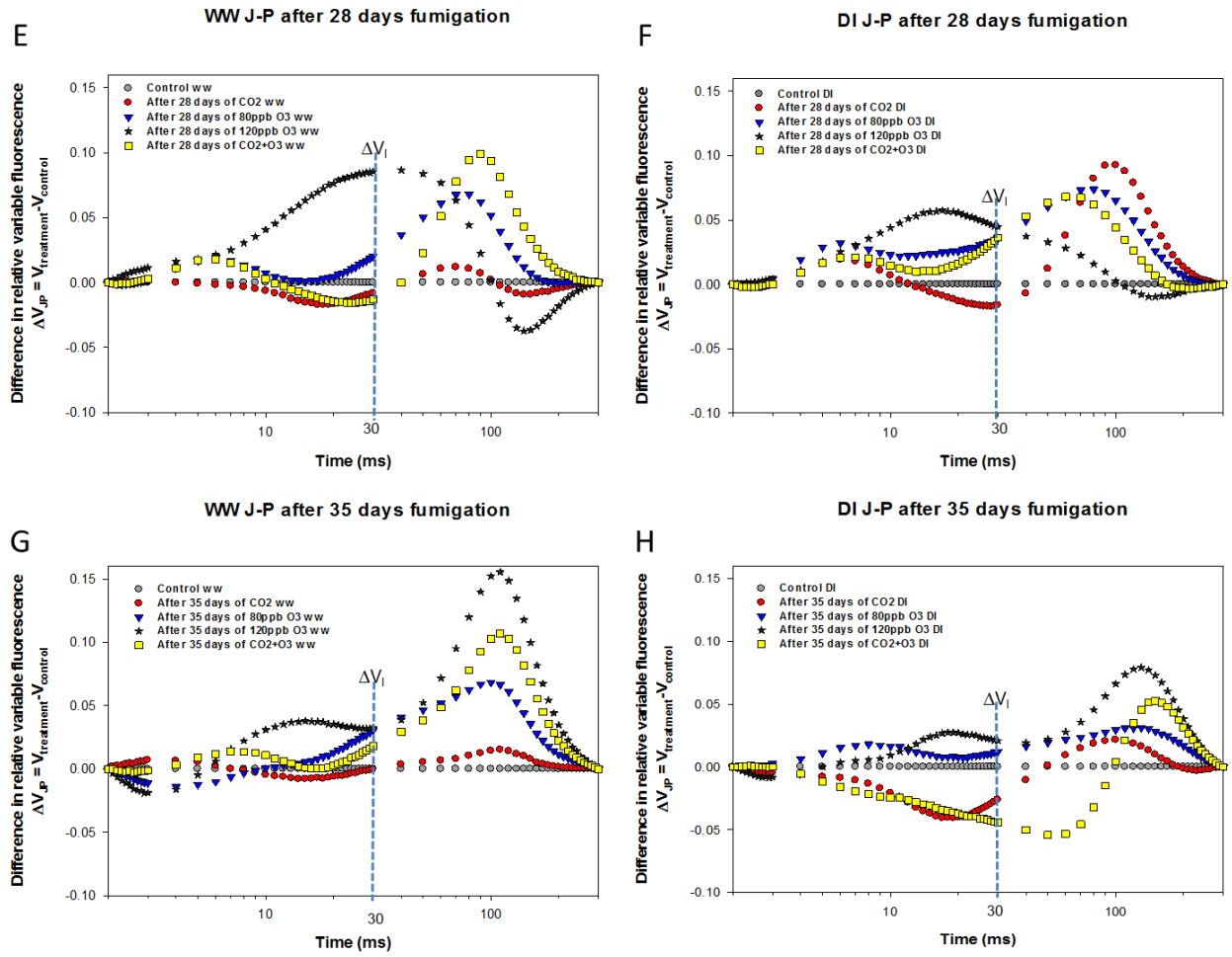


Figure 24: Changes in the difference of the relative variable chlorophyll *a* fluorescence transients (A-H) of a dark-adapted quinoa leaves normalised between F_J (2 ms) and F_P (300 ms), [$V_{JP} = (F_t - F_o)/(F_P – F_J)$, $\Delta V_{JP} = V_{treatment} – V_{control}$], indicating the accumulation and efficient utilisation of plastoquinone in the well-watered and drought-induced quinoa fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3.

4.2.4.3 Specific energy fluxes

The normalised JIP-test parameters are shown in a radar plot from 14 to 35 days after fumigation (Figure 25A-H). Chlorophyll *a* fluorescence parameters, maximum quantum efficiency (φ_{P0} or F_v/F_m), performance index (PI_{ABS}) and total photosynthetic performance index (PI_{total}) were determined in the well-watered and drought-induced quinoa fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3.

4.2.4.3.1 Maximum quantum efficiency (φ_{P0} or F_v/F_m)

Quinoa plants subjected to O_3 stress and elevated levels of CO_2 under drought conditions resulted in the changes of several JIP parameters. The F_v/F_m was less sensitive to water deficit and O_3 stress and elevated CO_2 as there were no significant differences between these treatments. The F_v/F_m values decreased from 28 to 35 days after fumigation in the plants treated with elevated CO_2, 120 ppb O_3 and CO_2 + O_3 when compared to the values observed from 14 to 21 days after fumigation in both well-watered and drought-induced treatments (Figure 25A-H). Well-watered plants fumigated with 80 ppb O_3 had lower F_v/F_m values (0.841 to 0.849) from 14 to 21 days (Figure 25A and C) and increased F_v/F_m values (0.846 to 0.853) from 28 to 35 days after fumigation (Figure 25F and H). Hence, the increase in F_v/F_m ratio between these treatments was caused by the increase in F_m (maximum fluorescence) values (Figure 25A-H), which is an indication that the non-photochemical quenching coefficient (q_N) was reduced and/or photochemical quenching coefficient (q_P) was increased (Pellegrini *et al*., 2011).

4.2.4.3.2 Specific fluxes per reaction center (RC)

The specific flux or activity of active PSII reaction centers of ABS/RC, TR_o/RC, ET_o/RC, DI_o/RC and RE_o/RC were relatively low from 14 to 21 days after fumigation in the well-watered plants (Figure 25A and C). However, significant increase of these parameters were observed in the well-watered plants after 28 days of fumigation compared to the control and drought-induced plants treated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 (Figure 25F). The ABS/RC parameter reflects a total absorption of the PSII antenna chlorophylls per active reaction center (RC). The increase in this parameter, relative to the control could mean that a fraction of the RCs is inactivated or the apparent antenna size is increased. The JIP analyses of quinoa plants fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 suggest that high values of ABS/RC indicate a significant increase in the absorption of the photon flux per active RCs. Studies suggest that the latter could only mean a potential increase in the antenna size and not a structural increase of antenna size of the biochemical complex (Kalaji *et al*., 2014; Gururani *et al*., 2015). The increase of the ABS/RC (decrease of the active RCs) values was accompanied by increased trapping of an excitation energy (TR_o/RC), which resulted in the increase in reduction of Q_A to Q_A^-.

Generally, the well-watered quinoa fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 had high ET_o/RC compared to the drought-induced plants (Figure 25A-H), indicating a thermal activation of the dark reactions (Gururani *et al*., 2015). More so, RE_o/RC for the drought-stressed plants fumigated with 80 ppb O_3 had the greatest reduction higher than the control after 35 days of fumigation (Figure 25H). This parameter also increased in the well-watered plants fumigated with elevated CO_2 and CO_2 + O_3, indicating that the elevated CO_2 positively influenced the structure and functionality of the PSI. Progressive increase in ABS/RC and DI_o/RC indicates that more energy was dissipated as heat in both treatments (Figure 25E-H).

4.2.4.3.3 Phenomenological fluxes per excited cross section (CS)

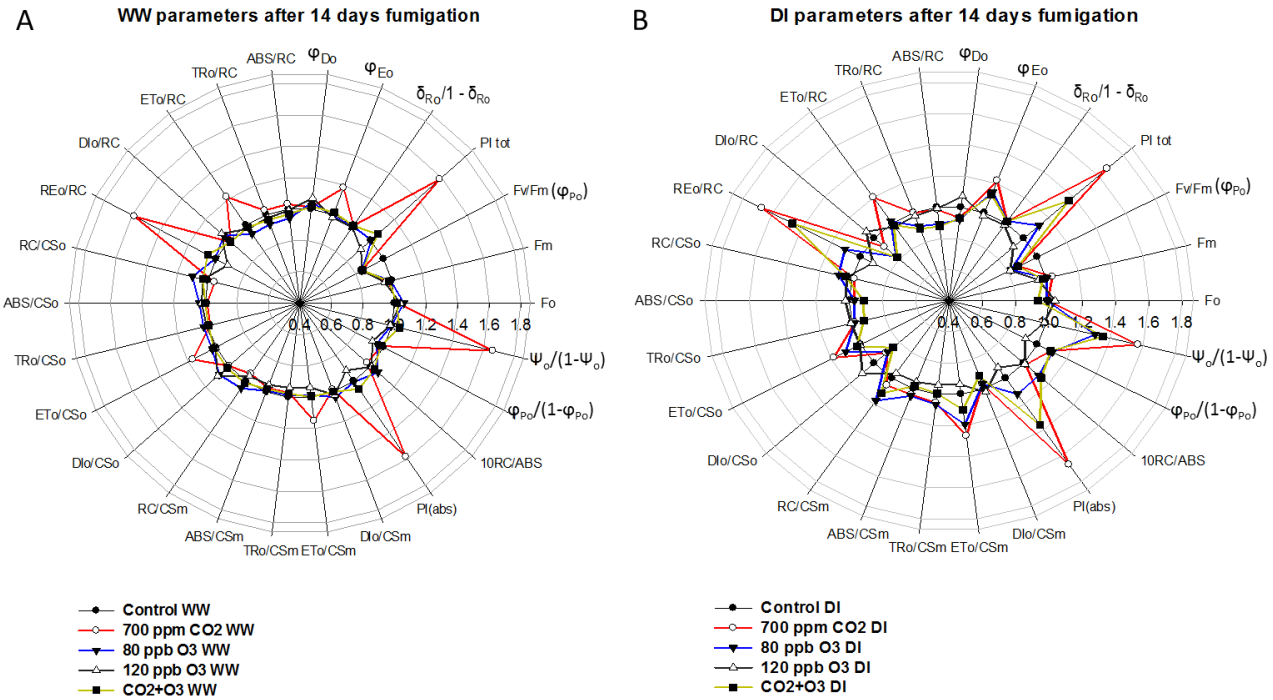
The values for phenomenological fluxes per excited cross section (CS) for ABS/CS and TR_o/CS declined from 14 to 28 days after fumigation and increased after 35 days of fumigation in both well-watered and drought-induced plants. However, ET_o/CS increased from 14 to 35 days after fumigation for both well-watered and drought-induced plants (Figure 25A-H). Moreover, drought-induced plants had higher values for specific flux per reaction center and phenomenological flux per excited cross section compared to the well-watered plants.

4.2.4.3.4 Performance indexes (PI_{ABS} and PI_{total}) and the partial parameters

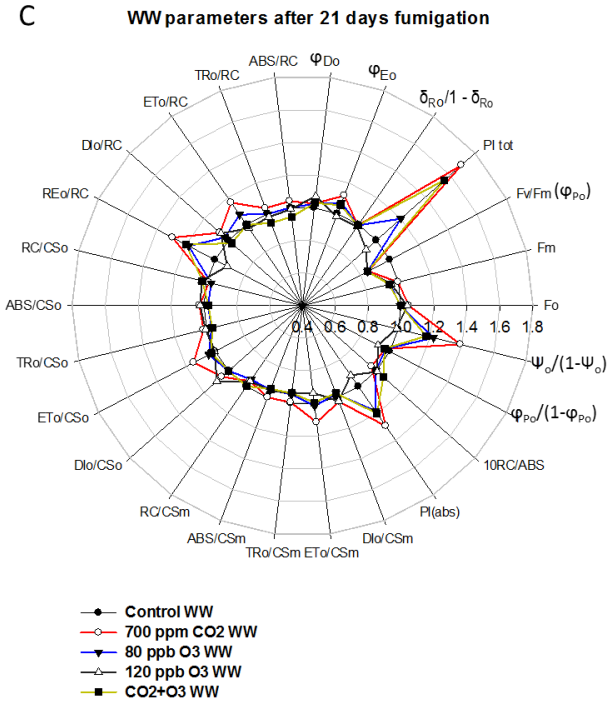
The performance indexes (PI_{ABS} and PI_{total}) is a measure of the plant's overall performance as influenced by drought, elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3. These parameters are used to quantify the PSII behaviour and measures photosynthetic efficiency up to the reduction of the PSI end-electron acceptors, respectively. The PI_{ABS} slightly decreased from 14 to 21 days after fumigation in both treatments when compared to the control (Figure 25A-D). However, the PI_{ABS} parameter started to increase after 28 days of fumigation for both treatments, but plants treated with elevated CO_2 had significant ($P \leq 0.05$) higher PI_{ABS} values when compared to the control (Figure 25E-H). Various driving components of the PI_{ABS}, density of operative photosystems (reaction centre per chlorophyll, RC/ABS), efficiency of energy trapping (ψ_o/(1 - ψ_o) and conversion of the excitation energy to the electron transport (φ_{Po}/(1 - φ_{Po}) had the same trend for the well-watered and drought-induced quinoa fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3. The RC/ABS and (φ_{Po}/(1 -

φ_{Po}) parameters were reduced in the well-watered and drought-induced plants for all treatments (Figure 25A-H). The parameter (ψ_0) symbolises the efficiency with which a trapped exciton transfers the electron to the photosystem electron transport chain further than Q_A^-. The value for (ψ_{Eo}) induced gain in the electron capacity, which was indicated by an increase in the value of ($\psi_o/1 - \psi_o$) in both well-watered and drought-induced plants fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3.

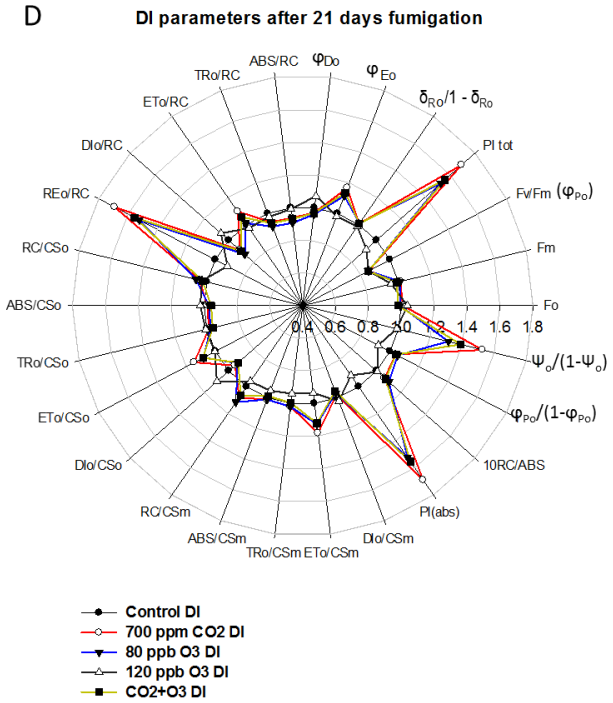
The performance index (PI_{total}) measures the plant's performance up to the reduction of the PSI end-electron acceptors. The PI_{total} declined from 14 to 35 days after fumigating plants with 80 ppb O_3 and 120 ppb O_3 in both well-watered and drought-induced conditions (Figure 25A-H). The PI_{total} also represent partial potential for energy conservation and was strictly connected to the plant overall growth rate and survival under stress conditions. However, quinoa plants fumigated with 80 ppb O_3 and 120 ppb O_3 exhibited a decrease in both PI_{ABS} and PI_{total} after 28 days of fumigation for both well-watered and drought-induced plants (Figure 25E-H and Figure 26A-D).



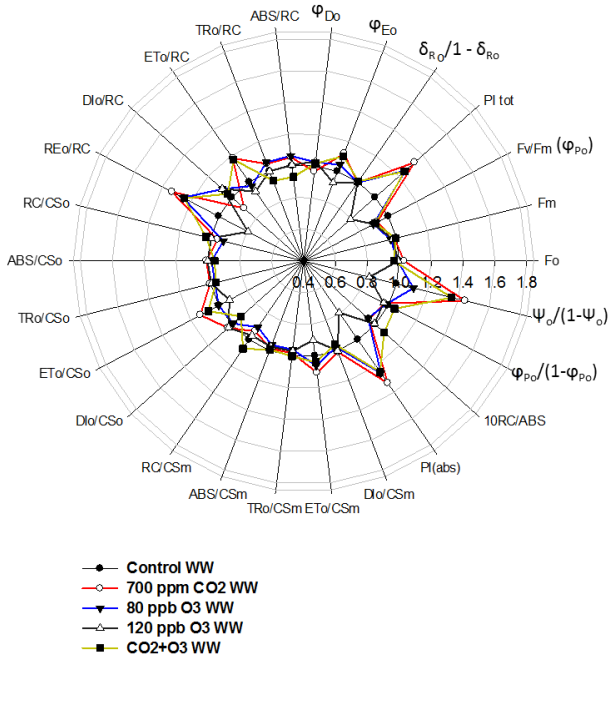
C


WW parameters after 21 days fumigation
ABS/RC
φDo
φEo
δRo/1 - δRo
PI tot
Fv/Fm (φPo)
Fm
Fo
Ψo/(1-Ψo)
φPo/(1-φPo)
10RC/ABS
PI(abs)
DIo/CSm
ETo/CSm
TRo/CSm
ABS/CSm
RC/CSm
DIo/CSo
ETo/CSo
TRo/CSo
ABS/CSo
RC/CSo
REo/RC
DIo/RC
ETo/RC
TRo/RC
0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8
Control WW
700 ppm CO2 WW
80 ppb O3 WW
120 ppb O3 WW
CO2+O3 WW

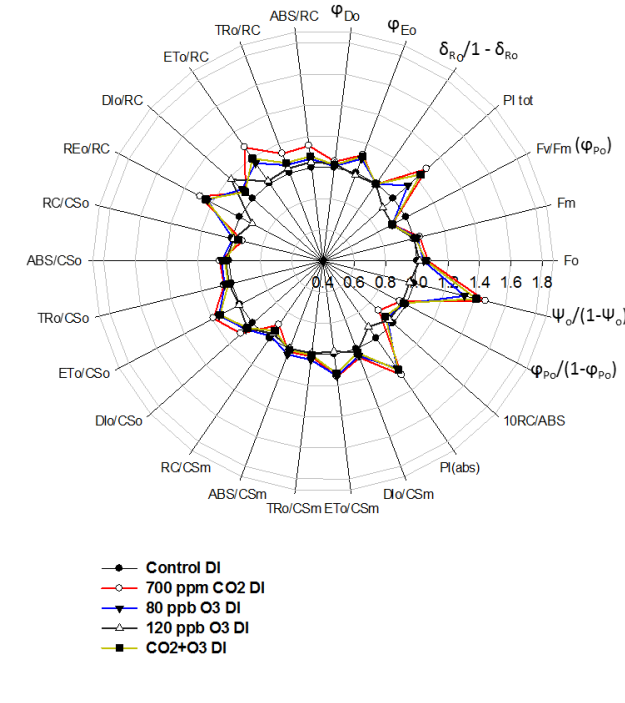
D


DI parameters after 21 days fumigation
ABS/RC
φDo
φEo
δRo/1 - δRo
PI tot
Fv/Fm (φPo)
Fm
Fo
Ψo/(1-Ψo)
φPo/(1-φPo)
10RC/ABS
PI(abs)
DIo/CSm
ETo/CSm
TRo/CSm
ABS/CSm
RC/CSm
DIo/CSo
ETo/CSo
TRo/CSo
ABS/CSo
RC/CSo
REo/RC
DIo/RC
ETo/RC
TRo/RC
0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8
Control DI
700 ppm CO2 DI
80 ppb O3 DI
120 ppb O3 DI
CO2+O3 DI

E


WW parameters after 28 days fumigation
ABS/RC
φDo
φEo
δRo/1 - δRo
PI tot
Fv/Fm (φPo)
Fm
Fo
Ψo/(1-Ψo)
φPo/(1-φPo)
10RC/ABS
PI(abs)
DIo/CSm
ETo/CSm
TRo/CSm
ABS/CSm
RC/CSm
DIo/CSo
ETo/CSo
TRo/CSo
ABS/CSo
RC/CSo
REo/RC
DIo/RC
ETo/RC
TRo/RC
0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8
Control WW
700 ppm CO2 WW
80 ppb O3 WW
120 ppb O3 WW
CO2+O3 WW

F


DI parameters after 28 days fumigation
ABS/RC
φDo
φEo
δRo/1 - δRo
PI tot
Fv/Fm (φPo)
Fm
Fo
Ψo/(1-Ψo)
φPo/(1-φPo)
10RC/ABS
PI(abs)
DIo/CSm
ETo/CSm
TRo/CSm
ABS/CSm
RC/CSm
DIo/CSo
ETo/CSo
TRo/CSo
ABS/CSo
RC/CSo
REo/RC
DIo/RC
ETo/RC
TRo/RC
0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8
Control DI
700 ppm CO2 DI
80 ppb O3 DI
120 ppb O3 DI
CO2+O3 DI

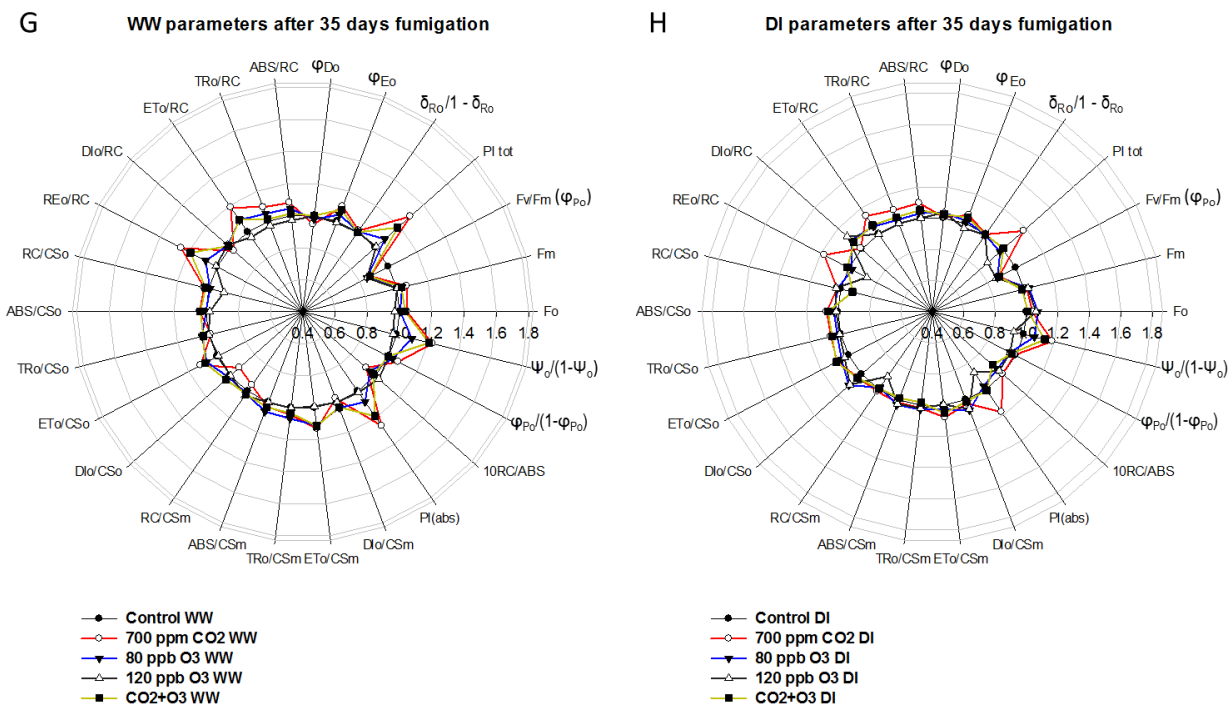


Figure 25: Radar plots (A-H) depicting possible changes in the JIP-test parameters, structure and function of the photosynthetic apparatus of quinoa fumigated with 700 ppm CO_2, 80 ppb O_3, 120 ppb O_3 and 700 ppm CO_2 + 80 ppb O_3 recorded after 14 to 35 days of fumigation in quinoa subjected to the well-watered (WW) and drought-induced (DI) conditions. The deviation of the behaviour pattern from the control treatment demonstrates the fractional impact of the treatment to the fluorescence parameters.

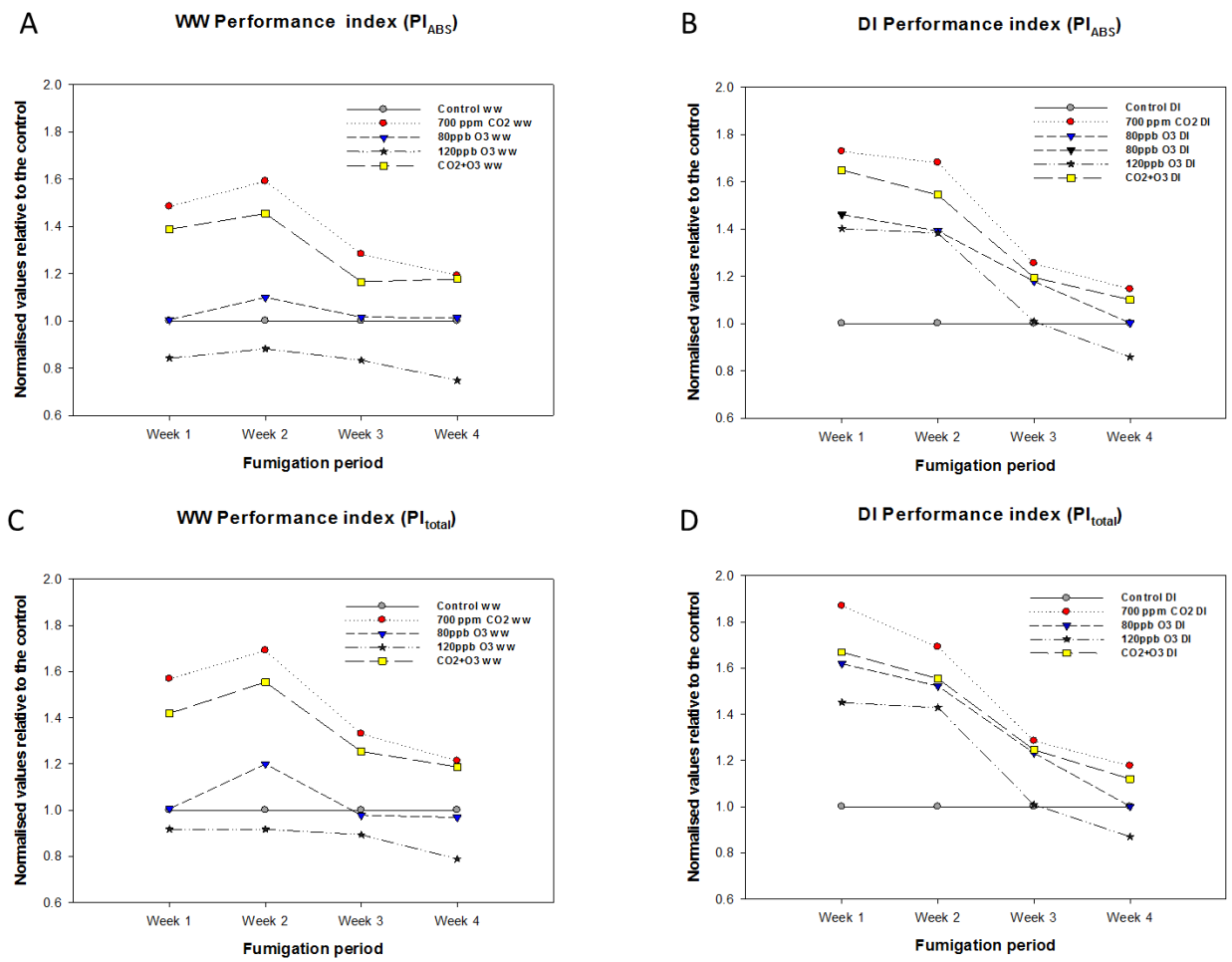


Figure 26: Normalised average photosynthetic performance index ($PI_{ABS,total}$) (A-D) plots of the well-watered (WW) and drought-induced (DI) quinoa fumigated with elevated CO_2 and 80 ppb O_3, 120 O_3 and CO_2 + O_3, relative to the control plants. The deviation of the behaviour pattern from the control demonstrates the fractional impact of the treatment to the chlorophyll *a* fluorescence.

4.2.5 Flowering

The development of the inflorescence primordia was observed to be 12 to 13 days earlier for the well-watered quinoa plants fumigated with elevated CO_2 in comparison with the control plants, drought-induced plants and plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3. Flowering time was accelerated by 9 days on the plants fumigated with elevated CO_2 under well-watered conditions when compared to the control plants and plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 (Figure 27). The number of days to flowering of the drought-induced plants was delayed by 13

days when compared to the well-watered plants (Figure 27). The number of flowers on the panicle varied between treatments. The number of flowers of the well-watered plants fumigated with elevated CO_2 were much more when compared to the control, the drought-induced plants and plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 (data not shown).

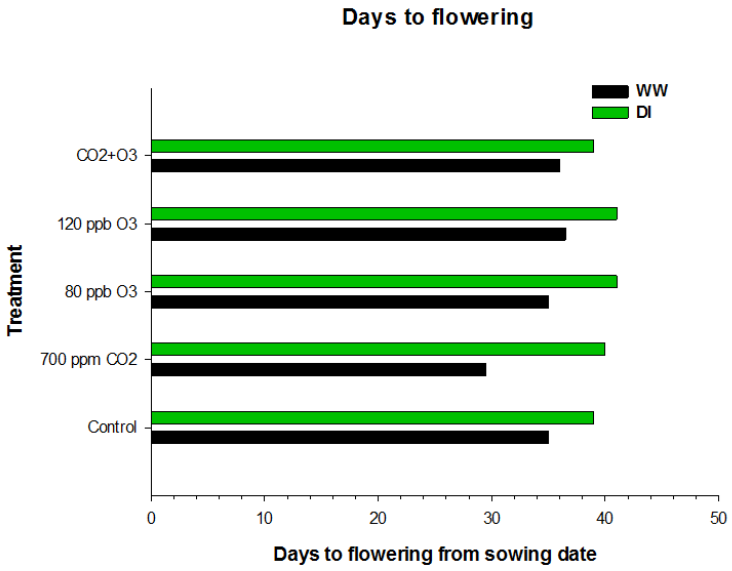


Figure 27: Number of days to flowering in the well-watered (WW) and drought-induced (DI) quinoa plants fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3, relative to the control plants.

4.2.6 Specific leaf area

The total leaf area differed significantly ($P \leq 0.05$) between the well-watered quinoa plants grown under elevated CO_2 and the control conditions. The total leaf area of the drought-induced plants were significantly ($P \leq 0.05$) lower when compared to the well-watered plants for all treatments (Figure 28A-B and Table 15). However, under elevated CO_2 conditions the well-watered quinoa had significant ($P \leq 0.05$) larger flag leaves compared to the plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3. The flag leaves of the well-watered quinoa fumigated with elevated CO_2 stayed green for longer period compared to the control plants, drought-induced plants and the plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 (Figure 17A-G). A significant ($P \leq 0.05$) decrease in the total leaf area was observed in the well-watered plants fumigated with

80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 when compared to the plants fumigated with elevated CO_2. However, a slight decline in the specific leaf area was observed in quinoa grown in the control environment as the plants were getting old (Figure 28A). Similar patterns were observed in parallel with the plants fumigated with elevated CO_2 at the later stage of the plants.

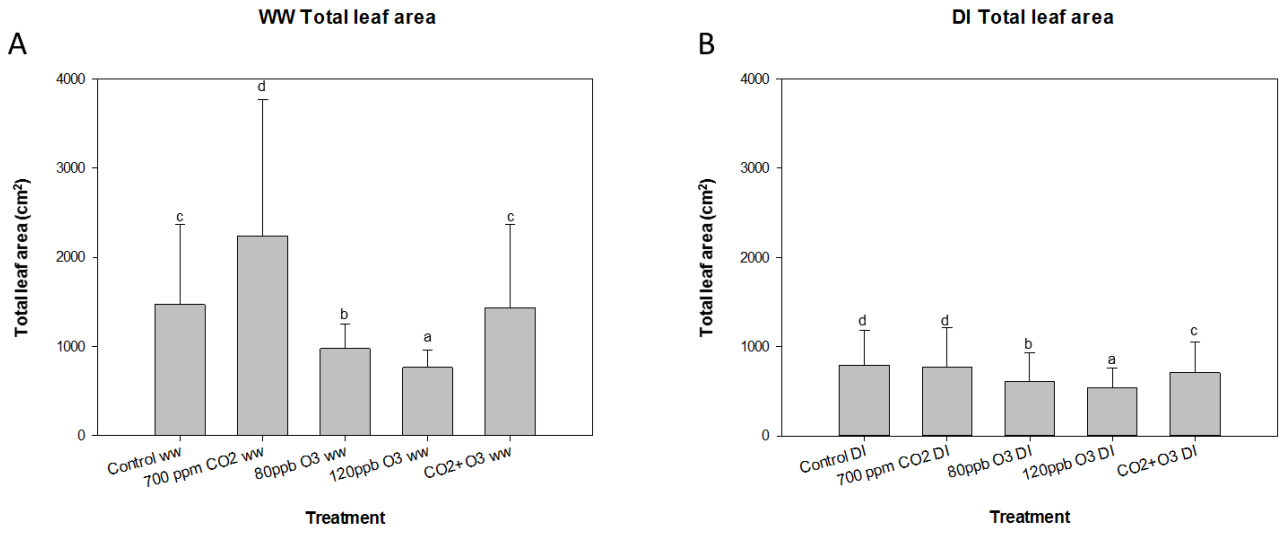


Figure 28: Average total leaf area plot of (A) well-watered (WW) and (B) drought-induced (DI) quinoa plants fumigated with elevated CO_2 and O_3 and a combination of both, relative to the control plants. Different letters in each column indicate significant differences ($P \leq 0.05$) between treatments.

4.2.7 Plant growth

Plant height of the well-watered plants fumigated with elevated CO_2 was significantly ($P \leq 0.05$) higher when compared to the control, plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3. Drought-stressed plants fumigated with elevated CO_2 had significantly ($P \leq 0.05$) higher plant height when compared with other treatments under similar conditions (Figure 29B). The plant height of the well-watered plants fumigated with 80 ppb O_3 and 120 ppb O_3 were reduced by 15% ($P \leq 0.05$) and 23% ($P \leq 0.05$), respectively, relative to the control (Figure 29A). Plants fumigated with elevated CO_2 increased in the plant height by 20% ($P \leq 0.05$) after 35 days of fumigation when compared to the control (Table 14).

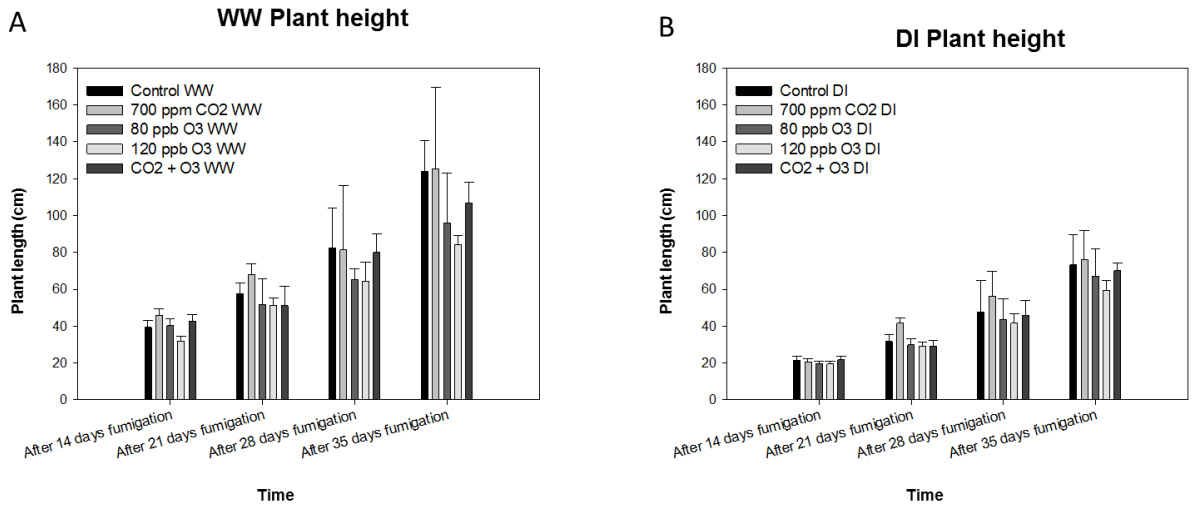


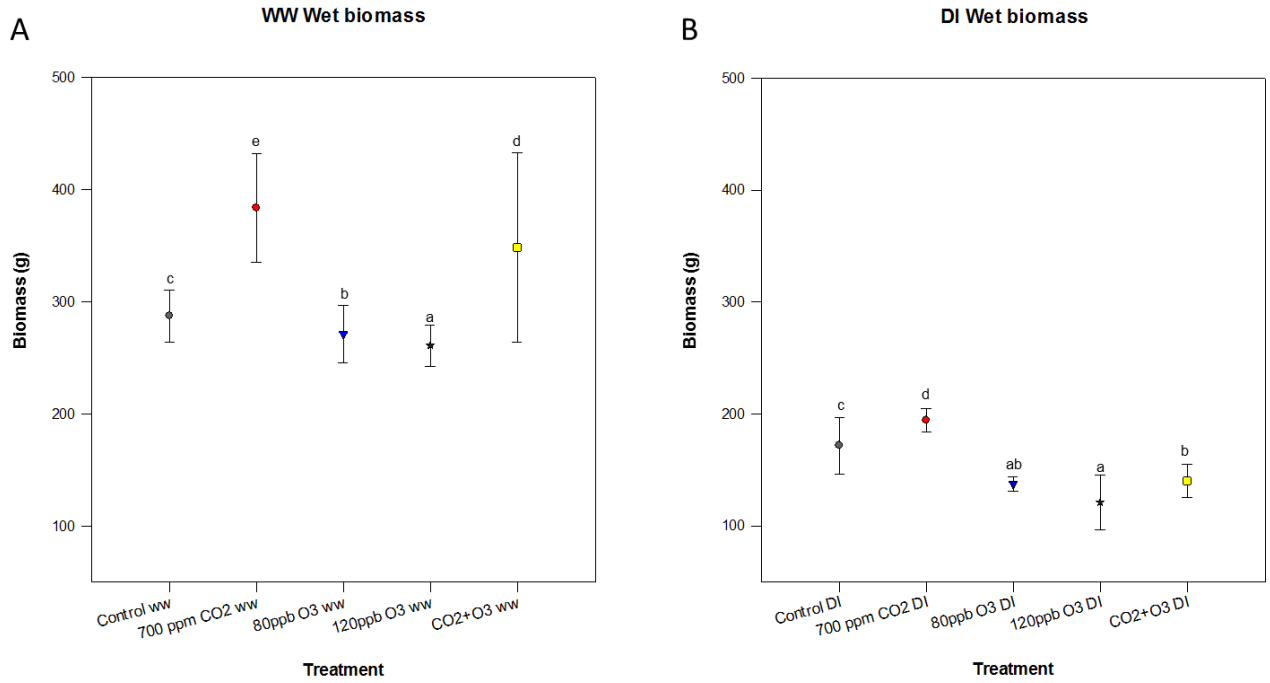
Figure 29: Average plant height of the well-watered (WW) (A) and drought-induced (DI) (B) quinoa plants fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3, relative to the control plants during the period of experiment.

Table 14: Average plant height (cm) of the well-watered (WW) and drought-induced (DI) quinoa fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3, relative to the control plants. Data shown as mean ± standard deviation. The measurements were taken every week; different letters indicate significant differences ($P \leq 0.05$).

Treatment	Fumigation period	Control	700 ppm CO_2	80 ppb O_3	120 ppb O_3	CO_2+O_3
Well-watered	After 14 days	40.20±3.67b	45.85±3.62d	39.35±3.55b	31.93±2.78a	42.78±3.61c
	After 21 days	57.75±5.74b	67.900±6.10c	51.150±10.24ab	51.38±3.85a	51.75±13.85ab
	After 28 days	82.33±21.52c	81.35±34.85d	65.18±6.00a	64.50±10.19a	80.05±10.18b
	After 35 days	123.85±17.02d	125.53±44.24e	96.05±11.14b	84.40±4.59a	107.03±26.91c
Drought-induced	After 14 days	21.35±2.16c	21.73±1.85c	19.70±1.43a	19.43±1.59a	20.55±1.70b
	After 21 days	31.65±3.56b	41.53±3.11c	29.30±3.14ab	29.20±1.98a	29.88±3.10ab
	After 28 days	47.80±16.84b	56.23±13.59c	43.43±11.43a	41.63±5.26a	45.80±7.98ab
	After 35 days	73.20±16.25c	76.25±15.76c	67.03±14.80b	59.38±5.56a	70.10±3.96b

4.2.8 Biomass

Biomass accumulation significantly ($P \leq 0.05$) varied in the well-watered quinoa fumigated with elevated CO_2 when compared to the control, drought-induced plants and the plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 (Figure 30A-D). The biomass was markedly affected by the elevated CO_2 levels and O_3 stress effect and the interaction of the drought stress. The interaction of drought stress with 80 ppb O_3 and 120 ppb O_3 reduced the biomass by 55% ($P \leq 0.05$) and 68% ($P \leq 0.05$), respectively. Under well-watered conditions elevated CO_2 significantly ($P \leq 0.05$) increased the plant fresh and dry weight (Table 15). The total above ground fresh weight of the well-watered plants fumigated with elevated CO_2 increased by 70% ($P \leq 0.05$) in comparison to the control and plants fumigated with 80 ppb O_3 and 120 ppb O_3 (Figure 30A). Similar trends in the total above ground dry biomass were observed in the well-watered plants fumigated with elevated CO_2 when compared to these treatments (Figure 30C). Elevated CO_2 also increased the biomass of the drought-stressed plants by 42% ($P \leq 0.05$), relative to the control (Figure 30B and D).



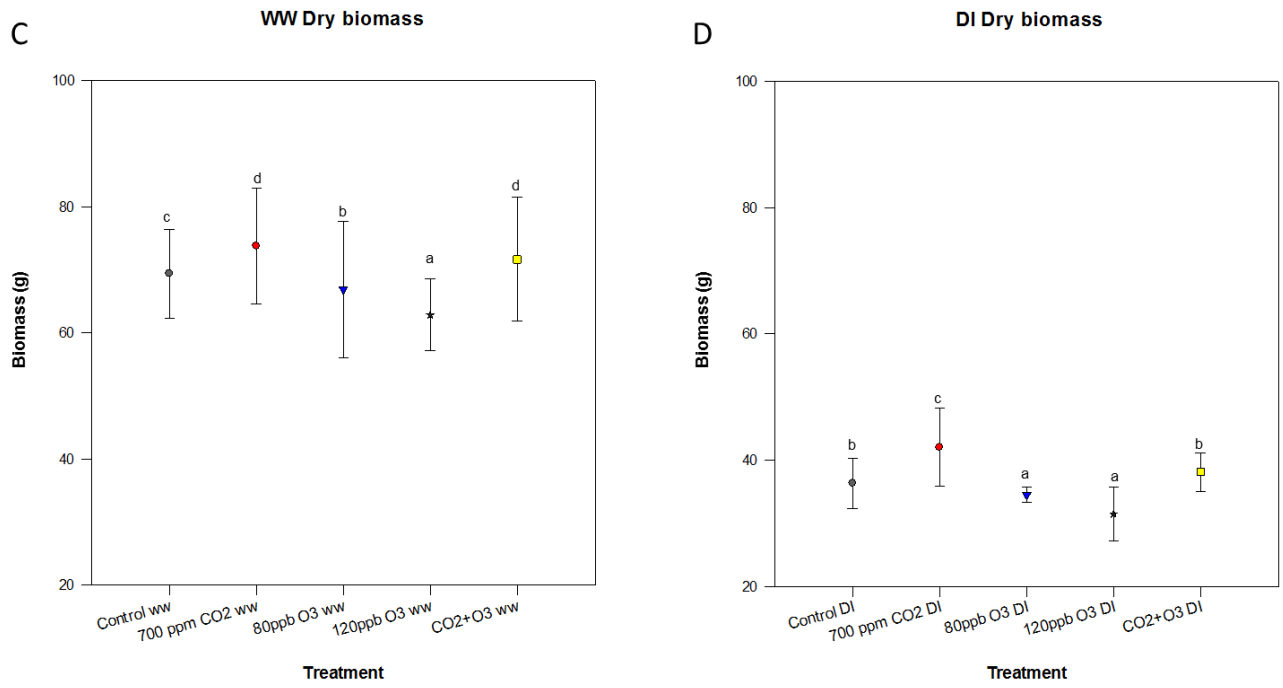


Figure 30: Average wet and dry biomass plots of the well-watered (WW) (A and C) and drought-induced (DI) (B and D) quinoa plants fumigated with elevated CO_2 and O_3 and combination of both, relative to the control plants during the period of harvest in all treatments. Different letters on each column indicate significant differences ($P \leq 0.05$) between treatments.

4.2.9 Seed filling and yield

Seed filling was significantly ($P \leq 0.05$) accelerated by 15 to 20 days in the well-watered quinoa fumigated with elevated CO_2 when compared to the control, drought-induced plants and the plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3. There was a fast subsequent seed development and maturation in the well-watered quinoa fumigated with elevated CO_2 (data not shown). The seed yield component was determined at the final harvest. Yield component of the plants subjected to fumigation treatments and drought simultaneously were reduced significantly ($P \leq 0.05$). The overall seed yield (grams per plant) decreased significantly by 44% ($P \leq 0.05$), 50% ($P \leq 0.05$) and 33% ($P \leq 0.05$) for the well-watered plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3, respectively, relative to the control (Figure 31A). The seed yield was drastically reduced by 61% ($P \leq 0.05$), 67% ($P \leq 0.05$) and 39% ($P \leq 0.05$) in drought-stressed plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3, respectively

(Figure 31B), when compared to the control. However, the seed yield increased by 71% ($P \leq 0.05$) and 35% ($P \leq 0.05$) under well-watered conditions and the drought-induced quinoa fumigated with elevated CO_2, respectively, relative to their controls (Figure 31A-B and Table 15).

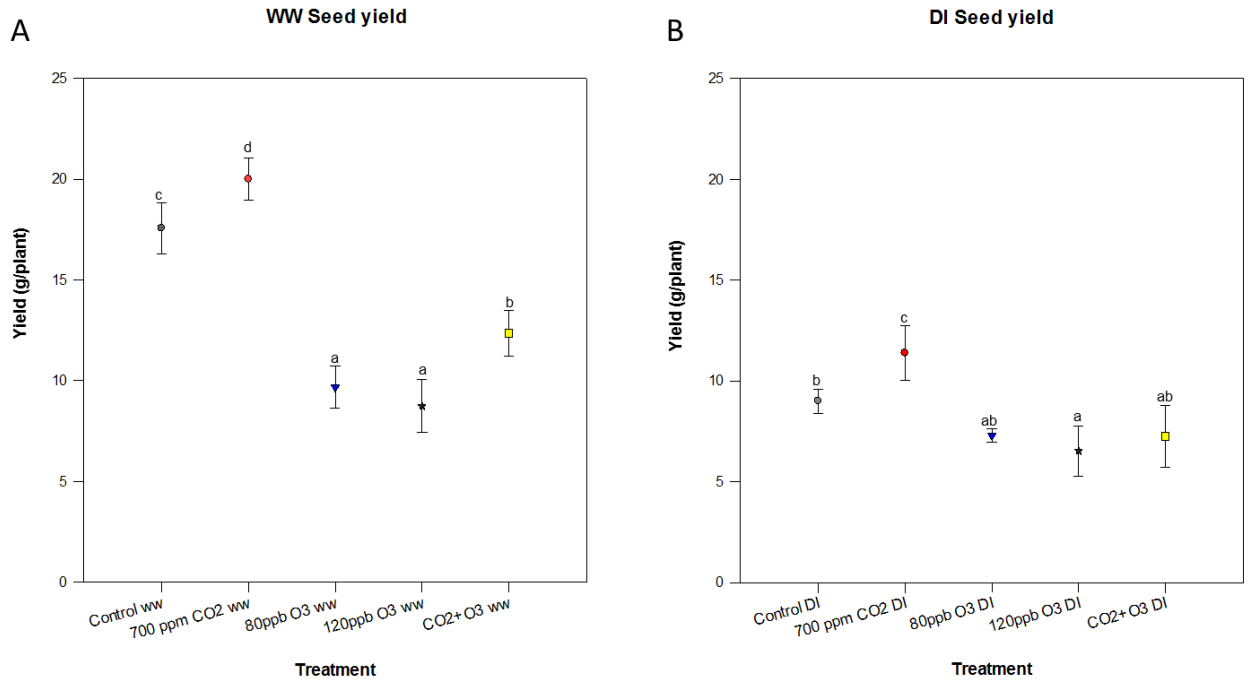


Figure 31: Average quinoa seed yield plots of the well-watered (WW) (A) and drought-induced (DI) (B) quinoa plants fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3, relative to the control plants during the period of harvest. Different letters on each column indicate significant differences ($P \leq 0.05$) between treatments.

Table 15: Total leaf area, wet and dry biomass and seed yield of the well-watered (WW) and drought-induced (DI) quinoa plants fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3, relative to the control plants. Data shown as mean ± standard deviation. Different letters indicate significant differences ($P \leq 0.05$).

Treatment	Parameter	Control	700 ppm CO_2	80 ppb O_3	120 ppb O_3	CO_2+O_3
Well-watered	Total leaf area (cm^2)	1470.75±894.56c	2240.00±1527.99d	976.50±276.41b	763.50±199.05a	1434.75±930.15c
	Wet biomass (g)	287.34±23.21c	383.57±48.42e	271.10±25.56b	260.97±18.29a	348.59±84.72d
	Dry biomass (g)	69.38±7.01c	73.75±9.17d	66.88±10.78b	62.83±5.79a	71.65±9.81d
	Seed yield (g/plant)	17.56±1.27c	19.99±1.05d	9.68±1.04a	8.73±1.32a	12.35±1.15b
Drought-induced	Total leaf area (cm^2)	794.00±385.53d	768.25±442.34d	604.25±329.33b	534.75±223.86a	709.00±345.55c
	Wet biomass (g)	171.67±25.22c	194.10±10.49d	137.42±6.30ab	120.97±24.63a	140.15±14.63b
	Dry biomass (g)	36.34±4.03b	42.02±6.17c	34.48±1.21a	31.48±4.33a	38.14±3.05b
	Seed yield (g/plant)	8.99±0.60b	11.38±1.36c	7.29±0.32ab	6.52±1.25a	7.25±1.53ab

CHAPTER 5: DISCUSSION

5.1 Soil water status

A linear relationship existed between the soil moisture and temperature in all water regimes. The same trend was also observed in the pots with the well-watered and drought-induced plants fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and 700 ppm CO_2 + 80 ppb O_3 (Figure 2A-C and Figure 16A-C). At the same time, the soil moisture content was significantly higher when the soil temperature dropped and vice versa (Figure 2A-B). The results demonstrate a good correlation between the soil conductivity and temperature (Figure 2B and C), as the increase in the soil temperature resulted in increased soil electrical conductivity. However, the latter only occured when the soil moisture content was high (Figure 2A-B and Table 1). The concomitant increase in the soil moisture content and temperature triggered a direct increase in the soil electrical conductivity (Figure 2A-C and Figure 16A-C). However, the prolonged increase in the soil temperature can cause a deterioration in the soil water content due to high evaporation of the soil moisture (Davidson *et al*., 1998; Brevik *et al*., 2006). These results also suggest that the decrease in the soil electrical conductivity under drought stress conditions restricted plants to get the minimal water, but did not prevent plants to extract the available moisture in the soil (Martinez *et al*., 2008). A positive relationship between the soil conditions and the crop growth characteristics, for example, the leaf area index (LAI), plant height, biomass and seed yield was observed. The same correlations were observed by Hakojärvi *et al*., 2013 and Stadler *et al*., 2015. Decreased soil moisture and soil electrical conductivity at 10% (WR 1) and 20% (WR 2) field capacity and in the drought-induced plants fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 (Table 1 and Table 7) resulted in a reduced LAI (Figure 14A and Figure 28A-B), plant height (Figure 13 and Figure 29A-B), biomass (Figure 14B-C and Figure 30A-D) and seed yield (Figure 15 and Figure 31A-B). Therefore, soil conditions has a notable influence on the crop growth and yield.

5.2 Responses of quinoa to different water regimes

5.2.1 Stomatal conductance (g_{H2O})

A difference in the stomatal conductance was observed between the different water regimes (Figure 3A and C). Reduction in the stomatal conductance due to a reduction in the soil water content, as mentioned by López-Climent *et al*. (2008) in *Phaseolus vulgaris,* correlated with this study. Quinoa plants with the lowest stomatal conductance values directly corresponded to the water regime with the 10% field capacity (Figure 3A-C and Table 2). As the soil water potential increased, so did the stomatal conductance. In similar studies, where quinoa was subjected to different water regimes, a decline in the stomatal conductance was observed when the soil water potential decreased (Vacher, 1998; Liu *et al.*, 2004, 2005a). The closure of the stomata is a mechanism to protect the plant against excessive water loss (Dizes, 1992). The decline in the stomatal conductance and the photosynthetic rate seems to be common in the plants that are under water stress and saline conditions (Miyashita *et al*., 2005; López-Climent *et al*., 2008). Thus, closing of the stomata in quinoa might have resulted in a decline in the absorption of CO_2. This decline could result in a decline in the photosynthetic processes. This further explains that the significant ($P \leq 0.05$) decrease in the stomatal conductance observed at the 10% field capacity (WR 1) indicate that the cultivar studied is not tolerant to water stress even though the stomatal conductance was relatively stable as reported by Vacher (1998). Hence, it was demonstrated that at 75% field capacity (WR 3) there was sufficient water to mitigate the water stress in all growth stages of quinoa (Figure 3A-C). The reduction in the stomatal conductance in drought-stressed plants which was accompanied by a significant reduction in the LAI (Figure 14A), plant height (Figure 13), PI_{total} (Figure 10A-C Figure 11B), biomass (Figure 14B-C) and seed yield (Figure 15) could be an indication of decreased photosynthesis.

5.2.2 Chlorophyll *a* fluorescence

5.2.2.1 OJIP transient

Analysing the OJIP-transient revealed that water deficit stress influenced the shape of the transient. The most prominent changes in the fluorescence intensity of the OJIP-transient were between 2 ms (J-step) and 300 ms (F_m). Water deficit conditions had the

most noteworthy reduction in the fluorescence intensity between 2 ms and 300 ms (Figure 4A-C). These changes in the fluorescence intensity are associated with the restriction in the flow of electrons between PSII and PSI and a decrease in the plants' ability to reduce $NADP^+$ to NADPH (Oukarroum *et al*., 2012).

5.2.2.2 Differences in relative variable fluorescence (∆V)

5.2.2.2.1 ΔV_L-Band

Normalisation of the fluorescence curves between O (0.02 ms) and K (0.3 ms) (Figure 6A-C) revealed positive ΔV_L-bands of the plants subjected to drought stress (10% field capacity) and moderate drought stress (20% field capacity). The identification of the positive ΔV_L-bands are an indication of a decrease in the energetic grouping of the PSII units and less stability of the system (Strasser *et al*., 2004; Zhu *et al*., 2005; Oukarroum *et al*., 2007; Strasser *et al*., 2007; Yusuf *et al*., 2010; Redilas *et al*., 2011a). These results are not in agreement with that of Redillas *et al*. (2011a), which found the appearance of a negative ΔV_L-band on a drought resistant rice cultivar. It can therefore be assumed that the quinoa cultivar used is not drought tolerant. Decreased energetic connectivity is a partial protective mechanism necessary to increase energy dissipation in order to improve the utilisation of the excitation energy during the non-photochemical quenching processes (Redillas *et al*., 2011a). However, the well-watered plants in water regimes 3 and 4 revealed negative ΔV_L-bands (Figure 6A-C) from the beginning of the experiment indicating an increase in the energetic grouping of the PSII units. Several studies have indicated that higher energetic connectivity in the photosynthetic machinery leads to a better utilisation of the excitation energy and higher stability of the system (Zhu *et al*. 2005; Oukarroum *et al*., 2007; Strasser *et al*., 2007; Redilas *et al*., 2011a). This further explains that quinoa require more water to grow optimally as indicated by the plants at 75% and 90% field capacity (WR 3 and 4), respectively (Figure 6A-C).

5.2.2.2.2 ΔV_K-Band

The occurrence of a positive ΔV_K-band is an indication of the reduced efficiency of the OEC complex to split water and to provide electrons to the P_{680} reaction center (Oukarroum *et al*., 2012) or it is an indication of an increase in the antennae size (Yusuf

et al. 2010) of the PSII. A negative ΔV_K-band is an indication of a decrease in the reduction rate of Q_A to Q_A^-, which could also be an indication that there is no availability of non-water electron donors to the OEC (Gururani *et al*., 2012; Kalaji *et al*., 2014; Gururani *et al*., 2015).

Generally, quinoa plants subjected to drought stress exhibited a positive ΔV_K-band from the beginning to the end of the experiment (Figure 7A-C), indicating an increase in the reduction rate of Q_A to Q_A^- and that non-water electron donors were accessible to the OEC (Gururani *et al*., 2012; Kalaji *et al*., 2014; Gururani *et al*., 2015). The increase in the variable fluorescence in water regimes 1 and 2 was due to the decrease in the ET beyond Q_A^- (Haldiman and Strasser, 1999), which is induced by the accumulation of reduced Q_A^- (Strasser and Strasser, 1995). The appearance of a pronounced positive ΔV_L- and ΔV_K-bands at 10% and 20% field capacity suggests that quinoa plants are sensitive to water stress when the soil water potential reaches 0.0343 and 0.259 m^3/m^3 VWC, respectively. These results also suggest that the OEC was disrupted as the flow of electrons to the RCs of the PSII was decreased under drought stress when soil volumetric water content reaches 0.0343 and 0.259 m^3/m^3 in water regime 1 and 2, respectively (Figure 2A and Table 1). This could also lead to a decrease in the fraction of Q_A^-. The impairment on the donor side of the PSII has been supported by a positive ΔV_K-band appearing at 0.3 ms (Figure 7A-C).

5.2.2.2.3 ΔV_J-Band

The occurrence of the positive ΔV_J-band is associated with the accumulation of Q_A^-, indicating that electrons are not entering the electron transport chain (Ranjan *et al*., 2014). Hence, the occurrence of a negative ΔV_J-band is an indication of the more effective movement of electrons into the electron transport chain (Schansker *et al*., 2003; Gururani *et al*., 2015). The negative ΔV_J-band could also be an indication that the OEC function and Q_A^- fraction was decreased. At the same time, the formation of a negative ΔV_J-band could be the result of the reduced accumulation of electron carriers like plastoquinonol and plastocyanin (Schansker *et al*., 2003; Ranjan *et al*., 2014). These results were in agreement with Kalaji *et al*. (2014), which demonstrated that the occurrence of the negative ΔV_J-bands were more prominent in drought-stressed plants than the well-watered plants (Figure 8A-C). This indicates that drought stress hampered

the OEC function of quinoa by decreasing the flow of electrons to the RCs of the PSII, thus decreasing the concentration of Q_A^-. However, damaged OEC activity can be compensated for by non-water electron donors (Kalaji *et al*., 2014; Gururani *et al*., 2015).

5.2.2.2.4 ΔV_I-Band

The significant ($P \leq 0.05$) increase in the variable fluorescence between 2 ms and 300 ms in water regime 1 and 2 and a decrease in water regimes 3 and 4 reveals the pool size of the final electron acceptors of the PSI (Yusuf *et al*., 2010). The increase in the amplitude of the variable fluorescence of water regimes 1 and 2 (Figure 9A-C) indicated that the structure and function of the PSI in quinoa was negatively influenced by drought stress. This also indicates that the PSI in quinoa is influenced by plant water potential to regulate the pool size of the final electron acceptors on the acceptor side of the PSI. The highest amplitude of the variable fluorescence (Figure 9A-C) at 10% and 20% field capacity is an indication of the slow reduction rate, while the lowest amplitude of the variable fluorescence at 75% and 90% field capacity (Figure 9A-C) indicate a faster reduction rate of the end acceptors of the PSI (Redillas *et al*., 2011a). These results further indicate that the decrease in the pool size was not related to the regulation of the reduction rate of the PSI electron acceptors. Similar results were reported by Redillas *et al.* (2011a, 2011b) in rice plants (*Oryza sativa*) grown under water deficit and nitrogen-limited conditions, respectively. More so, the appearance of a positive ΔV_I-bands in drought-stressed plants could be due to the inhibition of ferredoxin-$NADP^+$ oxidoreductase (FNR) activity. This enzyme is responsible for the final step of linear electron flow transferring electrons from ferredoxin-$NADP^+$ (Schansker *et al*., 2003; Kalaji *et al*., 2014).

5.2.2.3 Maximum quantum efficiency (F_v/F_m)

A statistically non-significant decrease in the maximum quantum yield (F_v/F_m) of the PSII occurred when quinoa plants were subjected to water stress (Figure 10A-C). Strasser *et al*. (2004) has reported that an F_v/F_m of 0.750 is considered a boundary value for a fully functional PSII. However, quinoa maintained a high F_v/F_m (0.839 to 0.853) at 10% field capacity (Figure 10A-C), which could be an indication that quinoa plants were able to use and move electrons into the electron transport chain. Lepeduš

et al. (2012) reported a 0.742 F_v/F_m which is below boundary line for Mo17 maize inbred line subjected to water stress. In this study it was demonstrated that the F_v/F_m showed less sensitivity to water stress as there were no significant differences between the water regimes. The increase in F_v/F_m ratio was caused by the increase in F_m (maximum fluorescence) values (Figure 10A), which is an indication of reduced non-photochemical quenching coefficient (q_N) and/or increased photochemical quenching coefficient (q_P) (Krause and Weis, 1991; Muller *et al*., 2001; Finazzi *et al*., 2006; Pellegrini *et al*., 2011). Studies indicate that the decrease in F_v (variable fluorescence) values represent thylakoid membrane damage, whereas decrease in F_m values (Figure 10B-C) indicate the inhibition of the PSII activity (Rai and Agrawal, 2008).

However, high F_v/F_m observed in this study at water regimes 1 and 2 (Figure 10A-C), suggests that there was no damage to the PSII structure. The increase in F_v values further indicate that the thylakoid membrane was not damaged, whereas decrease in F_m values (Figure 10C) indicates that the PSII activity was inhibited in water stressed plants. The decrease in PI_{total} values of drought-stressed plants (Figure 10A-C) further indicate that the PSII activity was inhibited and drought stress caused a structural and/or functional damage to the PSI. The increase in the fluorescence intensity was due to the reduction in quinone A (Q_A), which determine the shape of the OJIP curve (Shinkarev and Govindjee, 1993). Studies also indicate that at J to P rise all Q_A molecules are completely reduced, due to the reduction of the electron transport chain caused by traffic jam of electrons on the PSI acceptor side (Schansker *et al*., 2005).

On the basis of the presented findings of the F_v/F_m this study clearly demonstrated that this parameter is less sensitive to water stress and cannot be used for assessing drought stressed plants. The same observations using the F_v/F_m for studying fluorescence behaviour for stressed plants have also been reported elsewhere (van Heerden *et al*., 2003; Oukarroum *et al*., 2007; Redillas *et al*., 2011a).

5.2.2.4 Specific fluxes per reaction center (RC)

The JIP-test parameters are useful to identify plants with tolerance to various environmental stressors and permits the understanding of processes related to the energy flux in the electron transport chain (Redillas *et al*., 2011b). The most commonly used parameters to evaluate stress on the plants are specific flux of absorption

(ABS/RC), trapping (TR_o/RC), electron transport (ET_o/RC), dissipation (DI_o/RC) and reduction flux of the final PSI electron acceptors per RC (RE_o/RC). Water deficit stress triggered a significant ($P \leq 0.05$) increases in the values of specific fluxes or activity of active PSII reaction centers of ABS/RC, TR_o/RC, ET_o/RC and DI_o/RC. This is an indication that the electron transport from Q_A^- to the electron acceptors was not disturbed (Redillas *et al*., 2011b). The increase in the ABS/RC, TR_o/RC, ET_o/RC and DI_o/RC were relatively high at water regimes 1 and 2 compared to water regimes 3 and 4 (Figure 10A-C). These results suggest that the increase in the ABS/RC and TR_o/RC per active RC increased in relation to the inactivation of some RCs. Furthermore, drought stress triggered the photosynthetic adjustments as indicated by an increase of the antenna size of the active RCs (RC/ABS) causing a decrease in the photochemical efficiency (φ_{P0}) or (F_v/F_m) and PI_{ABS} and and maintenance of high PI_{total} (Figure 10A-C). At the same time, the down regulation of the PSII was evident in the drought-stressed plants, which showed a marked increase in DI_o/RC (Figure 10A-C) indicating that the energy of dissipation was enhanced in the inactive RCs. These results further indicate that the down regulation was triggered by a decrease in ψ_{Eo}, φ_{Eo}, ET_o/RC, RE_o/RC and RC/CS_o of the performance indices (Figure 10A-C). Diversion of more excited energy to heat dissipation is a photoprotective role of the non-photochemical quenching (Horton *et al*., 1996; Bussotti *et al*., 2011), as the excited chlorophylls in the core antenna of the closed RCs can generate radicals that can induce photoinhibition during high-light fluxes (Long *et al*., 1994). These results suggest that the energy dissipation was enhanced in order to protect quinoa stressed leaves from the photo-oxidative damage, thereby dissipating excess absorbed light into heat. The high values of the specific fluxes of ABS/RC, TR_o/RC and ET_o/RC at water regimes 1 and 2 (Figure 10A-C) indicate increase in the absorption of photon flux per active RCs, reduction of Q_A to Q_A^- and thermal activation of the dark reactions in the PSII. The link between these energy fluxes (ABS/RC, TR_o/RC and ET_o/RC) is a clear indication that a drought-stressed quinoa utilized energy more efficiently. Similar response of specific energy fluxes to water regimes 1 and 4 (Figure 10A-C) suggests that the well-watered and drought-stressed plants were affected at the same part of the photosynthetic system. Hence, water stress negatively influenced the structure and functionality of the PSI as reflected by a decreased reduction flux RE_o/RC (Figure 10A-C). Similar results were reported by Kalaji *et al.* (2014) in maize and tomato plants monitoring nutrient deficiency effects on

the PSI and Redillas *et al*. (2011a) on the non-transgenic rice plants under drought conditions.

5.2.2.5 Phenomenological fluxes per excited cross section (CS)

The decrease in the phenomenological fluxes per excited cross section (CS) for ABS/CS, TR_o/CS at water regimes 1 and 2 (Figure 10A-C) reflect an increased density of the inactive RCs in drought-stressed plants. The decrease in the TR_o/CS and ET_o/CS (Figure 10A-C) suggests that the active RCs were converted into the inactive RCs, thereby reducing the efficiency of trapping and reducing the PSII activity. These could be an indication that drought-induced plants were susceptible to photoinhibition. Inactivation of the RCs is considered a down-regulation mechanism that enables plants to dissipate excess absorbed light (Flexas and Medrano, 2002). More so, the increased phenomenological fluxes per cross section in both well-watered and drought-induced quinoa stimulated a notable increase in the PI_{ABS} (Figure 10A-C).

5.2.2.6 Performance indexes (PI_{ABS} and PI_{total}) and the partial parameters

The PI_{ABS} and PI_{total} are considered good indicators of the changes in the photosynthetic activity as they are sensitive to the environmental stressors that damage the photosynthetic apparatus in the plants (Krüger *et al*., 1997; Strauss *et al*., 2006; Stirbet and Govindjee, 2011). The PI_{ABS} parameter also provides a complete and quantitative information on the performance of the plants (Strasser *et al*., 2004). The results indicate that the PI_{ABS} values of drought-stressed plants were decreased significantly ($P \leq 0.05$) from the beginning of the experiment to the end (Figure 10A-C and Figure 11A). This further indicates that the dark reactions after reduction of Q_A^- are sensitive to drought stress as indicated by a decrease in the (ψ_o/(1 – ψ_o) (Figure 10A-C). Similar results were reported by Redillas *et al*. (2011a) in the non-transgenic rice plants under drought conditions.

The PI_{total} index is the most sensitive parameter of the JIP-test and efficient tool to quantify stress in the plants as it takes into account all main photochemical processes of the PSII reaction center complex. It can measure the partial energy conservation potential to the final PSI electron acceptors (Tsimilli-Michael and Strasser, 2008; Yusuf *et al*., 2010). Results showed that a significant ($P \leq 0.05$) decrease in the PI_{total} values

occurred at water regimes 1 and 2 from the onset of the drought stress (Figure 10A-C and Figure 11B). These are the indications that severe water stress deactivates the PSII RCs, thereby inducing the downstream reaction of the plastoquinone A (Q_A) in the photosynthetic transport chain. Hence, the increase in the PI_{ABS} and PI_{total} at water regimes 3 and 4 (Figure 10A-C and Figure 11B) were associated with the increase in the leaf electron transport capacity.

Consequently, these findings indicate that quinoa was more sensitive to severe drought stress (10% field capacity) when compared to the plants under well-watered conditions (control, 75% and 90% field capacity). Analysis of the JIP-test allowed the quantification of the photosynthetic parameters, thereby conferring complete and quantitative information on the changes that occur in the PSII and PSI functions.

5.2.3 Days to flowering

Flowering is a crucial development stage in the life cycle of any plant and determines the seed number (Geerts *et al*., 2006b). The duration of the stage from sowing date to visible flower buds varied markedly between the drought-induced and well-watered plants. Elevated drought stress prolonged the flowering date by an additional 4 days from the sowing date, which suggest that it had a negative effect on the phenological development of quinoa. A delay in flowering on the drought-induced plants in response to pre-anthesis drought stress can be due to the retarded growth, thereby increasing the plant growth cycle (Geerts *et al*., 2006b). However, temperature and photoperiod effects on flowering varied seasonally. The well-watered plants flowered within 31 days in spring and 44 days in winter from sowing date. The drought-induced plants flowered within 36 days during spring and 45 days during winter from sowing date (Figure 12). The latter also suggest that solar radiation induced early flowering in quinoa (Bertero *et al*., 1999a), as the short day and long night in winter could have caused a delay in the flowering. Elevated pre-anthesis severe water stress (10% field capacity) prolonged the plant growth cycle and substantially increased the time to anthesis and physiological maturity.

5.2.4 Plant growth, specific leaf area and biomass

Water stress induces reduction in the plant specific leaf area which negatively affects the plant photosynthetic capacity and exhibit growth reduction, protein degradation, reduction in respiration and biomass production (Li and Li, 2005). However, González *et al*. (2011) observed that there was no significant reduction in the leaf area in quinoa during water stress. Contrasting results were obtained in this study, as there were significant differences in the leaf areas of the drought-induced and well-watered plants. Hence, the 56% ($P \leq 0.05$) decrease in the leaf area at 10% field capacity in this study could be due to the use of a different cultivar (Figure 14A and Table 6), as cultivars respond differently to the environmental stressors. More so, the 52% ($P \leq 0.05$) decrease in the plant height at 10% field capacity suggests that quinoa is susceptible to severe water stress (Figure 13 and Table 5).

A pronounced decrease in dry weight values was observed in the drought-stressed plants compared to the well-watered counterparts (Figure 14B-C and Table 6). The ability of the quinoa plants to regulate the opening of the stomata during growing season determines the amount of CO_2 assimilate and biomass accumulation (Vacher, 1998; Shabala, 2013). A marked decline in the stomatal conductance reduces water loss during water stress, which enables quinoa to reduce net CO_2 assimilation and transpiration rate, thus minimally reduces the plant dry weight (Munns, 2002; Shabala, 2013). Consistent results were obtained in this study as there was a marked decline in the stomatal conductance of the drought-stressed plants (Figure 3A-C and Table 2), which resulted in reduced dry weight (Figure 14C). This further suggests that the drought avoidance mechanism that occurs after flowering (Geerts *et al*., 2008) appears to be insufficient in mitigating the effects of water stress. Retardation in the plant growth by 52% ($P \leq 0.05$) (Figure 13) and reduction in a leaf area by 56% ($P \leq 0.05$) (Figure 14A) and dry biomass by 46% ($P \leq 0.05$) (Figure 14C) at 10% field capacity might be ascribed to a depletion of assimilated carbon during respiration to produce metabolic energy needed to increase and maintain biomass (Bhargava *et al*., 2007). These results are in agreement with the results reported by Geerts *et al*. (2006b) who observed a reduction in the plant growth and dry biomass by 43% in quinoa under drought conditions.

5.2.5 Seed filling and yield

Quinoa yield reduction can be severe when the plants experience drought during flowering and seed filling stages (Garcia *et al*., 2003). However, severe water stress that is only concentrated in the vegetative stages can stabilize quinoa yield (Geerts *et al*., 2008). Consistent results were obtained in this study, as the plants that were induced to a continuous severe water stress (10% field capacity) produced a poor seed yield (Figure 15 and Table 6). The reduction in the photoassimilating surface due to a partial leaf dropping and stunted plants (Figure 13) and decreased stomatal conductance (Figure 3A-C and Table 2) could be the main contributing factors that reduced the seed yield by 46% (Figure 15 and Table 6) in the plants exposed to 10% field capacity. Studies also indicate that a reduction in the stomatal conductivity and leaf area disturbs the gaseous exchange and photoassimilating surface which drastically reduces yield (McDowell *et al*., 2008; Kudoyarova *et al*., 2013).

Moderate water stress (20% field capacity) was beneficial as it stabilized grain yield at higher level (10% higher) when compared to the 10% field capacity (Figure 15). Studies also suggest that the ability of quinoa to temporarily double the photosynthetic rate ratio over transpiration during water stress when leaf water potential is very low causes quinoa to reach a comparable yield to the well-watered plants and higher water use efficiency (Geerts *et al*., 2008). This study clearly demonstrated that quinoa subjected to a 10% field capacity during the pre-flowering, flowering and grain filling stages resulted in a reduced total grain yield (Figure 15). Similar results were reported on quinoa under drought stress (Vacher, 1998; Munns, 2002; Geerts *et al*., 2006b, 2008; Shabala, 2013).

5.3 The effects of O_3 on quinoa and its interaction with elevated CO_2 levels and drought

5.3.1 General observation of O_3 damage on quinoa leaves

The detrimental effects of O_3 on the plants are well documented in the literature (Pell *et al*., 1997; IPCC, 2013) on various crops. Typical symptoms of O_3 damage on the plants include reduction of the leaf area, necrosis on leaves and early leaf senescence. Chronic exposure of quinoa leaves to 80 ppb O_3 and 120 ppb O_3 indicated that the canopy leaves were less sensitive to 80 ppb O_3 compared to 120 ppb O_3 (Figure 17D and F). The bottom leaves showed signs of O_3 damage at both 80 ppb O_3 and the 120 ppb O_3 (Figure 17E and G). The most likely reason why the canopy leaves showed less signs of O_3 damage is that these leaves were exposed for a shorter period to O_3 compared to the bottom leaves. Visible foliar injury by 80 ppb O_3 was also suppressed by elevated CO_2 treatments (Figure 17C), possibly because reductions in the stomatal conductance (Figure 18A-H) decreased the flux of O_3 into the leaves and/or the production of the antioxidants was increased, as suggested by Fiscus *et al.* (2005).

5.3.2 Stomatal conductance (g_{H2O})

From the stomatal conductance data it became apparent that high levels of O_3 fumigation induced an increase in the stomatal conductance in both well-watered and drought-induced plants fumigated with 120 ppb O_3 (Figure 18A-H). In an experiment where quinoa plants were subjected to elevated CO_2 levels and 80 ppb O_3 separately and CO_2 + O_3, showed that the stomatal conductance was significantly ($P \leq 0.05$) decreased only in the presence of elevated CO_2 (Figure 18A-H). The observed decline in the stomatal conductance under elevated CO_2 levels can be attributed to the increase in the internal CO_2 concentration. An increase in the internal CO_2 concentration will result in the closure of the stomata (Wullschleger *et al*., 2002; Ainsworth and Rogers, 2007).

Studies indicate that water stress reduces the stomatal conductance, but high ozone levels may reduce the sensitivity of the stomata closure during soil drying (Centritto *et al*., 2003; McLaughlin *et al*., 2007; Wilkinson and Davies, 2009, 2010). Consistent results were obtained in this study as the drought-induced plants fumigated with 80 ppb

O_3 and 120 ppb O_3 increased the stomatal conductance by 33% ($P \leq 0.05$) and 56% ($P \leq 0.05$), respectively, relative to the control (Table 8). Similar stomatal conductance responses to O_3 by both the well-watered and the water deficit treatments is an indication that the stomata were possibly severely damaged by 120 ppb O_3. These results are in agreement with the results reported by Biswas and Jiang (2011) who observed an increase in the stomatal conductance in O_3-sensitive *Triticum aestivum* L. subjected to water stress. This further supports the observation that the quinoa cultivar used is sensitive to high levels of O_3.

Elevated CO_2 induces stomata closure that will result in less O_3 that will be taken up by the plant and thereby ameliorating the effects of O_3 damage (McKee *et al*., 1997). Consistent results were obtained in this study as there were no significant differences in the stomatal conductance of the control plants and plants fumigated with CO_2 + O_3 under well-watered conditions (Figure 18C and G). These findings demonstrate that elevated CO_2 reduced the negative effects of O_3 on the stomatal conductance and photosynthetic processes.

5.3.3 Chlorophyll *a* fluorescence

5.3.3.1 OJIP transient

The exposure of quinoa to drought stress, elevated CO_2, O_3 and CO_2 + O_3 treatments induced significant physiological, phenological and developmental changes. The OJIP transient curves were responsive to the water deficit and O_3 stress and elevated CO_2 as shown in figure 19A-H and table 9. Water deficit and O_3 stress negatively influenced the OJIP transient as demonstrated by the reduction in the fluorescence intensity between 2 ms and 300 ms. In addition, the significant ($P \leq 0.05$) decrease in the fluorescence rise at the J, I, and P steps of quinoa fumigated with 80 ppb O_3 and 120 ppb O_3 (Table 9 and 10) is an indication that the ET on the donor side or the acceptor side of the PSII was damaged. Damaged ET will result in the restriction of the flow of electrons between the PSII and PSI and a decrease in the plants ability to reduce $NADP^+$ to NADPH (Oukarroum *et al*., 2012).

5.3.3.2 Difference in relative variable fluorescence (∆V)

5.3.3.2.1 ΔV_L-Band

In-depth analysis of the relative variable fluorescence transient reveals the appearance of the negative ΔV_L-bands in both well-watered and drought-induced plants fumigated with elevated CO_2, 80 ppb O_3 and CO_2 + O_3 after 14 to 21 days of fumigation (Figure 21A-D). This is an indication that there was an increase in the energetic groupings of the PSII units, relative to the control. Hence, plants fumigated with elevated CO_2 had the biggest amplitude of the variable fluorescence and plants fumigated with 80 ppb O_3 had smallest amplitude of the variable fluorescence (Figure 21A-D). Other studies have indicated that the higher energetic connectivity in the photosynthetic machinery leads to a better utilisation of the excitation energy and higher stability of the system (Zhu *et al*. 2005; Oukarroum *et al*., 2007; Strasser *et al*., 2007; Redilas *et al*., 2011a). Therefore, the findings in this study indicated that the flow of energy to P_{680} was not disrupted after 14 to 21 days of fumigation in both well-watered and drought-induced treatments. The presence of the positive ΔV_L-bands (Figure 21F and H) after 28 to 35 days of fumigation in all drought-induced treatments is an indication that drought stress negatively affected the energetic grouping of the PSII units and cause change in the thylakoid membrane structure (Oukarroum *et al*., 2007).

5.3.3.2.2 ΔV_K-Band

Generally, the well-watered plants fumigated with elevated CO_2 maintained a negative ΔV_K-band from 14 to 35 days after fumigation, which is an indication of a decrease in the reduction rate of Q_A to Q_A^- (Figure 22A, C, E and G). In addition, drought-induced quinoa fumigated with elevated CO_2 also exhibited a negative ΔV_K-band from 14 to 21 days after fumigation (Figure 22B and D). This could also mean that there was no availability of the non-water electron donors to the oxygen evolution complex (OEC) (Gururani *et al*., 2012; Kalaji *et al*., 2014; Gururani *et al*., 2015) for drought-induced quinoa fumigated with elevated CO_2 during this period.

The positive ΔV_K-band that was revealed from 28 to 35 days after fumigation for all drought-induced treatments (Figure 22F and H) is an indication that the efficiency of the OEC to split water and provide electrons to a P_{680} reaction center was reduced

(Oukarroum *et al*., 2012) or it is an indication of an increased antennae size (Yusuf *et al*. 2010) of the PSII. The increase in the variable fluorescence for all drought-induced treatments from 28 to 35 days after fumigation (Figure 22F and H) was due to the decrease in the ET beyond Q_A^- (Haldiman and Strasser, 1999), which is induced by the accumulation of a fraction of reduced Q_A^- (Strasser and Strasser, 1995).

Oukarroum *et al*. (2007) suggested that the appearance of ΔV_L and ΔV_K-bands can be a good indicator for early detection in the physiological instabilities before the appearance of the visual damage caused by the stress. Thus, more pronounced positive ΔV_L and ΔV_K-bands of the well-watered plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 (Figure 21A, C, E and G and Figure 22E and G) and all treatments of the drought-induced plants (Figure 21B, D, F and H and Figure 22F and H) suggests that these plants are more sensitive to these treatments.

5.3.3.2.3 ΔV_J-Band

The decrease in the OEC function and Q_A^- fraction is reflected by a negative ΔV_J-bands of the well-watered plants fumigated with 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 (Figure 23C, E and G). Similar response were observed in all drought-induced plants fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 (Figure 23B, D, F and H). This is an indication that the drought stress further contribute to a decrease in the oxygen evolution and Q_A^- fraction. The formation of a negative ΔV_J-band could be the result of the reduced accumulation of the electron carriers like plastoquinone and plastocyanin (Schansker *et al*., 2003; Ranjan *et al*., 2014). These results were in agreement with Kalaji *et al*. (2014), which demonstrated that the occurrence of the negative ΔV_J-bands were more prominent in the drought-stressed plants than well-watered plants (Figure 23A-H). These results also indicated that water and O_3 stress hampered the OEC function of quinoa by decreasing the flow of electrons to the RCs of the PSII, thus decreasing the concentration of Q_A^- (Figure 23B, D, F and H). This is an indication that the interaction of O_3 and drought stress aggravate the disruption of the OEC function by decreasing the flow of electrons to the RCs of PSII, thus decreasing the concentration of Q_A^-. However, damaged OEC activity can be compensated by the non-water electron donors (Kalaji *et al*., 2014; Gururani *et al*., 2015).

5.3.3.2.4 ΔV_I-Band

The significant ($P \leq 0.05$) increase in the variable fluorescence between 2 ms and 300 ms of the well-watered plants fumigated with 80 ppb O_3 and 120 ppb O_3 after 28 days of fumigation (Figure 24E), reveals an increase in the pool size of the final electron acceptors of the PSI (Yusuf *et al*., 2010). However, the significant ($P \leq 0.05$) decrease in the variable fluorescence of the plants fumigated with elevated CO_2 and CO_2 + O_3 (Figure 24E), reveals a decrease in the pool size of the final electron acceptors of the PSI (Yusuf *et al*., 2010). Thus, an increase in the variable fluorescence of the drought-stressed plants fumigated with 80 ppb O_3 and 120 ppb O_3 (Figure 24F and H) is an indication that quinoa is negatively influenced by water and O_3 stress to regulate the pool size of the final electron acceptors on the acceptor side of the PSI. This is evident from the decrease in the pool size of the final electron acceptors of the drought-stressed plants fumigated with 80 ppb O_3 and 120 ppb O_3 (Figure 24F and H). The highest amplitude of the variable fluorescence (Figure 24A-H) in both well-watered and drought-stressed plants fumigated with 80 ppb O_3 and 120 ppb O_3 is an indication of a slow reduction rate of end acceptors of the PSI (Redillas *et al*., 2011a). The lowest amplitude of the variable fluorescence in the plants fumigated with elevated CO_2 and CO_2 + O_3 for both treatments (Figure 24A-H) indicate fastest reduction rate of end acceptors of the PSI (Redillas *et al*., 2011a). These results further indicate that the decrease in the pool size was not associated with the regulation of the reduction rate of the PSI electron acceptors, but rather a functional damage to the PSI (Redillas *et al*., 2011b). Similar results were reported by Redillas *et al.* (2011a, 2011b) in the rice plants (*Oryza sativa*) grown under water deficit and nitrogen-limited conditions, respectively. The appearance of the positive ΔV_I-bands in the plants fumigated with 80 ppb O_3 and 120 ppb O_3 in both treatments (Figure 24E-H) could be due to the inhibition of ferredoxin-$NADP^+$ oxidoreductase (FNR) activity. This enzyme is responsible for the final step of a linear electron flow transferring electrons from ferredoxin-$NADP^+$ (Schansker *et al*., 2003; Kalaji *et al*., 2014).

5.3.3.3 Maximum quantum efficiency (φ_{P0} or F_v/F_m)

Maximum quantum yield (F_v/F_m) fluctuated from 14 to 35 days after fumigation in both well-watered and drought-induced treatments without any statistical significance

variation (Figure 25A-H). Studies indicate that high values of the F_v/F_m suggest that the PSII structure was not damaged (Guidi *et al*., 2002; Rai and Agrawal, 2008). The high values of the F_v/F_m observed in this study suggest that the PSII structure of quinoa fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 (Figure 25A-H) was not damaged. A statistically non-significant difference in the F_v/F_m values between these treatments is an indication that this parameter cannot identify changes in the PSII quantum yield under stress conditions (Pereira *et al.*, 2000; Lepeduš *et al*. 2012). However, the decrease in the PI_{total} values of the plants fumigated with 80 ppb O_3 and 120 ppb O_3 (Figure 25A-H) was a clear indication that the PSII activity was reduced, which also resulted in the structural and/or functional damage of the PSI. The increase in the F_v/F_m ratio of the drought stressed plants and plants fumigated with 80 ppb O_3 and 120 ppb O_3 was caused by the increase in the F_m (maximum fluorescence) values (Figure 25A-H), which is an indication that the q_N was reduced and/or q_P was increased (Pellegrini *et al*., 2011). High values of the q_N is an indication that the plant is able to dissipate energy in the form of heat which is a strategy to avoid photoinhibition of the PSII antenna complexes (Shinkarev and Govindjee, 1993; Pellegrini *et al*., 2011). The q_N indicate the level of the non-radiative energy dissipation in the light harvesting complex II (LHC II) of PSII. This LHC II is ascribed to prevent over-reduction of the electron transfer chain and provides protection from photodamage (Krause and Weis, 1991; Muller *et al*., 2001; Finazzi *et al*., 2006).

However, other studies demonstrated that an increase in F_o (minimum fluorescence) is an indication that the PSII reaction centers are damaged or deactivated (Guidi *et al*., 2002). Similar results were observed in this study showing an increase in F_o of both well-watered and drought-induced treatments fumigated with 80 ppb O_3 and 120 ppb O_3 (Figure 25A-H), which is an indication that the PSII reaction centers of quinoa were damaged.

Studies also demonstrated that O_3 damages the PSII structure and blocks the photosynthetic electron transport chain connecting the PSII and PSI, which lead to the reduction of F_v/F_m ratio (Rai and Agrawal, 2008). However, the stable F_v/F_m ratio from 14 days after fumigation (Figure 25A-H) indicates that the PSII structure was not damaged and the photosynthetic electron transport chain connecting the PSII and PSI was not blocked.

The overall maximum quantum yield of the primary PSII photochemistry (F_v/F_m) decreased slightly in the well-watered plants fumigated with elevated CO_2 and CO_2 + O_3 after 14 days of fumigation (Figure 25A). Mulholland *et al*. (1997) observed the same in the wheat, which they further suggest was caused by a high content of active Rubisco and the absence of shade-acclimation by the canopy leaves.

Other studies have indicated that F_v/F_m of the rice plants taken on the older (16^{th} leaf stage) leaves recovered faster from 60 ppb O_3 damage compared to the younger plants. The reason for this is because the leaves become thicker with increasing height in the leaf position (Hoshikawa, 1989; Kobayakawa and Imai, 2011). However, the decrease of the F_v/F_m can be correlated with the amount of O_3 flux into the plant that is fully controlled by the rate of the stomatal conductance (Fiscus *et al*., 2005; Griffiths and Helliker, 2013). Contrasting results were obtained in the present study in both well-watered and drought-induced quinoa, as there was no significant difference in the F_v/F_m values after fumigation with 700 ppm CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 (Figure 25A-H). In addition, the findings of this study could not verify if the statistically non-significant increase of the F_v/F_m was due to quinoa's recovery from O_3 damage or not. Therefore, non-significant difference of the F_v/F_m values observed confirms that the F_v/F_m is less sensitive to water and O_3 stress and elevated CO_2 levels. This study is in agreement with the findings observed by Pellegrini *et al*. (2011) in *Melissa officinalis* fumigated with 200 ppb O_3. Other studies further indicated that the F_v/F_o is a better measure for O_3 stress than the F_v/F_m, as it can identify small differences in the PSII quantum yield under stress conditions (Pereira *et al*., 2000). Based upon these findings it can be concluded that the F_v/F_m is less sensitive to water and O_3 stress and elevated CO_2 levels and cannot be used for assessing plants subjected to these conditions.

5.3.3.4 Specific fluxes per reaction centre (RC)

The decrease in the specific fluxes per reaction center (RC) of ABS/RC, TR_o/RC and ET_o/RC, in both the well-watered and drought treatments, is an indication that the photochemical processes associated with the PSII and electron transport were influenced by drought stress and O_3 treatments from 14 to 21 days after fumigation (Figure 25A-D). These stressors also negatively influenced the functionality of the PSI as a result of the decrease in the reduction of the end electron acceptors as expressed

per reaction center (RE_o/RC). However, a significant ($P \leq 0.05$) increase in the RE_o/RC after 28 to 35 days of fumigation with 80 ppb O_3 (Figure 25E-H) indicates the recovery potential of electron transport beyond Q_A^- to the inter-systems of electron acceptors. The former clearly demonstrated that the O_3 stress and the interaction of O_3 and drought stress triggered a down regulation of the PSII photochemistry. This down regulation consisted of a reduction in the ψ_{Eo}, φ_{Eo}, ET_o/RC, RE_o/RC and RC/CS_o of the performance indices, in parallel with an enhanced dissipation of absorbed energy per RC, DI_o/RC (Figure 25 A-H). The enhanced heat dissipation per reaction center (DI_o/RC) during down regulation is associated with a photoprotective role (Bussotti *et al*., 2011; Gururani *et al*., 2015). Similar results have been reported in the tomato plants (Guidi *et al*., 2001), *Melissa officinalis* (Pellegrini *et al*., 2011).

5.3.3.5 Phenomenological fluxes per excited cross section (CS)

The decrease in the phenomenological fluxes per excited cross section (CS) of ABS/CS, TR_o/CS of the well-watered plants fumigated with 80 ppb O_3, 120 ppb O_3 and for all drought-induced treatments reflect an increased density of the inactive RCs (Figure 25A-H). The decrease of TR_o/CS and ET_o/CS suggests that the active RCs were converted into the inactive RCs, thereby reducing the efficiency of trapping and reducing the PSII activity. This could be an indication that the well-watered plants fumigated with 80 ppb O_3, 120 ppb O_3 and all drought-induced treatments were susceptible to photoinhibition. Inactivation of the RCs is considered a down-regulation mechanism that enables plants to dissipate excess absorbed light (Flexas and Medrano, 2002). Hence, the decreased phenomenological fluxes per cross section of the well-watered plants fumigated with 80 ppb O_3, 120 ppb O_3 and all drought-induced treatments stimulated a notable increase in the φ_{Do}, TR_o/RC and PI_{ABS} and decrease in the RC/CS_o and ET_o/CS (Figure 25A-H and Figure 26A-B). Reduced phenomenological fluxes per active RCs caused by drought and O_3 stress (Figure 25A-H) is an indication that few electrons were transferred to the PSII RC. The quantum yield of the primary photochemistry (F_v/F_m) remained almost constant in both treatments, which is an indication that it is less sensitive to drought and O_3 stress. The reduced energy cooperativity (expressed by positive ΔV_L) and increased reduction rate of Q_A (expressed by positive ΔV_K) in these treatments (Figure 21A-H and Figure 22E-H) further indicate

that quinoa is not tolerant to drought and O_3 stress. Similar results were reported in *Melissa officinalis* (Pellegrini *et al*., 2011).

5.3.3.6 Performance indexes (PI_{ABS} and PI_{total}) and the partial parameters

The PI_{ABS} values decrease from 14 to 21 days after fumigation in both treatments (Figure 25A-D) and increased again after 28 to 35 days of fumigation (Figure 25E-H) with no significant variation. Hence, the PI_{ABS} of the CO_2 fumigated plants increased as the trial progressed (Figure 26A-B). The ameliorative effect of the elevated CO_2 on O_3 was evident as the PI_{ABS} values of O_3 fumigated plants was consistently lower than the plants fumigated with CO_2 + O_3. However, the well-watered quinoa demonstrated tolerance to O_3 damage at low concentrations. As a result, the PI_{ABS} values of 80 ppb O_3 fumigated plants peaked after 28 days of fumigation and were consistently the same as the control plants (Figure 25E and G). A decrease in the PI_{total} values in the plants fumigated with 80 ppb O_3 from 14 to 21 days after fumigation was followed by a significant ($P \leq 0.05$) increase after 28 to 35 days of fumigation (Figure 26C-D). The former not only resulted in the loss of the PSII activity, but also caused structural and/or functional damage to the PSI. The latter indicate the recovery of the PSII activity preventing the structural and/or functional damage to the PSI of the drought-induced plants and the 80 ppb O_3 fumigated well-watered plants. A decline in the PI_{total} may be due to the concomitant increase in the stomatal conductance at the corresponding O_3 levels of both well-watered and drought-stressed plants. Significant ($P \leq 0.05$) increase of the PI_{total} in the plants fumigated with CO_2 + O_3 (Figure 25C-H) is an indication of the ameliorative effect of the elevated CO_2 levels on O_3. The increase in the PI_{ABS} and PI_{total} in the plants fumigated with CO_2 + O_3 was associated with an increase in the leaf electron transport capacity (Figure 25C-H and Figure 26A-D). These results are in agreement with the findings of Krüger *et al*. (1997); Strauss *et al*. (2006) and Stirbet and Govindjee (2011) who reported that PI_{ABS} and PI_{total} are sensitive to the environmental stressors that damage the photosynthetic apparatus in plants.

5.3.4 Days to flowering

Several experiments have indicated that elevated CO_2 can induce early flowering in both short day and long day plants (He *et al*., 2005; Springer and Ward, 2007). It was therefore not surprising that the elevated CO_2 shortened flowering time with 9 days

under well-watered and water deficit conditions when compared to their controls (Figure 27). Early flowering is an adaptation strategy that confers to the plant the opportunity to accumulate resources to avoid dry or cold period for an improved seed yield and quality (Roux *et al*., 2006). The flowering intensity was significantly ($P \leq 0.05$) higher on the well-watered quinoa plants fumigated with elevated CO_2. Drought-stressed plants had lower flowering intensity compared to the well-watered plants for all treatments (Figure 27). However, most flowers were aborted on the plants fumigated with 80 ppb O_3 and 120 ppb O_3. Similar results were reported in *Phytolacca Americana* (He *et al*., 2005) in *Capsicum annuum* (Springer and Ward, 2007) and in *Campanula rotundifolia* and *Scabiosa columbaria* (Hayes *et al*., 2012).

5.3.5 Specific leaf area

The increase in the individual and total leaf area of the well-watered quinoa fumigated with elevated CO_2 (Figure 28A) increased the ability of the plants to capture more solar radiation per unit mass which can enhance the plant growth (Chen and Setter, 2012). The development of new branches of the well-watered plants fumigated with elevated CO_2 resulted in a continued increase of the leaf area during flowering phase. This conferred to CO_2 fumigated plants an opportunity to produce more biomass and seed yield due to more leaves on tertiary branches. A significant ($P \leq 0.05$) decrease of the leaf area of the drought-stressed plants (Figure 28B) and plants fumigated with 80 ppb O_3, 120 ppb O_3 and $CO_2 + O_3$ (Figure 28A) resulted in the inability of the plants to capture enough solar radiation to enhance the plant growth. As a result of the reduced leaf area (Figure 28A-B) and damaged leaves (Figure 17D-G) of the plants fumigated with 80 ppb O_3 and 120 ppb O_3, photosynthesis was potentially inhibited leading to a retarded plant growth (Figure 29A-B). Similar results were reported in *Melissa officinalis* (Pellegrini *et al*., 2011), wheat (Biswas and Jiang, 2011) and in potatoes (Högy and Fangmeier, 2009).

5.3.6 Plant growth

Elevated CO_2 induced significant changes in the growth of the well-watered quinoa, as the plants fumigated with elevated CO_2 were 20% taller, relative to the control plants and 77% taller when compared to the plants fumigated with 80 ppb O_3 and 120 ppb O_3 (Figure 29A and Table 14). This increase was followed by a significant ($P \leq 0.05$)

increase in the tertiary branches. Therefore, high numbers of tertiary branches might act as potential sinks, conferring quinoa superior photosynthesis under elevated CO_2 conditions. This morphological component of growth of the well-watered quinoa fumigated with elevated CO_2, suggests improved utilisation of carbohydrates supply from the source tissues as a result of the improved photosynthetic rate to supply the growing sink demand (Högy and Fangmeier, 2009; Rasineni *et al*., 2011b). The accelerated growth, increased metabolic activity and the sink strength probably conferred to the well-watered quinoa fumigated with elevated CO_2 an opportunity to escape the photosynthetic down-regulation (Körner, 2006). The decrease of the plant height by 15% ($P \leq 0.05$) and 23% ($P \leq 0.05$) of the plants fumigated with 80 ppb O_3 and 120 ppb O_3, respectively, is a clear indication that O_3 reduces the plant height under well-watered and drought-stressed conditions (Figure 29A-B). These results are in agreement with the findings observed in *Melissa officinalis* (Pellegrini *et al*., 2011), wheat (Biswas and Jiang, 2011) and in potatoes (Högy and Fangmeier, 2009).

5.3.7 Biomass

The development of the tertiary branches and reproductive tissues of the well-watered quinoa fumigated with elevated CO_2 resulted in a 70% ($P \leq 0.05$) increase of shoot biomass (Figure 30A and C and Table 15). This is an indication that CO_2 fumigated quinoa was capable of efficiently regulating the carbon capturing and storage compared to the other treatments. The present study clearly demonstrated that elevated CO_2 enhanced the plant biomass productivity (Figure 30A and C) and increased the seed yield (Figure 31A) of quinoa under well-watered conditions. Hence, elevated CO_2 also promoted a biomass production by 42% ($P \leq 0.05$) in plants subjected to water stress (Figure 30B and D and Table 15) when compared to other drought-stressed treatments. It is widely accepted that water stress and elevated CO_2 may reduce biomass by reducing the stomata opening and limit CO_2 uptake (Fiscus *et al*., 2005; McLaughlin *et al*., 2007). As a result, the main objective of this study was to test whether drought stress and elevated CO_2 would offer quinoa better protection against high levels of O_3. However, different results were observed in the drought-induced plants fumigated with 80 ppb O_3 and 120 ppb O_3. These plants showed a 55% ($P \leq 0.05$) and 68% ($P \leq 0.05$) reduction in the plant biomass (Figure 30B and D), respectively. Retardation of the plant growth and reduction in a biomass due to O_3 exposure might be ascribed to the

diversion of the photoassimilates for repair and detoxification or decrease in the photosynthetic carbon gain (Leadley *et al*., 1990; Pell *et al*., 1997; Kangasjärvi *et al*., 2005). It was evident that drought aggravates the O_3 damage. Hence, elevated CO_2 was able to ameliorate the negative effects of O_3 under well-watered conditions (Figure 30A-D). Similar results were reported in wheat fumigated with O_3 (Pleijel *et al*., 1999; Biswas and Jiang, 2011) and in potatoes (Högy and Fangmeier, 2009).

5.3.8 Seed filling and yield

Seed yield increased by 71% ($P \leq 0.05$) on quinoa fumigated with elevated CO_2 under well-watered conditions (Figure 31A and Table 15) and seed filling was accelerated by 15 days outperforming the other treatments. Quinoa fumigated with elevated CO_2 revealed greater carbon partitioning to the grain, as demonstrated by a high grain yield (Figure 31A-B). The increased seed yield of the well-watered quinoa can be related to consistent development of tertiary branches and flowers observed on the well-watered quinoa fumigated with elevated CO_2. O_3 significantly ($P \leq 0.05$) decreased grain weight and yield per plant (Figure 31A-B). Reduction in the seed yield for all drought-induced plants fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 (Figure 31B) can be attributed to both seed weight and number of seeds per plant. The present study also indicated that the seed yield was dependent on water stress and the concentration of O_3 (Figure 31B and Table 15). These results are in agreement with the findings observed in wheat (Pleijel *et al*., 1999; Biswas and Jiang, 2011)

5.4 Summary

The JIP-test parameters changed under water and O_3 stress and elevated CO_2 in this study. The PSII electron transport chain was clearly influenced by these conditions. These results also confirmed that the JIP-test can be used as a sensitive method for measuring the effects of water and O_3 stress and elevated CO_2 levels in the quinoa leaves, and is a good tool for investigating differences in the physiological behaviour of the PSI and PSII. In addition, for the evaluation of water and O_3 stress in quinoa, we propose that the reduction of the JIP-test parameters of ψ_{Eo}, φ_{Eo}, ET_o/RC, RE_o/RC, RC/CS_o, PI_{ABS} and PI_{total} and an enhanced dissipation of absorbed energy per RC, DI_o/RC can be used to assess these conditions. At the same time, elevated CO_2 levels triggered the increase of ψ_{Eo}, φ_{Eo}, ET_o/RC, RE_o/RC, RC/CS_o, PI_{ABS} and PI_{total} and

decrease in DI_o/RC. More so, these parameters can identify the plants grown under normal conditions from those grown under stress conditions and permits an understanding of the processes related to energy flux in the electron transport chain. The exposure of quinoa to drought and O_3 stress, elevated CO_2 and CO_2 + O_3 treatments induced significant physiological, phenological and developmental changes. Elevated CO_2 could prevent O_3-induced damage in the photosynthetic efficiency of quinoa. The proposed hypothesis at the onset of the study states that the PSII parameters will be more tolerant to water and O_3 stress than the PSI parameters. The results indicated that the proposed hypothesis were substantiated as the exposure of quinoa to water and O_3 stress induced significant decrease in the chlorophyll *a* fluorescence, flowering, plant height, leaf area, seed yield and biomass. In addition, elevated CO_2 induced significant increases in these parameters. Water stress and elevated CO_2 induced a significant decrease in the stomatal conductance. However, O_3 has induced a significant increase in the stomatal conductance under well-watered and drought-induced conditions. These changes can play a crucial role in agriculture as a whole by providing immediate answers in remediating the effects of the environmental stressors before they are irreparable. Further studies are required to explain the physiological basis caused by the effects of these conditions on the OJIP transients.

5.5 Conclusion

Severe water stress induces down-regulation of the photochemical activity in quinoa by deactivating the PSII RCs. The JIP-test is a sensitive method for quantifying the effects of water and O_3 stress and elevated CO_2 levels in quinoa and is a good tool for investigating the differences in the PSII and PSI behaviour. The ΔV_L and ΔV_K-bands are good indicators to identify the physiological disruptions in the PSII before the appearance of the visual damage caused by the stress. Severe drought stress up to anthesis can result in a postponement of the anthesis and physiological maturity of quinoa. Restrained water conditions is not likely to cause this effect.

The exposure of quinoa to drought stress, elevated CO_2, O_3 and CO_2 + O_3 treatments induced significant physiological, phenological and developmental changes. The positive ΔV_K-band and ΔV_L-band exhibited by the drought-stressed plants fumigated with elevated CO_2, 80 ppb O_3, 120 ppb O_3 and CO_2 + O_3 indicated that quinoa was

susceptible to drought stress and the interaction of these treatments. Results clearly showed that elevated CO_2 could ameliorate O_3-induced damage in the photosynthetic efficiency of quinoa. The variable positive photosynthetic responses of the well-watered quinoa under elevated CO_2 and CO_2 + O_3 indicate that quinoa can produce good yields under these conditions. The elevated CO_2 ameliorated the negative effects of O_3 on the plants that were fumigated with CO_2 + O_3. Elevated CO_2 increased the PI_{ABS} and PI_{total} in quinoa. Quinoa's tolerance threshold level to drought stress was at 20% field capacity and below 80 ppb O_3. O_3 fumigation increased the stomatal conductance (g_{H2O}) under well-watered and drought-induced conditions indicating that the stomata were possibly severely damaged by O_3.

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ANNEXURES (TOC_HEADING)

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