The effects of drought, waterlogging and heat stress on tomatoes (*Solanum lycopersicon* L.*)*

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Abstract of the thesis submitted to fulfilment of the requirements for the Degree of Doctor of Philosophy.

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Field production of tomatoes generally involves exposure to various sub optimal environmental conditions and environmental stress factors including high temperature, scarcity of water or excessive water. This study investigated morphological, physiological and biochemical responses to water stress (water deficit and waterlogging) of two tomato (*Solanum lycopersicon* L.) cultivars ('Best Boy Bush' and 'Scoresby Dwarf') at three developmental stages (vegetative, flowering and fruiting) in the glasshouse, and at the fruiting stage in a kinetic study in the field. These cultivars, together with the cultivar 'Soprano' were further investigated in a heat stress experiment (at 40/30 °C day/ night temperatures).

Generally, growth parameters including leaf length and leaf area as well as plant biomass accumulation were reduced by the three stress factors. The reduction in growth-related traits was more pronounced at the earlier stages of plant development (i.e. the vegetative stage). The impact of heat stress was characterised by a significant increase in the number of abscised flowers (5.4 fold) and of flowers with stigma tube elongation (3.5 fold), as well as the prevention of fruit set. Trials to examine physiological responses to water stress showed reductions in plant water status (leaf relative water content and leaf water potential) and leaf gas exchange (photosynthesis, stomatal conductance and transpiration).

Osmotic adjustment was observed under water deficit by virtue of a reduction in adjusted osmotic potential and an increase in proline production. For instance, adjusted osmotic potential decreased by 29%, and proline levels increased by 48% in the leaves and by 65% in the roots in plants subjected to drought stress in the glasshouse study. A reduction of osmotic potential (-91%) and an accummulation of free proline (from Day 2 to Day 8) were also observed in drought treated plants in the field trial. In contrast, free proline levels decreased under waterlogging.

Tomato plants responded to both water stress treatments with the accumulation of hydrogen peroxides, which resulted in oxidative stress. High levels of H_2O_2 were observed in leaves and roots of water-stressed plants in the glasshouse and field trials. However, the H_2O_2 levels in the roots of plants subjected to waterlogging were slightly lower in the glasshouse trial. As a consequence of the elevated oxidative load (H_2O_2 production), there was greater damage to lipids, proteins and DNA in water-stressed plants. The activities of enzymatic antioxidants were all increased under drought stress but were inactivated in plants at the reproductive developmental stage under waterlogging conditions in the glasshouse trial. As a result, activities

of superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase and glutathione peroxidase all increased under drought stress, but decreased under waterlogging following the accumulation of H_2O_2 and the occurrence of oxidative stress.

In the field experiment, the activities of these enzymatic antioxidants increased two days after exposure to water stress and continued to rise as the period of water stress increased. In contrast, enzyme activity reached a plateau five days after the start of waterlogging. The production of non-enzymatic antioxidants such as ascorbate and glutathione increased in tissues of plants subjected to water deficit under both growing conditions. Levels of these antioxidants were either slightly increased or significantly decreased in plants subjected to waterlogging in both growing conditions (glasshouse and field). Waterlogging induced hypoxia in plants as measured by increasing ADH activity in the roots of waterlogged plants. ADH levels began rising from Day 2 and continued to rise with duration of the waterlogging period. The activities of glyoxalase enzymes increased in parallel with an accumulation of methylglyoxal in both leaf and root tissues.

Ascorbic acid levels and total antioxidant capacity both increased in tomato fruits sampled from drought stressed plants. However, these antioxidants decreased in tomato fruits harvested from plants subjected to waterlogging. Total carotenoid content was reduced in the pericarp of 'Best Boy Bush' fruits grown under water deficit and waterlogging, but not in the pericarp of 'Scoresby Dwarf' fruits. The biological activity of these water stressed tomato fruits was assessed using an *in vitro* gastrointestinal digestion coupled with Caco-2 cell cultures. Caco-2 cell viability under oxidative stress was improved when the cells were pre-treated with digested tomato fruit grown under water stress.

There were significant cultivar differences in many stress responses under glasshouse conditions, with lower levels of oxidative damage and increased protective responses of the antioxidant apparatus in ‘Scoresby Dwarf’ under water stress. There were also indications of heat stress tolerance in the cultivar ‘Scoresby Dwarf’, which had lower numbers of abscised flowers and lower incidence of flowers with elongated stigma tubes in response to high temperature stress, compared to ‘Best Boy Bush’.

Taken together, this study provides a guide for the identification of stress tolerant genotypes and improves the understanding of the importance of biochemical changes in plants experiencing oxidative stress. The findings can be used for the selection and development of stress tolerant tomato cultivars, and suggest merit for the application of targeted drought treatments to boost the production of antioxidant phytochemicals in tomato fruits that are of benefit for human health.

Keywords: Tomato, water deficit, waterlogging, high temperature, enzymes, antioxidants, reactive oxygen species, oxidative stress, methylglyoxal, nutrional quality, bioactive.

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List of Abbreviations

Abbreviation	Parameter
ABA	Abscisic acid
ADH	Alcohol dehydrogenase
AHC	Agglomerative hierarchical clustering
AOP	Adjusted osmotic potential
APOX	Ascorbate peroxidase
AsA	Ascorbate or ascorbic acid
BBB	'Best Boy Bush'
Caco-2	Caucasian colon adenocarcinoma cell
CAT	Catalase
Con	Control
DHA	Dehydroascorbate
DHAR	Dehydroascorbate reductase
DM	Dry matter
DNA	Deoxyribonucleic acid
Dr	Drought
DW	Dry weight
F_v	Chlorophyll fluorescence
FW	Fresh weight
GLOX	Glyoxalase
GPOX	Gluathione peroxidase
GR	Glutathione reductase
GSH	Reduced form of glutathione
GSSG	Disulfide glutathione
HCA	Heatmap clustering analysis
H_2O_2	Hydrogen peroxides
LA	Leaf area
LH	Polyunsaturated fatty acids
LOO▪	Lipid peroxyl radicals
LOOHs	Lipid hydroperoxides
LRWC	Leaf relative water content

LPOX	Lipid peroxidation
MDA	Malondaildehyde
MDHAR	Monodehydroascorbate reductase
MG	Methylglyoxal
NOAA	National oceanic and atmospheric administration
$OH^{\bullet -}$	Hydroxyl radical
$^1O_2$	Singlet oxygen
$O_2^{\bullet -}$	Superoxide anion
PCA	Principle component analysis
PCs	Protein carbonyls
PDM	Percentage of dry matter
PSII	Photosystem II
QAC	Quaternary ammonium compounds
ROS	Reactive oxygen species
RSR	Root:shoot ratio
SBD	'Scoresby Dwarf'
SLA	Specific leaf area
SOD	Superoxide dismutase
SPN	'Soprano'
TAC	Total antioxidant capacity
TDR	Time domain reflectometry
TW	Turgid weight
WL	Waterlogging
WMO	World meteorological organization

Chapter 1
Introduction

The tomato (*Solanum lycopersicon* L.) is one of the world's most important vegetables and the second most important crop in New Zealand following potatoes (http://www.freshfacts.co.nz, 2010). Tomatoes are cultivated widely and global production has increased about 300% over the last four decades (Heuvelink, 2005). Tomato production systems can be viewed as belonging to either open field production systems or production systems conducted under some form of a protective structure such as a glasshouse. Field grown tomatoes are commonly found in tropical, subtropical and warm temperate climates. Field produced tomatoes are frequently exposed to unfavourable environmental conditions such as waterlogging or excess water caused by heavy rains or cyclones, droughts and high temperatures. These environmental stresses are major causes of crop yield losses worldwide. Mahajan and Tuteja (2005) reported that the combination of all environmental stresses has, at times, claimed about 50% of global major crop production.

A water deficit or drought is, globally, the most common stress condition and it is increasingly of concern worldwide (Mahajan & Tuteja, 2005; Reddy et al.,2004). Plant productivity is predominantly influenced by water availability. Tomatoes are very sensitive to drought stress, initially during vegetative development and, later, when the tomato is reproductive (Wudiri & Henderson, 1985). Several studies have investigated the effect of water deficit on individual aspects of tomato morphology, physiology and biochemistry (e.g: Liu et al., 2010; Pervez et al., 2009; Wudiri & Henderson, 1985). However, there are very few studies on the effects of severe drought stress through the critical developmental stages (early vegetative growth, flowering and fruiting stage) of tomato plants.

Flooding and waterlogging are also considered to be major stress factors. It is estimated that about 13% of the global land area and 16% of the tomato areas in production worldwide are prone to the risk of flooding and waterlogging (Ahsan et al., 2007; Cramer et al., 2011). The primary effect of waterlogging in plants is oxygen deprivation. When there is a low oxygen environment in the root zone, there is a change from aerobic to anaerobic conditions. Root metabolism in an anaerobic environment yields less energy for plant functions (Horchani et al., 2008; Sairam et al., 2008). Bradford and Hsiao (1982) reported that a plant's physiological response to waterlogging is very similar to the response induced by a water deficit. It is well

documented that waterlogging promotes petiole epinasty and the formation of adventitious roots (Bradford & Dilley, 1978; Horchani et al., 2008; Jackson & Campbell, 1976). However, there is little comparative research on the effects of waterlogging and drought in plants exposed to these water stress factors at the same time.

The likelihood and frequency of extreme climatic events occurring in the same geographical region are predicted to increase in response to global climate change (Climate-Charts.com, 2007). Hence, studies are warranted that directly compare the effects of such events on crop plants. Furthermore, there is a recognition of a global rise in temperature and, consequently, an increasing number of heat stress studies have been conducted on tomatoes (Camejo et al., 2005; Sato et al., 2000; Wahid et al., 2007). The daily temperature in regions termed tropical and subtropical has increased to above 30°C in the summer season (Climate-Charts.com, 2007). This temperature regime exceeds the optimal growing range for tomato, which is from 13-25°C (Heuvelink, 2005). This elevated temperature range poses a threat to tomato production in these regions. Heat stress has significant effects on the morphology and physiology of a tomato, especially during the reproductive stage of growth. Nevertheless, little research has been conducted to examine the effects of high day- and night-time temperatures in tomatoes.

Stress factors such as water deficit, waterlogging and heat stress induce oxidative stress in plants. Oxidative stress occurs in plant cells when the production of reactive oxygen species (ROS) is excessive (Sharma et al., 2012). ROS, including singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet -}$), are constantly produced as by-products of metabolic reactions (Foyer & Noctor, 2011; Sharma et al., 2012). Oxidative stress can induce lipid peroxidation, protein oxidation and DNA damage in plants (Arora et al., 2002; Blokhina & Fagerstedt, 2010). To protect themselves against these toxic ROS, plants have evolved antioxidant defence mechanisms (Gill & Tuteja, 2010; Sharma et al., 2012). These include both enzymatic and non-enzymatic antioxidants. Antioxidant enzymes in tomato plants subjected to water deficit include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APOX) and glutathione reductase (GR) (Sanchez-Rodriguez et al., 2012; Tahi et al., 2008; Zgallai et al., 2006). In addition, the presence of non-enzymatic antioxidants such as ascorbate and glutathione have also been reported in tomato plants under oxidative stress induced by a water deficit (Murshed et al., 2013; Sanchez-Rodriguez et al.,2012). Research on the antioxidative systems of waterlogged tomato plants is limited. However, the presence of some specific enzymes and antioxidants in hypoxic tomatoes have been investigated (Horchani et al., 2010a; Lin et al., 2004). The study of Lin et al. (2004) suggested that APOX activity and

the total ascorbate content were increased by waterlogging stress, whereas SOD, CAT, and an oxidised form of glutathione (GSSG) were not affected. Reductions in total ascorbate and dehydroascorbate (DHA) under waterlogging conditions were suggested by Horchani et al., (2010b). However, there has been no research that compares the antioxidative systems of drought stressed and waterlogged tomato plants.

Besides ROS, there is another highly toxic compound produced under stress called methylglyoxal (MG). MG is a side-product of various metabolic pathways. MG can inhibit cell proliferation, increase protein oxidation, react with the guanyl nucleotide in DNA and inactivate antioxidant defence systems (Hoque et al., 2012; Mostofa & Fujita, 2013; Yadav et al., 2005). MG is catalysed by glyoxalase enzymes (glyoxalase I and glyoxalase II) and has been widely studied in microbes and animals, but very little is known about this compound in plants (Hoque et al., 2012; Yadav et al., 2005).

In plants, apart from the enzymatic and non-enzymatic antioxidants, there are other ROS scavenging compounds, some of which have been classified as phytochemicals (Sanchez-Rodriguez et al., 2011). These phytochemicals include carotenoids, ascorbic acid or vitamin C and total antioxidants. The accumulation of carotenoids in plants is mainly determined by plant genotype and environmental conditions (George et al., 2004; Kopsell & Kopsell, 2006). In tomato fruits, 80-90% of carotenoids are lycopene and 7-10% are β-carotene (Frusciante et al., 2007; Rosales et al., 2011). Ripe tomato fruits have also been known to show high antioxidant activity, which can protect biological systems from any harmful effect caused by excessive oxidant accumulation (Arnao et al., 2011). Total antioxidant activity is generally separated into water-soluble or water-insoluble components (Rodriquez-Roque et al., 2013; Vallverdu-Queralt et al., 2012). Total phenolics and vitamin C are predominantly water-soluble antioxidants, whereas water insoluble antioxidants is mainly contributed to by carotenoids (Guil-Guerrero & Rebolloso-Fuentes, 2009; Novarro-Gonalez et al., 2011; Toor & Savage, 2005). A reduction in total carotenoid levels in the fruit pericarp of waterlogged tomato plants was reported by Horchani et al., (2010a).

Recently, the bioavailability of these phytochemicals has gained increasing research interest. Several studies have integrated a new approach for measuring bioavailability using the *in vitro* gastrointestinal digestive model, coupled with the Caco-2 cell culture model (Liu et al., 2004; Thakkar et al., 2007). To the best of the author's knowledge, there have been no studies regarding the biological activity of phytochemicals in tomato fruits subjected to water stress.

Therefore, this thesis reports the main effects and interactions of drought and waterlogging on tomatoes (Figure 2.2.1.a). An additional chapter also explores heat stress effects in tomatoes. Following the literature review (Chapter 2), the experimental design and the treatment conditions are described in Chapter 3, before examining the growth and morphological effects caused by water stress (drought and waterlogging) and the corresponding stress responsiveness of tomatoes. Chapter 4 describes the physiological effects caused by water stress in tomatoes. Chapter 5 details the corresponding biochemical effects and responses, including stress markers and antioxidative defence systems, observed when tomatoes are water stressed in the glasshouse trail. Chapter 6 examines the activity of glyoxalase enzymes in response to the accumulation of methylglyoxal on top of biochemical attributes that have previously been investigated in the glasshouse trail (Chapter 5). The nutritional quality and the biological activity of tomato fruits from water stressed plants using an *in vitro* gastrointestinal digestion method coupled with a human cell-culture (Caco-2 cell) are also included in Chapter 6. In Chapter 7, the morphological and physiological effects of heat stress are investigated with the aim of examining the possible coping strategies shown by tomato plants under high temperature stress. The correlations of some key attributes using principle component analysis (PCA) are included in each result chapter. The similarity of biochemical attributes using heatmap clustering analysis (HCA) are presented in Chapter 5 and 6. The thesis concludes with a general discussion of the results and the identification of possible future research directions resulting from the findings of this study (Chapter 8).

The central research hypotheses are, as follows:

1). Morphological and physiological responses to water stress (both drought and waterlogging) and heat conditions in tomatoes will vary with different plant selections and developmental stages.

2). Detoxification of reactive oxygen species will occur under both water stress treatments, but there will be differences in the responses and kinetics of enzymatic and non-enzymatic antioxidants depending on the water stress treatment.

3).The nutritional quality of tomato fruits will be altered by water stress. Tomato fruits grown under water stress conditions will be high in bioactive substances, including total antioxidants, total ascorbate and total carotenoids. These compounds can benefit human health through the protection of cellular systems from oxidative damage.

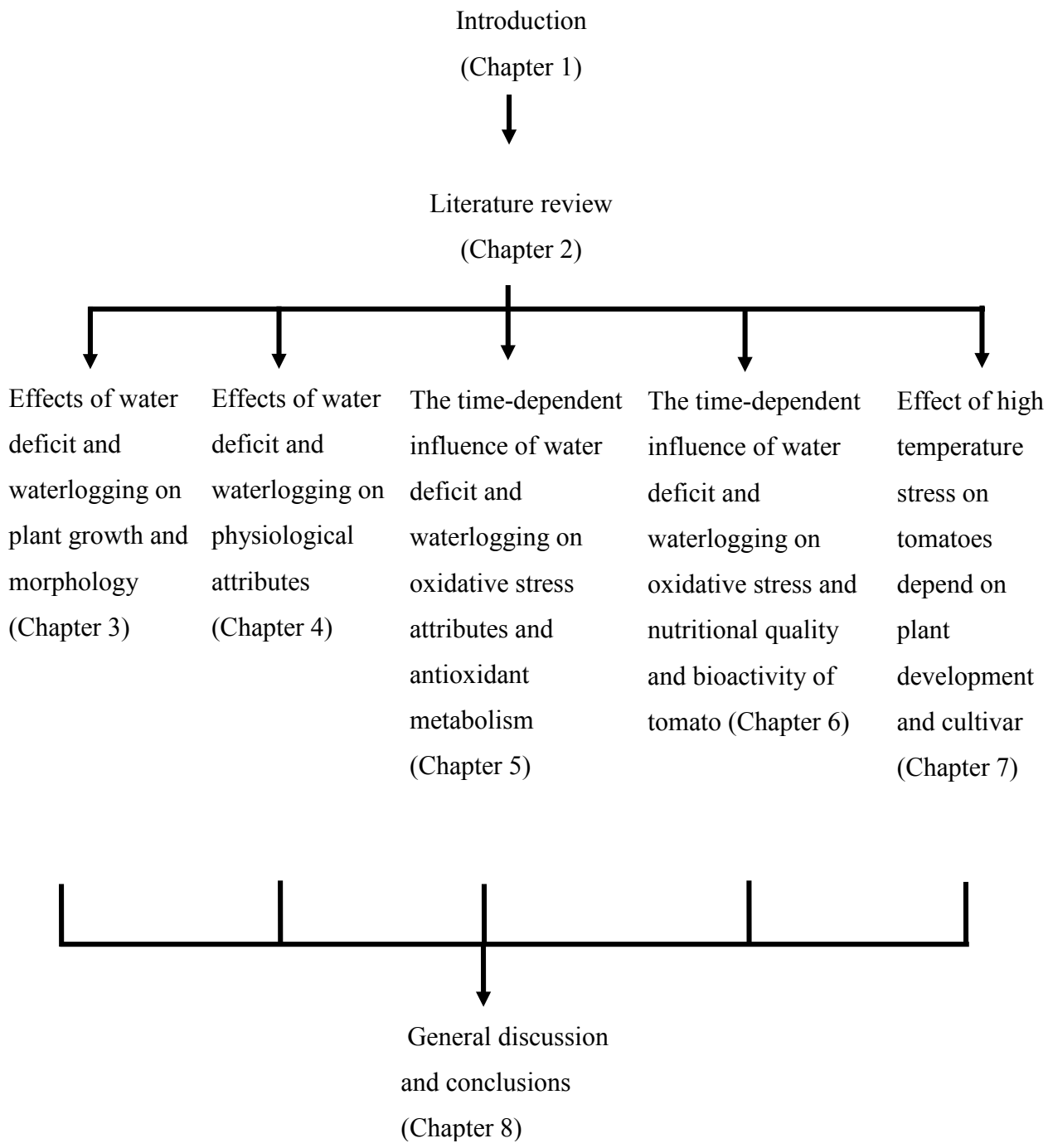

Figure 2.2.1.a Flow diagram of the thesis layout

Chapter 2
Literature review

2.1 Tomato

Tomato (*Solanum lycopersicon* L.) is one of the world most important vegetable crops and the second most important in New Zealand following potatoes (*New Zealand Horticulture - Facts & Figures*, 2012). The tomato originated from South America and has been widely cultivated from Asia to Europe and America (*Crop protection compendium: Solanum lycopersicum (tomato)*, 2010). Tomato belongs to the *Solanaceae* family, together with capsicum, eggplant and potato (Benton Jones, 2008; Hanson et al., 2000). Tomato production has gradually increased in the last decade despite the production area remaining almost the same *(Food and Agricultural Commodities Production, 2010)*. In 2009, the FAO production statistics for world tomato production were 153,884,368 tonnes. Asia accounted more than 50% of the world tomato production, followed by America, at about 18%, with Europe and Africa at 14.58% and 12.28%, respectively. Oceania (mainly Australia and New Zealand) shared less than 0.5% of the world tomato production (*Food and Agricultural Commodities Production*, 2010). The demand for tomato products has increased substantially to a consumption rate of 72.7 kg per capita per year (Benton Jones, 2008). Tomatoes are consumed fresh and processed (sauce, paste, juice and ketchup) (Benton Jones, 2008).

Tomato production systems can be viewed as belonging to either open field production systems or production systems conducted under some form of structure. Structures, which provide crop cover, provide opportunities for crop environment modification or control. Field grown tomatoes are commonly produced in tropical, subtropical and warm temperate climates with a sufficiently long growing season for fruiting and fruit maturation. In New Zealand, even though glasshouse production is increasing, the majority of tomato production is cultivated outdoors (*New Zealand Horticulture - Facts & Figures*, 2012).

Table 2.2.1.a Summary of growth and development of field grown semi-determinate tomato modified from (Benton Jones, 2008; Hanson et al., 2000; Morgan, 2008)

Growth and development stage tomato	Description
Germination	The optimum temperature for seed germination is between 22 and 24°C and it will take about four to six days. From germination to initial leaf formation is about 25-35 days.
Transplanting	Seedlings are ready to transplant when they have four to five leaves or are about four weeks old.
Vegetative	From transplanting to the initiation of flower buds will take about 20-25 days. This varies by tomato cultivar and environmental conditions.
Flowering	From flower initiation to fully open flowers and ready for fertilization will be approximately 20-30 days.
Fruit formation	From flowering to fruit set will take about 15-20 days.
Fruit maturity	From fertilization or fruitlet to harvest is about six to eight weeks or 40 -75 days.

2.2 General attributes of environmental stress

There are few places in the world where plants can escape from some form of environmental stress, such as water deficits, extreme temperatures, salinity, floods, high light intensity, UV, nutrient deficiencies, high levels of heavy metals or air pollution (Claussen, 2002; Taiz & Zeiger, 2010). Environmental stresses or abiotic stresses are a primary cause of crop yield reduction worldwide. Mahajan and Tuteja (2005) reported that a combination of all environmental stresses has resulted in about a 50% production loss globally for major crops. Among these factors, drought, waterlogging and high temperatures are probably the highest threats and they have been chosen for investigation in this study.

Many large global climate organizations such as the World Meteorological Organization (WMO), the National Oceanic and Atmospheric Administration (NOAA), and NASA have found that 2000 – 2009 was the warmest decade since instrumental measurements began in the 1880s (Zhao & Running, 2010). Global climate change with changing temperature and precipitation values has been blamed for the growing frequency and intensity of droughts and floods (Breshears et al., 2005; Lehner et al., 2006). Zhao and Running (2010) suggested that high air temperatures lead to high autotrophic respiration and low vapour pressure deficit which controls and increases surface evapo-transpiration; thus, less water is available for vegetative growth (Zhao & Running, 2010). Water is essential for plants, so plant growth can be limited by both water deficit and excessive water. Ninety seven per cent of water taken up by plants will be lost into the atmosphere and only 2% will be available to be used for plant cell expansion and 1% for photosynthesis (Taiz & Zeiger, 2010). In summary, these factors (frequency and intensity of droughts, floods and high temperatures) that lead to more environmental stresses in plants will pose serious threats to global agricultural production and food security. It is imperative to make plants ready for water deficits and waterlogging as well as high temperatures. However, this requires a better understanding of the coping mechanisms and adaptations by plants under these unfavourable conditions.

2.2.1 Drought

Drought is the most common stress factor in reducing crop yields. There are a number of different definitions for drought stress. Taiz and Zeiger (2010) suggested that drought is a meteorological term for a period of insufficient precipitation and results in a plant water deficit. Others state that drought is a moderate loss of water which leads to stomatal closure and a limitation of gas exchange (Jaleel et al., 2009). Drought is of increasing concern worldwide due

to increased evapotranspiration losses from plants and soils. Drought stress is closely associated with high temperature stress and, together, they can affect 64% of the global land area (Cramer et al., 2011). Water is a major factor influencing plant productivity, so when water is insufficient in the soil, and atmospheric conditions cause a continuous loss of water, drought stress occurs. In plants, water is essential for the photosynthesis reaction – a reaction process that is mainly affected by physiological pathways and environmental factors (Shao et al., 2007). Plants adapt to survive and to maintain their growth and development. Many mechanisms may be involved and these mechanisms may include drought avoidance and drought tolerance (Mishra et al., 2011; Reddy et al., 2004). Drought avoidance is the ability of plants to retain a high tissue water potential either through increased water absorption from roots or reduced evapo-transpiration from their aerial parts, while drought tolerance refers to the plant's ability to sustain normal functions even at a low water potential (Mishra et al., 2011; Reddy et al., 2004). Drought can affect the plants' morphology, physiology and biochemistry, leading to a reduction in plant growth and productivity (Thapa et al., 2001). Different plant species have different family-specific responses to cope with drought. However, there are some common responses such as slowed cell division and changes in gene expression leading to altered physiological reactions, for example, a reduction in photosynthesis (Bohnert & Jensen, 1996; Thapa et al., 2011).

Generally, a tomato plant requires about 400 to 600 mm of water within a 75-125 day growing period (Jensen et al., 2010). Jensen et al. (2010) and Shinohara et al. (1995) reported that tomatoes can tolerate drought to some degree but Wudiri and Henderson (1985) suggested that tomatoes were very sensitive to water stress particularly during vegetative development and the reproductive stage. When water stress was introduced, photosynthesis and transpiration might be inhibited but will gradually recover during on-going stress even under low leaf water potential (Shinohara et al., 1995). Sudden or severe water stress can delay or inhibit flowering and fruiting, whereas moderate water stress at the flowering stage may accelerate fruiting (Wudiri & Henderson, 1985). Drought stress may induce morphological, physiological, and biochemical changes in plants (Mishra et al., 2011) through reduction in tissue water content and water potential (Garcia et al., 2007; Shinohara et al., 1995).

2.2.2 Waterlogging

Flooding and waterlogging are also recognised as major, global, abiotic stresses. They stand alongside water deficits, salinity and extreme temperature as threats to yield realisation (Visser et al., 2003). Thirteen per cent of land area and 16% of production area worldwide are at a high risk of floods and waterlogging (Ahsan et al., 2007; Cramer et al., 2011). Soil is considered

waterlogged when the water content is 20% above field capacity and there is free standing water on the soil surface (Ahsan et a., 2007; Irfan et al., 2010). Field capacity is the maximum volume of water that a soil can hold by capillary action before water is drawn away by gravity (http://botanydictionary.org). The soil water potential is high at field capacity and the percentage of water that the soil can hold varies with texture, clay having a high field capacity and sand a low field capacity (http://botanydictionary.org). In Asia, where typhoons often cause heavy rains, waterlogging has become one of the most common environmental threats to crops (Ahsan et a., 2007). Poor drainage is also responsible for waterlogging in the field. Horchani et al. (2009) reported that more than one-third of the world's irrigated areas have inadequate drainage. Excessive water has negative impacts on plant growth and survival (Horchani et al., 2010a; Lopez & Rosario, 1983) as it significantly inhibits gas exchange between a plant and its environment due to the lower gas diffusion rate in water than in air (Armstrong, 1979).

The primary effect of waterlogging in plants is oxygen limitation or hypoxia (Lopez & Rosario, 1983; Sairam et al., 2008). Excessive water in the root zone, leads to plant injuries due to the blockage of oxygen and other gas exchanges between the soil and the atmosphere because water fills the pore spaces in the soil (Horchani et al., 2009). Horchani et al. 2010a reported that the tomato is an anoxic tolerant species, which means that tomato plants have some tolerance to an oxygen deficit. Lack of oxygen in the root zone causes a reduction of root hydraulic conductivity, thus, influencing water and nutrient uptake by the plants (Jackson et al., 2003) and also oxygen transport from the roots to the shoots (Wei et al., 2013). It has been suggested that plants take three basic steps in response to waterlogging (Wei et al., 2013). First, there is a reduction in signal transductions including carbohydrate assimilation and photosynthates utilisation. Secondly, there is the activation of fermentative pathways. Thirdly, there will be morphological changes (formation of aerenchyma and adventitious roots) (Wei et al., 2013). Several negative impacts on the morphology, physiology and biochemistry in tomato plants occur during, and are subsequent to, oxygen deprivation. Damage in plants depends on the intensity of stress, the susceptibility of the crop and the developmental stage (Horchani et al., 2010a; Lopez & Rosario, 1983).

2.2.3 High temperature stress

As previously stated, there is a trend of a continuing increase in global temperatures. Some studies have suggested that the temperature increase has become more pronounced in the Southern Hemisphere, including South America, New Zealand, Australia, some Pacific Islands and Indonesia (Easterling et al., 1997). Daily temperatures in the tropics and subtropics, such

as the Caribbean, parts of Africa, Iran, Pakistan and Southeast Asia, have also risen (Easterling et al., 1997). High temperature stress or thermal stress refers to increasing temperatures beyond the ambient level for a period of time that is long enough to cause permanent damage to growth and development of a plant (Wahid et al., 2007). High temperatures have several negative impacts on plants; for example, damage to cells and sub-cellular components as well as a reduction in photosynthesis and fruit set (Wahid et al., 2007). The primary effect of heat stress is the inhibition of photosynthesis, which can be temporarily or permanently damaged by the severity of high temperature stress (Salvucci & Crafts-Brandner, 2004a). In the field, high temperature stress is often accompanied by drought because if the plants receive an adequate supply of water the stomata will remain open despite the elevated temperature (Salvucci & Crafts-Brandner, 2004a). The cool down of leaf temperature through the evaporative cooling system (as water evaporates from the leaf) can be insufficient with the occurrence of water deficit conditions coinciding with heat stress (Salvucci & Crafts-Brandner, 2004b).

In the tropics and subtropics the average summer temperature range is between 30 and 35°C (Climate-Charts.com, 2007). These temperatures are above the optimal temperature growing range for tomatoes, which has been suggested as between 13 and 25 °C (Heuvelink, 2005; Preedy & Watson, 2008; Sato et al., 2000). This rise in temperature has posed a threat to the field production of tomatoes, which are commonly found in Asia. In New Zealand, despite the increase in glasshouse production, the majority of tomato production takes place outdoors (*New Zealand Horticulture - Facts & Figures*, 2012). Long exposure to high temperatures will injure several cell and sub cell components including membranes, proteins and lipids. Wahid et al. (2007) suggested that high temperatures cause harmful morpho-anatomical, physiological and biochemical changes in plants. The reproductive phase of tomato growth is the most susceptible (Preedy & Watson, 2008; Wahid et al., 2007). The other significant effects of thermal stress in tomato plants include reduction of photosynthesis, poor fruit set and altered physiological components.

2.3 Specific attributes of drought, waterlogging and heat stress

In general, the consequences of environmental stresses are the generation of reactive oxygen species (ROS), disruption in membrane stability, increasing protein denaturation, perturbation mechanisms and physical injury (Taiz & Zeiger, 2010). Water stress, including both drought stress and stress caused by waterlogging, cause similar damage in plants because they affect the same cellular processes and also induce oxidative stress (Taiz & Zeiger, 2010). Plants have

adapted to protect themselves from stress factors through various mechanisms. These mechanisms include long-term phenological and morphological adaptation, short-term avoidance and escape strategies (Taiz & Zeiger, 2006). However, if the plant's response is insufficient, there could be irreversible damage to cells, the destruction of functional and structural proteins and membranes, and cell death may result (Taiz & Zeiger, 2006; Wahid et al., 2007).

Table 2.2.3.a Summary of plant response to water stress

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2.3.1 Stress response observed through morphological differences

2.3.1.1 Modified plant growth

Generally, under stress, plant growth is reduced and this reduction is manifested as a reduction in stem elongation, leaf expansion and numbers of leaves (Sanchez-Rodriguez et al., 2010a) (Figure 2.3.1.a). Drought may cause a significant reduction in leaf area but root growth may be maintained in order to extract more water from deeper soil layers (Mishra et al., 2011; Pervez et al., 2009; Torrecillas et al., 1995). Poor growth in plants may be caused by the slower cell division. Slower cell division may be induced by a decline in cyclin-dependent kinase activity (Mahajan & Tuteja, 2005). However, Mahajan and Tuteja (2005) suggested that the reduction

in leaf expansion is not an effect caused by drought stress but it is a form of response demonstrating that plants have adapted to minimize transpiration. The adaptation is called leaf area adjustment (Mahajan & Tuteja, 2005).

Figure removed subject to copyrights

Figure 2.3.1.a Some causes of reduction in plant growth (Jaleel et al., 2009)

When plants are waterlogged, root biomass is greatly decreased. The root biomass decrease is caused by a reduction in anaerobic respiration and death of root tissue due to the prolonged root submergence (Aloni & Rosenshtein, 1982; Horchani et al., 2008). A special growth feature observed when plants are subjected to high temperature is that the newly produced leaves are smaller and have a vertical orientation and it has been suggested that this growth response helps plants minimise direct exposure to heat (Taiz & Zeiger, 2006). In general, after a period of exposure to severe stress, observers note wilting, leaf curling and rapid senescence in the old leaves, leading to plant death (Aloni & Rosenshtein, 1982; Sairam et al., 2008; Torrecillas et al., 1995).

2.3.1.2 Senescence and abscission

Waterlogging promotes rapid senescence of the old leaves (Aloni & Rosenshtein, 1982; Lin et al., 2004). This may result from either a reduction in leaf chlorophyll content or an accumulation of ROS in the leaves (Ahsan et al., 2007; Lin et al., 2004). Leaf senescence and abscission was also reported in plants under a water deficit and heat stress (Jaleel et al., 2009; Sanchez-Rodriguez et al., 2010a; Wahid et al., 2007). Horchani et al., 2008 reported that abscission of flowers and young fruits occurs when waterlogging occurs during reproductive development. This abscission response may be altered carbohydrate assimilation in plants and an altered

priority for fruit development resulting in less nutrient availability for flowers and young fruits (Horchani et al., 2008).

2.3.1.3 Development of petiole epinasty

Petiole epinastic growth and the development of adventitious roots are the typical consequences of waterlogging. Root submergence and a lack of oxygen in the rhizosphere can promote epinastic (downward) growth of tomato leaves (Aloni & Rosenshtein, 1982; Jackson & Campbell, 1976; Lopez & Rosario, 1983). The epinastic effects can be seen about four days after plants have been subjected to waterlogging (Jackson & Campbell, 1976). This response may be associated with oxygen deficiency in the root zone and ethylene production. High concentrations of ethylene were found in plant tissue extracted from epinastic petioles (Jackson & Campbell, 1976). Ethylene diffusion was blocked when the roots were submerged in water, thus, ethylene moved upwards and accumulated in the shoots (Bradford & Dilley, 1978).

2.3.1.4 Formation of adventitious roots

Many studies have suggested that the formation of adventitious roots may help plants to survive under excessive water conditions (Aloni & Rosenshtein, 1982; Jackson & Campbell, 1976; Bradford & Dilley, 1978). Jackson and Campbell (1976) reported that adventitious roots appeared on the base of a tomato stem about two days after plants were exposed to waterlogging. However, Horchani et al. (2008) and Lopez and Rosario (1983) observed that adventitious root appearance was seen about two weeks after plants were subjected to waterlogging. The appearance of adventitious roots may be induced by the plant growth regulator, ethylene (Sairam et al., 2008). Aloni and Rosenshtein (1982) reported plant turgor and growth can be recovered with the formation of adventitious roots. Adventitious roots are essential for the survival of plants when most of the roots rot after a period of root submergence (Aloni & Rosenshtein, 1982). The effect of waterlogging is more pronounced when plant growth is more advanced; for example, it is more pronounced at fruit set than in the earlier vegetative stages. The recovery of the root mass through the development of adventitious roots, could be affected by competitive demands for photosynthates by developing organs, such as fruits (Horchani et al., 2009).

2.3.2 Physiology

2.3.2.1 Plant water relationships

Water potential and leaf relative water content

It is very well understood that leaf water status is usually associated with stomatal conductance. The close correlation between leaf water potential and stomatal conductance occurs even under water deficit conditions (Reddy et al., 2004). Drought conditions cause a reduction in leaf tissue

water content and water potential (Calcagno et al., 2011; Shinohara et al., 1995). Yuan et al., 2010 reported a significant reduction in leaf relative water content (LRWC) in tomatoes under water stress.

Waterlogging induces a similar plant water status as drought stress. Aloni and Rosenshtein (1982) suggested that waterlogging promotes the development of a water deficit in the rhizosphere, despite the plant roots being completely submerged in water. This restriction in plant water supply is probably caused by an oxygen deficiency in the root zone, which then disturbs the metabolic activity of the roots (Aloni & Rosenshtein, 1982).

Plant water status is the most significant variable under heat stress. Wahid et al. (2007) reported that severe heat stress has a negative impact on root hydraulic conductance and leaf water relations in tomatoes even under optimal conditions for soil water supply and relative humidity.

Osmotic adjustment

Some plants have the ability to adopt coping mechanisms for water stress. Reducing water loss, which will help maintain water potential, is a means of overcoming water stress (Garcia et al., 2007; Sanchez-Rodriguez et al., 2010b). One coping mechanism is termed osmotic adjustment. This metabolic process involves the accumulation of compatible osmolytes or osmo-protectants such as organic solutes, amino acids, polyamines and quaternary ammonium compounds (QAC), and incurs energy costs (Mahajan & Tuteja, 2005; Sanchez-Rodriguez et al., 2010a; Torrecillas et al., 1995). The reduction of cellular water potential to values lower than the external water potential through a reduction in osmotic potential has also observed (Yoshiba et al., 1997). Torrecillas et al. (1995) reported that high solute accumulations caused a reduction in osmotic potential.

Under waterlogging conditions, a high accumulation of solutes was observed two days after the stress was experienced (Jackson et al., 2003). This flush of solutes is probably caused by the death of root cells through prolonged oxygen deprivation and the increased unrestricted entry of minerals as the dying roots failed to regulate selective ion uptake or created a more openly apoplastic pathway (Jackson et al., 2003).

Accumulation of free proline

Proline is an amino acid that contributes to osmotic adjustment (Claussen, 2005). The observable variation in proline has been shown to occur under a broad range of stress conditions including water stress, salinity, extreme temperature and high light intensity (Claussen, 2005; Thapa et al., 2011; Yoshiba et al., 1997). Because proline is involved in osmotic adjustment, it

accumulates at the same time as the other osmo-protectants (Claussen, 2005; Yoshiba et al., 1997). Proline has several major functions including mediating osmotic adjustment, protecting protein structures from denaturation, stabilising cell membranes by interacting with phospholipids, scavenging ROS and serving as energy and nitrogen sources (Claussen, 2005; Sanchez-Rodriguez et al., 2010a). Some authors have reported that high drought stress tolerant plants often have high proline concentrations. However, this was not observed by Sanchez-Rodriguez et al (2010a), who claimed that proline was just a stress symptom.

Under drought stress, proline biosynthesis and accumulation may be associated with the detoxification of ROS, a reduction in water potential and a reduction in photosynthesis rates (Reddy et al., 2004; Thapa et al., 2011). Proline accumulation in plants may be caused by either the activation of enzymes of proline biosynthesis (P5C synthase EC 2.7.2.11) or the inactivation of proline degradation (Reddy et al., 2004; Yoshiba et al., 1997). Reddy et al. (2004) suggested that proline can protect membranes and proteins even when LRWC was decreased during drought stress. Waterlogging can cause a reduction in free proline in the roots but increase free proline in the leaves (Lopez & Rosario, 1983). Aloni and Rosenshtein (1982) suggested that the production of proline in tomatoes under hypoxia can be divided in to two phases and was probably associated with water potential. Phase 1, proline accumulation begins approximately two days after waterlogging and continued rising for four days before diminishing. Phase 2, proline accumulated to a peak production on day 11 after the onset of waterlogging before decreasing.

2.3.2.2 Leaf gas exchange

Photosynthetic damage

Features of photosynthesis such as photosynthetic rate, stomatal conductance and intercellular CO_2 concentration are reported to be significantly lowered under both drought conditions and waterlogging conditions (Jackson & Campbell 1976; Mishra et al., 2011; Yuan et al., 2010). When there is a water deficit, the photosynthetic rate is lowered directly via a reduction in internal CO_2 supply or lowered indirectly through the inhibition of photosynthetic enzymes (Haupt-Herting & Fock, 2000). A reduction in the internal CO_2 supply is caused by stomatal closure, which typically occurs as part of the mechanism to avoid water loss by reducing transpiration (Calcagno et al., 2001; Haupt-Herting & Fock, 2000; Mishra et al., (2001). Inhibition of photosynthetic enzymes such as rubisco, ATP, photophosphorylation and ribulose-1, 5 bisposphate (RuBP) also contributes to lower photosynthesis rates (Calcagno et al., 2001; Haupt-Herting & Fock, 2000). A deficiency in internal CO_2 diffusion and the

inhibition of photosynthetic enzymes stimulate the oxidation of NADPH in the Calvin cycle. Therefore, $NADPH^+$, the primary acceptor of photosynthesis electrons, is not sufficiently available and photosynthetic rates are reduced (Haupt-Herting & Fock, 2000). An intercellular CO_2 deficiency can lead to an excessive reduction in various components of the electron transport chain and electrons get attached to oxygen at photosystem I. The processes described above, generated several reactive oxygen species (ROS) (Mahajan & Tuteja, 2005).

High temperature stress also causes a reduction in photosynthesis; however, the plants response to high temperature varies from the water stress response. Under water stress, stomatal conductance is induced by a hormone signal from the roots to the shoots and not from the leaves as occurs with high temperature stressed plants (Wahid et al., 2007). Leaf temperature is increased under high ambient temperature stress, leading to a reduction in the ability of the leaves to sustain gas exchange and as a consequent inhibition of photosynthesis (Wahid et al., 2007). Sato et al. (2000) reported that the Photosystem II (PS II) electron transport of tomato plants was severely affected after six hours of exposure to a temperature of 42°C. In addition, high temperatures caused a change in cellular CO_2 assimilation and a reduction in stomatal conductance (Camejo et al., 2006; Wahid et al., 2007). This may result from severe damage to PS II and a reduction in rubisco activity (Camejo et al., 2006; Salvucci & Crafts-Brandner, 2004a). Alternately, CO_2 assimilation and stomatal conductance reductions may have been caused by the presence of ROS (Pareek et al., 2010).

Conductance

Under drought stress, stomatal conductance may directly influence the evaporation of water from the guard cells (Sanchez-Rodriguez et al., 2010a). This process is termed hydropassive closure and it is generally accepted that it is regulated by the root-produced hormone, abscisic acid (ABA), which acts as an early signal of a soil water deficit (Jensen et al., 2010; Mahajan & Tuteja, 2005). ABA is produced by roots and transported through the xylem to the shoots, where it can cause a reduction in leaf expansion and restrict stomata opening (Reddy et al., 2004; Thapa et al., 2011).

Stomatal closure is considered to be a general response to root hypoxia (Horchani et al., 2009). However, the closure of stomata under waterlogging conditions is not regulated by ABA and, therefore, the mechanism is different from that accompanying drought (Jackson & Campbell, 1976). When roots are submerged in water, ABA transport through the xylem to the shoots is

inhibited. Thus, Jackson et al. (2003) suggested that there must be another unknown signal stimulating stomatal closure under waterlogging conditions.

Transpiration

Plants respond to water stress by closing their stomata in order to avoid water loss through transpiration (Haupt-Herting & Fock, 2000). Stomatal closure is one of the earliest shoot responses to stress (Bradford & Hsiao, 1982). Therefore, a reduction of transpiration could be seen just hours from the onset of both a water deficit and waterlogging and the reduction is more pronounced if stress is prolonged (Bradford & Hsiao, 1982; Campos et al., 2009; Haupt-Herting & Fock, 2000). Similarly, under high temperature conditions the rate of transpiration is likely closely related to stomatal closure (Romero-Aranda et al., 2001). Transpiration has been shown to cool leaves when plants are subjected to heat stress (Camejo et al., 2006). The temperature of plant leaves has been shown to decrease by about 5°C compared to the ambient air temperature (Camejo et al., 2006). Transpiration involves energy costs, which dissipate about 20- 30% of heat energy. Romero-Aranda et al. (2001) reported that a high transpiration rate is accompanied by stomatal closure in thermo-sensitive tomato cultivars. However, Camejo et al. (2006) stated that transpiration was found to increase in both heat sensitive and tolerant tomato cultivars.

Chlorophyll content and chlorophyll fluorescence

Little is known about either the presence or the response of chlorophyll pigments in tomato plants when drought stress is experienced. However, Sanchez-Rodriguez et al. (2012) reported that chlorophyll degradation was measurable in a sensitive tomato cultivar. This change might be considered as a drought response mechanism in order to minimise light harvesting by chloroplasts. Haupt-Herting and Fock (2000) reported a significant reduction in initial chlorophyll fluorescence (F_o) under drought stress. The maximum efficiency of PSII measured as (F_m - F_o) /F_m (F_m is maximum chlorophyll fluorescence) in dark adapted leaves was not affected by drought stress (Haupt-Herting & Fock, 2000).

In the early stages of waterlogging, the chlorophyll content in the leaf tissue of tomato plants is not modified (Horchani et al., 2009). However, chlorophyll degradation becomes more pronounced as the duration of the waterlogging induced stress and, ultimately, chlorosis and leaf senescence are observed (Ahsan et al., 2007; Lin et al., 2004). Horchani et al. (2008) reported that the chlorophyll content of the leaves present before root hypoxia was not affected, but the chlorophyll contents of leaves that emerged during stress were reduced by 23% compared with control plants.

Both chlorophyll content and chlorophyll fluorescence are degraded under high temperature stress. The degradation is more pronounced in temperature sensitive genotypes and under extremely high temperatures (Camejo et al., 2006; Salvucci & Crafts-Brandner, 2004a; Wahid et al., 2007). High temperature stress induces changes in chlorophyll fluorescence. F_o values increase while chlorophyll fluorescence (F_v) values decrease. Maximum photochemical efficiency of PS II in dark adapted leaves can be shown by the F_v / F_m ratio (Camejo et al., 2005). This ratio can be altered by heat stress. In addition, the chlorophyll content in tomato plants was reported to reduce during high temperature stress but this reduction was not statistically significant (Camejo et al., 2006).

2.3.3 Biochemistry

2.3.3.1 Reactive Oxygen species (ROS)

Drought, waterlogging and heat stress induce oxidative stress in plants (Blokhina & Fagerstedt, 2010; Kang et al., 2009; Thapa et al., 2011). Oxidative stress plays a significant role in lipid peroxidation (LPOX), cell death and leaf senescence, all of which are adverse events (Ahsan et al., 2007). Plants experience oxidative stress when the oxidative load, ROS are over produced in tissues and ROS need to be removed to maintain normal plant growth in these situations (Eshdat et al., 1997). ROS are constantly produced as by-products of various metabolic reactions including photosynthesis, photorespiration and respiration (Foyer & Noctor, 2011; Yoshimura et al., 2004). Therefore, ROS may be found in metabolically active cells, particularly, chloroplasts (Dat et al., 2000; Gill & Tuteja, 2010). The authors suggested that in chloroplasts, PS I and PS II are major sites for oxygen generation. Various compounds may be ROS agents. For example, singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet -}$) are ROS agents (Burritt & Mackenzie, 2003; Pareek et al., 2010; Yuan et al., 2010). ROS agents are universal in signalling cascades and destructive processes. Therefore, ROS may be taken as markers of oxidative stress (Blokhina & Fagerstedt, 2010). The chemical properties of ROS are, in general, highly reactive affecting many cell functions by causing lipid peroxidation, protein oxidation and damaging DNA (Burritt et al., 2002; Gill & Tuteja, 2010; Mahajan & Tuteja, 2005). Figure 2.3..3.a shows a summary of ROS damage to lipids, proteins and DNA under oxidative stress.

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Figure 2.3.3.a Summary of ROS damage to lipids, proteins and DNA under oxidative stress (Sharma et al., 2012)

Each type of ROS can be converted into other ROS compounds (Figure 2.3.3.b). For example, the dismutation of $O_2^{\bullet -}$ (a ROS compound) by a key enzyme, superoxide dismutase (SOD) results in O_2 and H_2O_2 (another ROS compound) (Burritt & Mackenzie, 2003; Kang et al., 2009; Pareek et al., 2010). However, a combination of $O_2^{\bullet -}$ and H_2O_2 in the presence of Fe^{2+} or Fe^{3+} results in the production of $OH^{\bullet -}$, which are highly toxic ROS to plant tissues. This process concomitantly generates lipid hydroperoxides (LOOHs) (Burritt & Mackenzie, 2003; Knight et al., 2001).

Lipid peroxidation is a very deleterious process and occurs in every living organism (Gill & Tuteja, 2010). LPOX can modify the structure of complex protein assemblies including biological membranes and lipoproteins. As a consequence, cellular functions are likely to be disrupted (Yoshimura et al., 2004). The probability of membrane LPOX and, therefore, cellular disruption, increases with the accumulation of oxygen in plant tissues (Blokhina & Fagerstedt, 2010). Therefore, LPOX is an index of oxidative damage under stress. To date, general LPOX can be measured by the content of LOOHs or malondialdehyde (MDA) (Griffiths et al., 2000; Hall & Bosken, 2009).

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Figure 2.3.3.b Conversions of ROS compounds into other ROS compounds. Energy transfer (e-) may be involved (Gill & Tuteja, 2010)

LOOHs are products of the oxidation of polyunsaturated fatty acids, whereas MDA is a secondary end-product of polyunsaturated fatty acid oxidation (Griffiths et al., 2000; Hall & Bosken, 2009). LOOHs can be eliminated by either ascorbic acid or glutathione peroxidase (GPOX) (Knight et al., 2001). However, if these compounds are not detoxified, they will be decomposed to lipid peroxyl radicals (LOO▪) in the presence of ions (Figure 2.3.3.c) (Hall & Bosken, 2009; Knight et al., 2001). LOO▪ will then propagate the production of additional LOOHs by reacting with neighbouring polyunsaturated fatty acids (LH).

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Figure 2.3.3.c Formation of lipid hydroperoxide (Hall & Bosken, 2009)

As mentioned earlier, ROS can cause protein oxidation. Carbonyl groups, including aldehyde and ketones, are produced on protein side chains, where they are oxidised by ROS (Dalle-Donne et al., 2003).

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Figure 2.3.3.d Protein oxidation (Gill & Tuteja, 2010)

The carbonyl groups consist of the products resulting from the oxidation of a number of amino acids, such as Arginine, Histidine, Lysine, Protein, Threonine and Tryptophan (Figure 2.3.3.d) (Gill & Tuteja, 2010). The carbonylation can result from either direct or indirect oxidation of amino acid side chains (Dalle-Donne et al., 2003; Knight et al., 2001). The carbonyl groups may be introduced into proteins via the secondary reaction of the nucleophilic side chains with either aldehyde or reactive carbonyl derivatives (Dalle-Donne et al., 2003). ROS are more likely to target proteins that contain sulphur-containing amino acid and thiol groups. ROS, particularly $^{1}O_2$ and $OH^{\bullet -}$, can remove an H atom from cysteine residues to form a thiol radical (Figure 2.3.3.d). Alternatively, oxygen can attach to a methionine residue to form methione sulphoxide derivatives (Gill & Tuteja, 2010). Carbonyl proteins are the most commonly used markers for the degree of protein oxidation (Dalle-Donne et al., 2003; Knight et al., 2001).

ROS are also major source of DNA damage under oxidative stress (Lin et al., 2007; Sharma et al., 2012). Sharma et al. (2012) suggested that DNA damage includes deoxyribose oxidation,

strand breakage, removal of nucleotides, modification of bases and DNA-protein crosslinks. Sugar and base moieties of DNA are vulnerable to oxidation by ROS. Hydroxyl radicals attack DNA by reacting with all purine and pyrimidine bases and also the deoxyribose backbone of the DNA structure (Gill & Tuteja, 2010; Sharma et al., 2012) whereas singlet oxygen mainly attacks guanine (Figure 2.3.3.e) (Gill & Tuteja, 2010).

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Figure 2.3.3.e DNA oxidation (Gill & Tuteja, 2010)

In addition, ROS can remove hydrogen from the deoxyribose of sugar moieties of DNA, leading to single-strand breaks. The damage to DNA results in various effects including reduced protein synthesis, destruction of cell membranes and damage to photosynthetic proteins that, therefore, affects plant growth and development (Gill & Tuteja, 2010; Tuteja et al., 2009). Although, there is an existing system to repair DNA such as direct reversal of the damage, replacement of bases and replacement of the whole nucleotide, severe oxidative stress can lead to permanent damage to DNA and, ultimately, result in cell death (Gill & Tuteja, 2010; Sharma et al., 2012).

Yuan et al. (2010) reported that drought stress promotes an overproduction of ROS, particularly H_2O_2. The concentration of the H_2O_2 in drought stressed tomato leaves was higher compared to non-stressed leaves (Sanchez-Rodriguez et al., 2012; Yuan et al., 2010). This study also suggested that high H_2O_2 concentrations in plant tissues lowered the biomass of plants (Sanchez-Rodriguez et al., 2012). Hypoxia induces the accumulation of ROS through on-going

light reactions in the leaves (Lin et al., 2004). Hydrogen peroxide concentrations in tomato plants subjected to waterlogging, change over time. In the period 24 h to 48 h, from the onset of waterlogging, ROS production increases (Ahsan et al., 2007). In the time period 72 h after waterlogging, ROS decreases, probably due to the activation of antioxidant enzymes (Ahsan et al., 2007). Likewise, heat stress trigger ROS production and also induces lipid peroxidation in tomato plants (Camejo et al., 2006; Ogweno et al., 2009). Ogweno et al. (2009) reported that the H_2O_2 content increased in the tissue of heat stressed leaves compared with optimal growing leaves, whereas Camejo et al., 2006 stated that the concentration of H_2O_2 decreased under heat stress. The reduction in H_2O_2 probably resulted from efficient scavenging as it was observed that the H_2O_2 reduction paralleled the increase of ascorbate peroxidase (APOX) (Camejo et al., 2006). An examination of plant genotype differences regarding H_2O_2 concentration showed that it was significantly reduced in sensitive genotypes (Camejo et al., 2006). Lipid peroxidation and carbonyl proteins increased in both tolerant and sensitive cultivars; however, levels of these compounds were higher in thermo-sensitive genotypes.

There has been no research relating to DNA damage in tomato plants under water stress. Lin et al., 2007 reported that the DNA of *Vicia faba* leaves was damaged under oxidative stress induced by cadmium stress. Both single and double-strand breakages were analysed using the Comet assay. In the Comet assay, DNA breakages migrate in the electric field from the nuclei toward the anode (Lin et al., 2007). Only the small DNA fragments that can travel through the electric field and these fragments can be measured as preventing DNA damage. The study reported that the damage to DNA increased with increasing cadmium concentration in parallel to the elevating ROS level. The author also suggested that enzyme antioxidants played an important role in DNA damage. For example, an increase in SOD activity with a reduction in catalase activity can lead to an increase in H_2O_2 production and, therefore, more DNA damage (Lin et al., 2007).

2.3.3.2 Antioxidant defence mechanism

Some studies suggest that the sequences of events that occur as plants respond to stress is as follows:

1). Increasing ROS production in plant tissue, which oxidises target molecules

2). Gene expression for antioxidant functions, leading to the promotion of antioxidative systems and various antioxidants to counter the potential damage of ROS.

3). ROS elimination from plant cells (Reddy et al., 2004).

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Figure 2.3.3.f A general scheme for scavenging of ROS. Adapted from Wahid et al., 2007

The antioxidative system includes enzymatic and non-enzymatic antioxidants (Kang et al., 2009; Sanchez-Rodriguez et al., 2010a).

Enzymatic antioxidants

Enzymatic antioxidants include specific enzymes such as superoxide dismutase, catalase, a peroxidase enzyme (glutathione peroxidase) and enzymes involved in the ascorbate-glutathione cycle (previously known as the Halliwell-Asada pathway). Some enzymes in this pathway are ascorbate peroxidase (APOX), gluthathione reductase (GR), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR) (Burritt & Mackenzie, 2003; Sanchez-Rodriguez et al., 2010a). The ascorbate-glutathione is an important pathway for plants in tackling several environmental stresses including drought, waterlogging, and heat (Lin et al., 2004). Table 2.3.3.a summarises some major ROS scavengers.

Table 2.3.3.a Major ROS scavenging antioxidant enzymes

Enzymatic antioxidants	Enzyme Code	Reaction catalysed
Superoxide dismutase (SOD)	EC 1.15.1.1	$O_2{\cdot}^- + O_2{\cdot}^- + 2H^+ \rightarrow 2H_2O_2 + O_2$
Catalase (CAT)	EC 1.11.1.6	$H_2O_2 \rightarrow H_2O + {}^1/_2O_2$
Ascorbate peroxidise (APOX)	EC 1.11.1.11	$H_2O_2 + AA \rightarrow 2H_2O + DHA$
Monodehydroascorbate reductase (MDHAR)	EC 1.6.5.4	$MDHA + NAD(P)H \rightarrow AA + NAD(P)^+$
Dehydroascorbate reductase (DHAR)	EC 1.8.5.1	$DHA + 2GSH \rightarrow AA + GSSG$
Glutathione reductase (GR)	EC 1.6.4.2	$GSSG + NAD(P)H \rightarrow 2GSH + NAD(P)^+$
Glutathione peroxidase (GPOX)	EC 1.11.1.9	$2GSH + 2LOO{\cdot} \rightarrow 2LOOH + GSSG$

Adapted from Gill and Tuteja (2010) and Hall and Bosken (2009)

SOD (EC 1.15.1.1) is the most effective intracellular enzymatic antioxidant and is ubiquitous in all aerobic organisms and in all cellular compartments prone to ROS (Gill & Tuteja, 2010). As mentioned earlier, SOD removes $O_2^{\cdot-}$ by catalysing its dismutation. Once $O_2^{\cdot-}$ is reduced to H_2O_2 and O_2, the risk of $OH^{\cdot-}$ formation is minimised (Arora et al., 2002; Burritt & Mackenzie, 2003; Gill & Tuteja, 2010). CAT (EC 1.11.1.6) is an enzyme known as a tetrameric haeme (Dat et al., 2000). Hydrogen peroxide is directly eliminated by CAT and yields water and O_2. CAT is crucial for H_2O_2 scavenging under stressful conditions as demonstrated by Sanchez-Rodriguez et al. (2012). This study suggested that low H_2O_2 concentration is often associated with high CAT activity in the leaf tissues of tomatoes under drought stress. Gill and Tuteja (2010) reported that one molecule of CAT can convert approximately six million molecules of H_2O_2 per minute. GR (EC 1.6.4.2) is a flavo-protein oxidoreductase and can act as an enzyme in the ascorbate-glutathione cycle (Gill & Tuteja, 2010). The defence system against ROS can be sustained by GR as it can effectively remove H_2O_2 (Arora et al., 2002; Shao et al., 2008). APOX (EC 1.11.1.11) is a potent enzyme in scavenging ROS and protecting cells in plants from damage by ROS. APOX is involved in the conversion of H_2O_2 to water in the ascorbate-glutathione cycle and uses ascorbate (two molecules) as electron donor (Arora et al., 2002; Grata˜o et al., 2008). This process concomitantly generates monodehydroascorbate (MDHA). MDHA has a very short life if it is not reduced to ascorbate or dehydroascorbate (DHA) (Noctor

& Foyer, 1998). MDHA can be reduced enzymatically by MDHAR using NADPH as an electron donor (Arora et al., 2002; Burritt et al., 2002). MDHAR (EC 1.6.5.4) is a flavin adenine deinucleotide (FAD) enzyme. MDHAR can also scavenge H_2O_2. DHAR (EC 1.8.5.1) can generate ascorbate from the oxidised state (DHA) and stimulate the cellular ascorbate redox state. Gill and Tuteja (2010) suggested that an over expression of DHAR can improve plant defence systems against various forms of abiotic stress. Both, MDHA and DHA can be recycled to generate a pool of ascorbate molecules. GPOX (EC 1.11.1.9) is a member of a large isozyme family that has previously been identified as an oxyradical scavenger in animals (Eshdat et al., 1997; Noctor et al., 2012). These isozymes have only been reported as being present and active in plants in the last few decades, so research attention has been recent and intensive regarding the role of these isozymes in plants (Eshdat et al., 1997). GPOX is selenium dependent and can be found in all cells (Noctor & Foyer, 1998; Noctor et al., 2012). There are several types of GPOX in plant cells based on their active-site motif (Foyer & Noctor, 2011). Yoshimura et al. (2004) suggested that there are at least four distinctive groups of GPOX; however, the phospholipid glutathione peroxidase (PHGPOX) may play a more crucial role in protecting membranes from oxidative damage than other forms of GPOX. PHGPOX has been reported in photosynthetic tissues of tobacco, citrus, Arabidopsis, sunflower, tomato and pea (Faltin et al., 2010; Yoshimura et al. 2004). PHGPOX can directly reduce H_2O_2 and phospholipid hydroperoxides (Yoshimura et al., 2004) as well as the complex hydroperoxyl lipids including bio-membrane lipid layers (Faltin et al., 2010).

Non enzymatic antioxidants

Ascorbate or ascorbic acid and glutathione are non-enzymatic antioxidants (Kang et al., 2009; Sairam et al., 2008). Ascorbic acid is an abundant, powerful, water soluble antioxidant. Ascorbic acid can react not only with $^1O_2$, $O_2^{\bullet-}$ and $OH^{\bullet-}$ but also with LOOHs (Jimenez et al., 2002; Lin et al., 2004; Shao et al., 2008). High levels of ascorbic acid have been found in photosynthetic cells and also in some fruits (Gill & Tuteja, 2010). The highest concentration has been reported in mature leaves with fully developed chloroplasts and a high chlorophyll content (Gill & Tuteja, 2010). High DHAR and GR also raise the total ascorbate in leaves (Sanchez-Rodriguez et al., 2012). Glutathione is a cellular antioxidant and can protect thiol containing enzymes (Burritt & Mackenzie, 2003). In plant tissue, reduced form of glutathione (GSH) is continuously oxidised to a disulphide form, GSSG (Noctor et al., 2012). This oxidised form of glutathione, GSSG, is recycled to the glutathione pool by the catalytic action of glutathione reductase using NADPH (Noctor et al., 2012). GSH is also found in the tissues of both green and red tomato fruits (Jimenez et al., 2002).

It has been suggested that the antioxidant defence mechanism active during drought stress is modulated by the plant's water potential (Yuan et al., 2010). Examination of the enzymes that remove H_2O_2 from tomato leaf tissue under drought stress revealed that SOD activity did not change, whereas CAT activity decreased in the tissue of sensitive tomato cultivars (Sanchez-Rodriguez et al., 2012). The activity of enzymes in the ascorbate-glutathione pathways, including APOX, MDHAR, DHAR and GR, increased in the leaf tissues of drought tolerant cultivars. The concentration of reduced ascorbate increased in the tissues of drought tolerant genotypes but reduced in the tissues of sensitive genotypes. The assay of total glutathione in tomato leaf tissue showed that this compound was higher in drought tolerant cultivars than drought sensitive cultivars. A water deficit also generated the reduction of DHA and GSSG in the tissue of sensitive tomato cultivars (Sanchez-Rodriguez et al., 2012). The concentrations of total ascorbate and DHA in tomato fruits were altered by water stress (Murshed et al., 2013).

There is little information regarding the antioxidative system in tomato where plants have been subject to waterlogging. Lin et al. (2004) reported that enzyme assays of hypoxic root tissues of tomato plants showed that only the contents of APOX and total ascorbate increased. The other enzymes and antioxidants including SOD, CAT, ascorbic acid and GSSG were not affected by root submergence. Reductions were reported in total ascorbates and reduced ascorbate in tissue of tomato fruit developed during prolonged root hypoxia (Horchani et al., 2010).

Ogweno et al. (2009) reported that the activity of SOD, APOX and GPOX increased in detached leaf tissues of tomatoes under high temperature stress. This finding was different from the finding of Camejo et al. (2006), who suggested that heat stress generated a reduction in SOD activity in leaf tissues. Additionally, the former study reported that CAT activity was lower in tissues of heat stressed plants compared with tissues of optimally grown plants. However, Camejo et al. (2006) reported that the activity of CAT was unchanged in heat sensitive genotypes but markedly increased in tolerant cultivars. This finding can probably be explained by the different growing temperatures used by the researchers, the former experiment used a temperature of 35°C, while the latter used a temperature of 45°C. Total ascorbate was greatly decreased in the sensitive genotype, which was probably contributed by a loss of both reduced and oxidized forms of ascorbate (Camejo et al., 2006). In the heat tolerant tomato cultivars, however, the reduction of total ascorbate was less pronounced. In contrast, the total glutathione content was reduced in the tissues of a heat sensitive cultivar and slightly increased in a heat tolerant cultivar (Camejo et al., 2006).

2.3.3.3 Methylglyoxal detoxification

Methylglyoxal (MG, CH_3COCHO) is a transition-state intermediate of two triose-phosphates, namely, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate of glycolysis system (Yadav et al., 2005a). MG is a side-product of several metabolic pathways; for example, glycolysis and lipid peroxidation (Hoque et al., 2012). It was suggested that methylglyoxal can catalyse oxygen molecules and result in superoxide anion at the photosystem 1 in chloroplast (Hossain et al., 2014). A high level of MG is toxic to cells as MG inhibits cell proliferation. MG toxicity increases protein degradation, adducting with guanyl nucleotide in DNA, and inactivating the antioxidant defence system (Hoque et al., 2012; Mostofa & Fujita, 2013; Yadav et al., 2005a).

MG is detoxified by glyoxalase enzymes using reduced glutathione as a cofactor under normal and stressful conditions (Hoque et al., 2012; Jain et al., 2002; Yadav et al., 2005b). The glyoxalase pathway comprising glyoxalase I (GLOX1, EC 4.4.1.5) and glyoxalase II (GLOX2, EC 3.1.2.6), has been widely studied in microbes and animals but very little is known in plants.

Figure removed subject to copyrights

Figure 2.3.3.g MG detoxification by glyoxalase enzymes (Vivero et al., 2013)

The glyoxalase system plays a significant role in improving plant tolerance against abiotic stresses because the activities of glyoxalse enzymes not only protect plants against MG but also

help to regenerate the glutathione pool, thereby increasing GSH based the detoxification system and decreasing lipid peroxidation (Figure 2.3.3.g) (Hossain et al., 2013).

Studies have reported that the level of MG in plant tissue increased under various stress factors. For example, high levels of MG were reported in both the roots and shoots of rice plants subjected to salt, drought and cold stress (Yadav et al., 2005b). Several studies have demonstrated the role of glyoxalase enzymes in MG detoxification. Hossain et al. (2013) reported that the activity of GLOX1 increased in mustard seedlings under a water deficit when compared with the control plants, but lower activity was observed in GLOX2. The author suggested that inactivation or degradation of proteolytic enzymes was probably a cause of a reduction of GLOX2 activity found in mustard seedlings under drought conditions. Similarly, Yadav et al. (2005b) and Hossain and Fujita (2010) suggested that only GLOX1 was important for detoxifying MG from plant tissue. This conclusion was drawn from a study where the MG level did not increase in response to stress in transgenic tobacco with overexpressing of GLOX1 (Yadav et al., 2005b). A different finding was reported by Mostofa and Fujita (2013). Their study reported that the activity of GLOX2 increased in rice seedlings subjected to copper stress and there were also further increases in the activity of this enzyme after a salicylic acid pre-treatment in the copper stressed rice seedlings. It was proposed that GLOX2 may help to regenerate reduced GSH, although the concentration of this antioxidant was not reported as increasing in parallel to GLOX2.

2.3.3.4 Alcohol dehydrogenase (ADH)

Alcohol dehydrogenase is an enzyme located in the fermentation pathway. Accumulation of ADH in tissues under hypoxia has been reported in many crops, including maize, Arabidopsis (Peng et al., 2001) and rice (Blokhina et al., 2003). Waterlogging of plant roots causes a reduction in cytosolic pH due to the formation of lactic acid during fermentation (Ashraf, 2012; Drew, 1997). The waterlogged plant can switch from lactate production to ethanol fermentation or from aerobic to anaerobic pathways upon a reduction in pH by the enzyme, alcohol dehydrogenase and the inhibition of lactate dehydrogenase (Ashraf, 2012; Drew, 1997; Straeten et al., 1991). ADH is a major anaerobic polypeptide. The presence of ADH is a good indicator of plant exposure to waterlogging (Straeten et al., 1991).

2.3.3.5 Phytochemicals of tomato fruits

Carotenoids

There are more than 600 carotenoids in nature (Etcheverry et al., 2012). About 40 dietary carotenoids are regularly consumed by humans (Kopsell & Kopsell, 2006). Carotenoids are C_{40} isoprenoid polygene compounds that form lipid soluble yellow, orange and red pigments in many fruits and vegetables (Etcheverry et al., 2012; Kopsell & Kopsell, 2006). Research has indicated that carotenoids may have a role in the prevention of certain cancers, heart disease, eye diseases and can act as an immuno-enhancer (Frusciante et al., 2007; Kopsell & Kopsell, 2006). In tomato fruits, 80-90% of the carotenoid content is lycopene (an antioxidant) and about 7-10% is β-carotene, which acts as pro-vitamin A (Frusciante et al., 2007; Rosales et al., 2011). However, Kotikova et al. (2011) reported, on average, lower levels of lycopene (67%) and higher levels of β-carotene (21%) when they assessed carotenoid content. Genotype and environmental factors are the most important determinants affecting carotenoid accumulation (Gorge et al., 2004; Kopsell & Kopsell, 2006). However, little attention has been given to investigating total carotenoid content in tomato fruits harvested from plants under environmental stress. Horchani et al. (2010a) reported that total carotenoids were reduced in the fruit pericarp of waterlogged tomato plants irrespective of the fruit growth stage (matured green, orange or red ripe). The authors suggested that prolonged root hypoxia might not affect all aspects of fruit ripening, although they did observe a reduction in total carotenoids after a period of waterlogging. High temperature can also cause a reduction in carotenoids (which were measured separately as lycopene and β-carotene) in the exocarp (skin) of cherry tomatoes (Rosales et al., 2006). UV-B stress reduced the total carotenoids content in both the skin and pericarp of tomatoes (cv. *Alisa Craig*) at the red-ripe stage (Becatti et al., 2009). A similar finding was observed by Maggio et al. (2007). The study suggested that the total carotenoid contents in tomato fruit were reduced under high temperature and high light intensity.

Total antioxidant activity

An antioxidant is defined as a substance that can protect biological systems from any harmful effects caused by excessive oxidants (Arnao et al., 2001). There are many compounds with this capability. Total antioxidant activity is determined by assessing the capacity of extracted sample compounds taken from the food matrix to suspend the oxidation process within a controlled system (Goerge et al., 2004). Total antioxidant activity is generally measured as the sum of hydrophilic antioxidant and lipophilic antioxidant activity (Rodriguez-Roque et al., 2013; Vallverdu-Queralt et al., 2012). There are differences in the proportions of hydrophilic and lipophilic antioxidant activity in the total antioxidant activity of tomato fruits. Kaur et al.

(2013) reported that hydrophilic or water soluble antioxidants contribute about 78% to 96% of total antioxidant capacity, whereas water insoluble (also termed lipophilic) contributes from 3.4% to 21% of total antioxidant activity. Cano et al. (2003), however, suggested a lower range of hydrophilic antioxidant (71% to 85% of total antioxidant activity). Ascorbic acid and total phenolics are the most predominant hydrophilic antioxidants. Lipophilic antioxidants are mainly attributed to carotenoids (Guil-Guerrero & Rebolloso-Fuentes, 2009; Toor & Savage, 2005). Toor and Savage (2005) reported that the skin of tomato fruits have higher antioxidant activity when compared with the pulp. This finding is in accordance with reports from Chandra et al. (2012) and Ozgen et al. (2012). Similarly, Cano et al. (2003) suggested that total antioxidant activity increased with the progressive fruit ripening stages due to an alteration in the lipophilic antioxidants. Total antioxidant activity can be affected by genetic (cultivar selection), cultural practices and environmental factors (Ozgen et al., 2012). However, to the best of the author's knowledge there has been no research on total antioxidant activity in tomato fruits under water stress (drought and waterlogging).

In vitro assessment of phytonutrient bioavailability

As previously stated, tomato fruits contain many substances, such as carotenoids, phenolics, microelements and vitamins, all of which can benefit human health. Bioavailability of these phytonutrients varies depending on several factors, including species and structure, composition and release from the food matrix, amount consumed and absorption in the intestinal tract (Kopsell & Kopsell, 2006). The term bioavailability can be defined as the fraction of ingested nutrients that can be absorbed, used or stored by the body during normal physiological functions (Etcheverry et al., 2012; Sensoy, 2013). Bioavailability can be determined by using either an *in vivo* or an *in vitro* method. *In vitro* models have gained popularity in research over *in vivo* procedures, possibly because *in vivo* methods are expensive and the values have often been highly variable between subjects (Rodriguez-Roque et al., 2013). *In vitro* models, which are based on human physiology, are cheaper, faster and provide better control of the experiment (Etcheverry et al., 2012). However, these *in vitro* methods cannot be substituted for *in vivo* studies. Etcheverry et al. (2012) suggested that there are four main *in vitro* methods for examining bioavailability of food: solubility, dialysability, gastrointestinal models and Caco-2 cell models. A coupled model using an *in vitro* simulation of gastric and intestinal digestion and a Caco-2 cell culture are frequently seen in recent publications regarding phytonutrient uptake (Kopsell & Kopsell, 2006; Lin et al., 2004). Some of these studies have adapted a three-step process (oral phase, gastric phase and intestinal phase) to

mimic the human digestive system (Figure 2.3.3.h) (Thakkar et al., 2007; Failla et al., 2009). The procedure begins with a preparation of a buffer solution containing salivary α-amylase. The solution should have a pH range between 6.5 and 6.8. The buffer is added to the homogenised food samples or ground plant tissues. The samples and buffer are allowed a 5-10 minute incubation period at 37°C. This step completes the "oral phase" of the procedure. In the gastric phase, porcine pepsin (pH 2 to 2.5) is added and the sample is incubated for an hour at 37°C to mimic the digestion in the human stomach. The intestinal phase involves the addition of pancreatin and bile salts (pH 6.5 to 6.9) to the mixture and a further incubation for two hours at 37°C. Liu et al. (2004) and Reboul et al. (2006), however, have excluded the oral phase from their studies. Once the digesta resulting from the mimic of the human digestive system have been prepared they can be used for examining nutrient uptake and transport using a Caco-2 cell culture.

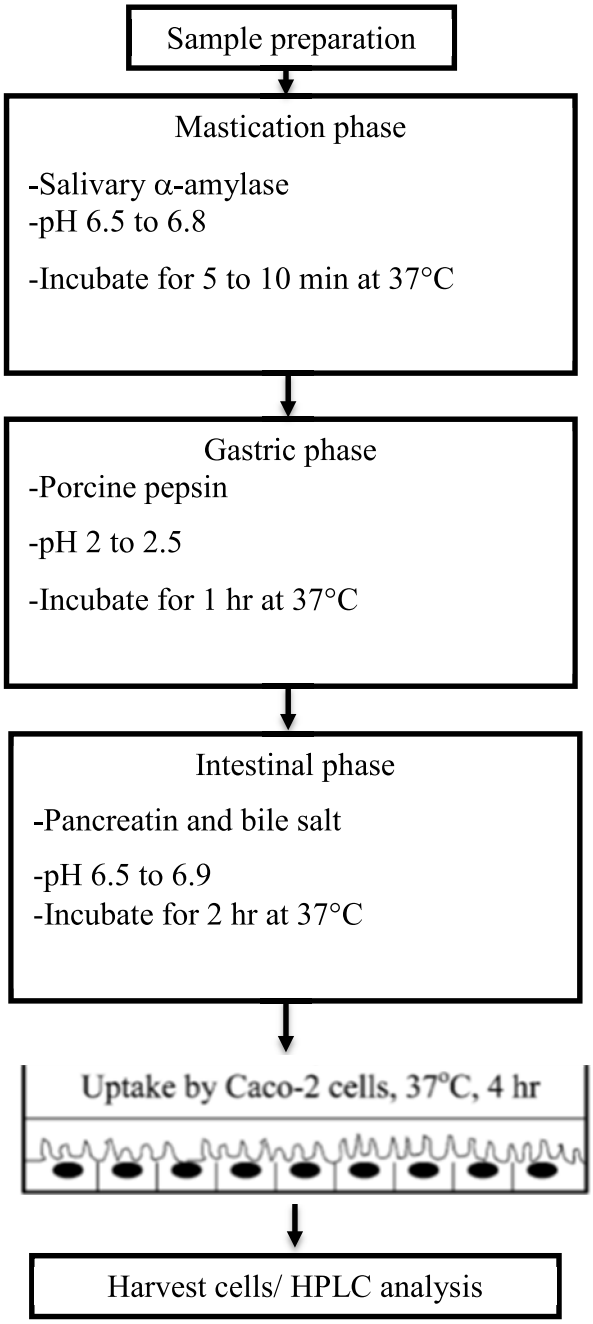


Figure 2.3.3.h A summary of an in vitro simulation gastric and intestinal digestion in couple with Caco-2 cell model adapted from (Liu et al., 2004; Rodriquez-Amaya, 2010)

A Caco-2 cell culture model or human Caucasian colon Adenocarcinoma cells belong to a human epithelial cell line derived from colon cells but these cells perform like intestinal cells in culture (Aherne et al., 2007; Etcheverry et al., 2012). Many Caco-2 cell culture models have been adapted for the samples under investigation. For example, Liu et al. (2004) measured the bioavailability of carotenoids from whole-food carrots and corn using two-day plated cells and two-week plated cells of monolayers of Caco-2 human intestinal cell line. The authors reported that there was no difference between these two different times. It was also suggested that Caco-2 cell uptake of the digesta will take between 2- 8 hours to reach a saturation level. Subsequently, the absorption potential of the sample can be measured via standard HPLC

methods and a prediction of bioavailability can be performed (Kopsell & Kopsell, 2006). There is little known about the bioavailability of the phytonutrients found in harvested tomato fruits taken from plants subjected to water stress. However, Horchani et al. (2010) reported that the amount of total carotenoids decreased in tissue of tomato fruits that developed during prolonged root hypoxia.

Plate 1. Tomato plants grown under water stressed conditions in the glasshouse

Chapter 3

Effects of water stress extremes on the growth and morphology of two tomato cultivars

Abstract

In the natural environment, tomato production might be exposed to extreme events including water deficit or excessive water. This chapter investigated plant growth and morphological responses of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf' at three critical stages of growth including vegetative, flowering and fruiting in the glasshouse and field conditions. The studies showed that most growth related traits were reduced under water stress. For example, water deficit caused a reduction in plant height (-24%), numbers of leaves (-35%) and of branches (-39%), leaf length (-20%) and stem diameter (-15%). Waterlogging also decreased most of these growth traits except in stem diameter, which increased by 9%. Generally, water stress caused a significant reduction in all dry matter traits at the earlier growth stages but increased the percentage of dry matter. The root: shoot ratio increased 2.4-fold under drought stress, but was reduced by 26% in plants subjected to waterlogging in the vegetative and flowering stages of the glasshouse experiment. Both treatments generated substantial plant damage, as shown by a high number of senesced leaves at all growing stages in the glasshouse and the field. Water deficit under glasshouse conditions caused reductions in reproductive characteristics and increased the percentage of dry matter of flowers and fruits. There were several intraspecific differences in water stress responses, suggesting higher stress resistance for the cultivar 'Scoresby Dwarf' compared to 'Best Boy Bush'. In conclusion, water deficit and waterlogging caused a number of similar growth effects but also differential morphological responses, some of which were cultivar-specific.

3.1 Introduction

Water is essential for plant growth and survival. Roots store nutrients, and absorb water and nutrients. An inadequate supply of water for root absorption or, alternatively, an excessive supply of water has negative effects on plants. While an insufficient supply of water will result in a water deficit, excessive amounts of water in the root zone tend to result in oxygen deprivation (hypoxia), reduced aerobic root respiration and reduced water uptake. Growth and morphological attributes can be used as indicators of plant responses to water stress. These attributes may be vegetative or reproductive in nature and may indicate stress responses appropriate to the different stages of growth.

Water deficit or drought is the most common stress factor in reducing crop yield. Tomatoes are sensitive to water deficit particularly in the early vegetative stage of growth and, again, in the reproductive stage of growth (Wudiri & Henderson, 1985). Drought stress can induce morphological, physiological and biochemical changes in plants because of the reduction in

plant tissue water content and water potential (Mishra et al., 2011). It is well documented that waterlogging can lead to a reduction of oxygen in the root zone with water displacing air in the spaces between the soil or media particles (Aloni & Rosenshtein, 1982; Sairam et al., 2008). Aloni and Rosenshtein (1982) suggested that this oxygen deprivation (hypoxia) results in a condition similar to a water deficit, despite the plant standing in water. Therefore, waterlogging and water deficit treatments may have similar effects on plants.

Research has shown that both water stress factors (drought and waterlogging) can cause a reduction in stem length, leaf expansion and the numbers of leaves produced (Aloni & Rosenshtein, 1982; Mishra et al., 2011). In addition, an acceleration in leaf senescence and the abscission of flowers and young fruits was reported in tomato plants subjected to both, drought stress and waterlogging (Aloni & Rosenshtein, 1982; Garcia et al., 2007; Mishra et al., 2011). Water deficit and waterlogging also can cause a reduction in general plant growth due to slower cell division (Horchani et al., 2008; Mahajan & Tuteja, 2005). Petiole epinasty and the formation of adventitious roots are specific responses observed when tomato plants are subject to waterlogging (Horchani et al., 2008; Jackson & Campbell, 1976; Sairam et al., 2008).

Generally, plant root growth is still maintained under a water deficit, allowing continued water absorption even when plant's water potential has dropped to a rate low enough to inhibit shoot growth, thus increasing the root:shoot ratio (Shao et al., 2008; Sharp et al., 2004). However, Torrecillas et al., 1995 reported that the root:shoot ratio of both wild and domesticated species of tomato was not affected by water stress. Furthermore, root growth under waterlogging treatments may be different from the root growth observed for plants subjected to drought stress, according to research by Horchani et al. (2008). These studies reported that the root:shoot ratio of tomato plants was reduced under hypoxic conditions, which may be the result of root cells dying.

Previous studies examined either drought or waterlogging responses in tomato (e.g. Ahsan et al., 2007; Jackson & Campbell, 1976; Sanchez-Rodriguez et al., 2010). However, there has been no research that has looked at these two stress factors as a direct comparative study. Furthermore, there have been no studies of tomato plant responses to these stress factors where the responses have been associated with the three plant developmental growing stages: vegetative, flowering and fruiting. Cultivar origin and location suggests that the cultivar, 'Scoresby Dwarf' (originating from Australia) may be tolerant to extreme environmental conditions such as high temperatures and scarcity of water. 'Scoresby Dwarf' has been used

commercially for about 30 years for field production. The tomato cultivar ‘Best Boy Bush’ is an F1 hybrid with a similar phenotype to 'Scoresby Dwarf', including a bushy growth habit and round shaped fruits. ‘Best Boy Bush’ was presumed to be more sensitive to environmental stress than 'Scoresby Dwarf' as it is a New Zealand home garden cultivar.

The objective of this chapter was to examine the effects of water stress (water deficit and waterlogging) of tomatoes on plant growth and morphological traits at three plant developmental growing stages and under two growing conditions, in the glasshouse and the field. It was hypothesised that both water stress extremes would slow plant growth and caused a reduction in plant biomass as well as a reduction in crop yields. In addition, it was hypothesised that there would be different responses between the two tomato cultivars 'Best Boy Bush' and 'Scoresby Dwarf'. A further aim was to test whether stress responses in these cultivars differed between glasshouse and field conditions.

3.2 Materials and methods

3.2.1 Glasshouse experimental design

This experiment was conducted in a glasshouse at the horticulture nursery at Lincoln University, Canterbury, New Zealand from 30th June to 15th December 2011. The experiment was laid out as a randomised complete block design, with three harvesting schedules, three levels of water stress (well-watered [control], drought and waterlogging) and two tomato cultivars (*Solanum lycopersicon* L. cv. 'Best Boy Bush' and cv. 'Scoresby Dwarf'). The drought treatment was designed to keep plants just above wilting point by withholding a nutrient solution for a period of time, until the plant started wilting, then irrigation was reintroduced (10% of total plant and pot weight every one or two days). The waterlogged plants were submerged in nutrient solution (about 2 cm above the substrate surface) by standing the plant pots inside plastic containers which were a little larger than the tops of the plants. The waterlogging treatment was started at the same time as re-irrigation in the drought treatment. The control plants were kept well-watered with applications of nutrient solutions. The application rate was based on individual determinations of the current total plant and substrate weights and then 25% of that weight was applied as a nutrient solution. Applications were made every one or two days depending on the weather conditions. Each pot contained one tomato plant and there were five replicated pots per treatment, giving a total of 90 pots for the whole experiment. Soil water status was quantified by TDR (See Chapter 4).

3.2.1.1 Plant materials and growth conditions

Tomato cv. 'Scoresby Dwarf' was purchased from Bristol Plants & Seeds (NZ) and tomato cv. 'Best Boy Bush' was purchased from Kings Seeds (NZ) Ltd. The tomato seeds were sown in polystyrene trays using potting mix (80% bark, 20% pumice and osmocote 16-35-0, horticultural lime and hydraflo as wetting agent). The tomato seedlings were grown under natural light conditions in the glasshouse. Five weeks after germination, the seedlings were transplanted into 4 L plastic pots (one seedling per pot), using a hydroponic media of vermiculite and perlite (1:1). Two weeks after transplanting, all plants received daily fertigation with nutrient solution. Average temperatures were 21 °C/17 °C day/night and relative humidity was about 52% (Appendix H).

3.2.1.2 Nutrient solution

All plants were fertigated with a half strength modified Hoagland nutrient solution based on Hoagland and Arnon (1938). This solution included macro nutrients (N 8 mM, K 3 mM, Ca 2 mM, P 1 mM, S 0.5 mM, Mg 0.5 mM), and micro nutrients such as Cl 25 μM, B 12.5 μM, Mn 1 μM, Zn 1 μM, Cu 0.25 μM, Mo 0.25 μM and Fe 8 μM). Because tomato plants required higher potassium and phosphorus nutrient levels for reproductive growth, the amount of K and P were increased to 8 mM and 3 mM, respectively (Sanchez-Rodriguez et al., 2010a).

3.2.1.3 Harvest periods

The experiment was conducted with three separate times of harvesting. Plants were harvested at one of the following growth stages: vegetative development, flowering or fruiting stage. In this experiment, the vegetative stage was at about 13 weeks from seed sowing and before the plants started flowering. The flowering stage was about 17 weeks from seed sowing when the tomato plants had visible buds or open flowers and, in some cases, some fruit set. The last harvest was undertaken when plants were at the fruiting stage which, in this case, was defined as the time when about 90% of plants were carrying one or more red fruits. This final stage was approximately 23 weeks after seed sowing.

First harvest, vegetative stage

Two weeks after transplanting, the drought treatment plants had water withheld until signs of incipient leaf wilting were apparent (20 days). The plants were then re-hydrated with Hoagland solution at a volume equal to 10% of the total pot and medium weights, in order to maintain plant survival and avoid permanent wilting. The fertigation was repeated every one or two days depending on the glasshouse conditions. Control plants continued to receive normal daily fertigation (25% of total pot and medium weight). When the re-hydration of the drought plants began, the waterlogged plants were submerged in Hoagland solution using enclosed plastic containers. The containers holding the waterlogged plants were maintained full of nutrient solution each day. The nutrient solution was replaced every three days. The water-stress treatments continued for two weeks (11-13 weeks after seed sowing).

Second harvest, flowering stage

The imposition of the drought and waterlogging treatments followed the same procedure as described above. The water stress treatment began when the plants reached 50% flowering. The water stress application was maintained for two weeks (15-17 weeks after seed sowing).

Third harvest, fruiting stage

The imposition of the drought and waterlogging treatments followed the same procedure described for the first and second harvest times (see above). The stress was introduced when the majority of the plants had some red fruits, at week 21 after seed sowing. The water stress experiments were carried out over a shorter period (10 days) than the previous growth stages, as it was observed that plants in the fruiting stage had increased sensitivity to water stress.

3.2.2 Field experimental design

This experiment was carried out at the horticulture research area of Lincoln University, Canterbury, New Zealand from 10th October 2012 to 20th March 2013. The design of this experiment was a randomised complete block design, with six harvesting time points, three levels of water stress (well-watered control, drought and waterlogging) and two tomato cultivars ('Best Boy Bush' and 'Scoresby Dwarf'). Each pot contained one tomato plant and there were ten replicated pots per treatment per time point giving a total of 360 plants. In addition, the experimental plants were surrounded by two rows of guard plants to reduce edge effects. The imposition of water stress treatment was started when most of the plants had at least one red ripe tomato fruit. Water was withheld from the drought treatment plants and re-applied (5% of tomato plant weight) to maintain plant survival (just above permanent wilting point). In addition, the tops of the pots holding the drought treatment plants were covered with plastic plates (with a small hole cut for the tomato stem) to divert precipitation water and dew away from the pots. The waterlogged plants were treated by standing the plant pots in slightly larger containers which were filled with water to the same level as the media in the plant pot. The well-watered control plants received water as required (about 25% of total plant and pot weight).

3.2.2.1 Plant materials and growth conditions

Seeds of the tomato cultivars 'Best Boy Bush' and 'Scoresby Dwarf' were sown in polystyrene trays using potting mix (80% bark, 20% pumice and osmocote exact 16-35-0, horticultural lime and hydraflo wetting agent). The tomato seedlings were grown under natural light conditions in the glasshouse. Five weeks after germination, the seedlings were transplanted in 4 L plastic pots (one seedling per pot), using a mixture of fertile soil from the I13 field in the plant science research area, Lincoln University) and sand (1:1) plus osmocote exact 16-35-0 slow-release fertilizer. Two weeks after transplanting into pots, the potted tomato seedlings were transported to the field site. The average maximum temperature during the experiment was 22 °C and the minimum was 11 °C and average rain fall was 30 mm per month (Appendix H).

3.2.2.2 Nutrient solution

From the flowering stage to fruit set, all plants were fertigated with a half strength modified Hoagland nutrient solution based on Hoagland and Arnon (1938), see 3.2.1.2. From fruit development until fruit harvesting, the application of nutrient solution was raised to full strength modified Hoagland solution and, because tomato plants require higher potassium and phosphorus levels for reproductive growth, the amount of K and P were increased to 8 mM and 3 mM, respectively (Sanchez-Rodriguez et al., 2010a).

3.2.2.3 Stress applications

The application of the stress treatments began when all plants had at least one red-ripe fruit. There were six harvesting schedules including Day 0 (before the application of water stress), Day 1 (24 hours after the imposition of water stress), Day 2 (48 hours after the imposition of water stress), Day 3 (72 hours after the imposition of water stress), Day 5 and Day 8. From Day 0, nutrient solution was withheld from drought stressed plants whilst nutrient solution was re-filled daily into containers holding the pots of waterlogged plants so that these plants were always submerged over the stress period.

3.2.2.4 Harvesting activities

Harvesting involved the recording of non-destructive measurements and then samples for various assessments and analysis procedures. All measurements and samples were taken on the same day and in as short a time as possible. Methods of plant growth and morphological measurements are described below:

Growth parameters

Plant growth measurements included counts of leaf, branch, flower and fruit numbers. Plant height was measured from the substrate surface to the top of the vegetative apex. Stem diameter was measured at the stem base above the substrate surface, while the root diameter was measured at the base of the tap root using digital LCD Calipers (Nokia (0) 6 Inch 150 mm Digital LCD caliper Micrometer Vernier Tool, UK Mobi Co., Ltd.). Root length was measured from the base of the tap root to the tip of the root.

Measurement of leaf components

The fresh lamina area of each plant was measured using an AM 300 field portable leaf area meter (OPTI-Sciences, USA). Two laminae per plant were selected for scanning. The laminae were taken from the third fully unfolded leaf. The specific lamina area was calculated from the ratio of lamina area to lamina dry matter (Hofmann & Campbell, 2011). Leaf length was

determined on the second fully unfolded leaf from the leaf tip to the intersection point with the stem. Leaf damage was scored visually on a percentage damage basis. Damage and defects assessed included the appearance of chlorosis, black spots on leaves, as well as leaf abscission. The number of senesced leaves was also counted.

Plant dry matter traits and percentage of dry matter

Plants were harvested and separated into leaves, stems, flowers, fruits and roots. The fresh weight of each was taken and the plant material was then dried at 70°C for 48 h to determine the dry matter (DM). The root to shoot ratio was calculated using the root dry matter and the above ground plant dry matter. Dry matter contents of leaves, stems, flowers, fruits, roots and of the total plant were measured by calculating the percentage of dry matter [PDM, (dry matter/fresh matter) x 100] (Hofmann & Campbell, 2011).

3.3 Statistical analyses

3.3.1 Analysis

Statistical analyses were conducted with the General Analysis of Variance (ANOVA) procedure in Genstat 14, to examine the main effects and their interactions. In addition to the overall interaction probabilities from ANOVA, comparisons between the interactions of the means were based on Tukey's 95% confidence intervals calculated in Genstat 14. If required, data were log-transformed to satisfy the homogeneity of variance assumptions in ANOVA. Where stated, the interactions LSDs at $P < 0.05$ were also used for this purpose in some cases. This LSD can still reveal significant differences among some means, even though the overall interaction term may be non-significant (Saville, 2003). All multiple comparisons were based on Tukey's 95% confidence intervals. Only significant results are presented.

Principal component analysis (PCA) was employed to further investigate the multiple comparisons of all traits. The PCA was performed in the multivariate analysis function in Genstat 14. The PCA scores were then used to conduct multiple comparisons and generate the PCA biplot. Based on the latent vectors and corresponding correlation comparisons, traits with significant correlations with the two principal dimensions were grouped at the end of each principal component, PC1 (x-axis) and PC2 (y-axis). Traits that associated with both principal components were placed at 45 degree angles between the axes. The PCA biplot of the effects of water deficit and waterlogging on plant growth and morphology was based on the relative changes induced by each of the two water treatments (ratios of water treatment: control) in the two cultivars at three developmental stages (Ballizany et al., 2012). Only attributes that showed significant correlations with either PC1 and/or PC2 are presented in the PCAs.

3.3.2 Data presentation

For conciseness, tables are used to show the statistical summaries of all main effects and their interactions with the water treatments, followed by corresponding tables showing the significant treatment-induced changes. These responses are then summarised in the text to assist readability. Results from the drought and from the waterlogging treatments are each expressed as percentage changes compared to the control (well-watered) plants. Again for conciseness, only significant results and representative graphs will be presented in this chapter. Supplementary information can be found in Appendix C, whereas the raw data are shown in Appendix A and B.

3.4 Results of the glasshouse studies

3.4.1 Plant growth and morphology

3.4.1.1 Main effects

Relative to the control (well-watered plants), growth related traits including plant height, leaf number, leaf length, branch number, root length, root diameter and SLA were all decreased in drought stressed plants (5-39%) and in plants subjected to waterlogging (5-15%) (except root length and diameter) (Table 3.4.1.a, b, Figure 3.4.1.a, b, c, d and Appendix C). Stem diameter was decreased by 15% under water deficit but increased by 9% under waterlogging. Using the well-watered control plants as a comparison, there was more damage and number of senesced leaves observed in plants subjected to drought (3.1- 6.2-fold) and waterlogging (3.2- 7.9-fold).

Table 3.4.1.a Summary of P values of the main effects and interactions with water treatment for plant morphology attributes in tomatoes

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage	Water x Cultivar x Devel. stage
Height (cm)	<.001	<.001	<.001	0.300	<.001	0.116
Leaf number	<.001	<.001	<.001	0.223	<.001	0.535
Leaf length	<.001	<.001	<.001	0.740	<.001	0.106
Branch number	<.001	<.001	<.001	0.358	<.001	0.688
Stem Diameter (mm)	<.001	<.001	<.001	0.640	<.001	0.859
Root length	0.018	0.238	<.001	0.756	0.192	0.368
Root diameter (mm)	<.001	<.001	<.001	0.485	<.001	0.196
Lamina Area (mm^{-2})	0.003	0.001	<.001	0.513	<.001	0.910
Specific lamina area	0.001	<.001	<.001	0.323	<.001	0.104
% Leaf damage	0.001	0.103	<.001	0.248	<.001	0.461
Senesced leaves	<.001	0.797	<.001	0.157	0.014	0.925

Raw data are shown in Appendix A

Averaged across the water treatments, 'Scoresby Dwarf' was 23% shorter in plant height, 9% shorter in leaf length, and 7% smaller in lamina area than 'Best Boy Bush' (Table 3.4.1.a, b, Figure 3.4.1.a, b, c, d and Appendix C). However, 'Scoresby Dwarf' had a higher number of leaves (42%), branches (28%) and SLA (7%) compared to 'Best Boy Bush'. Likewise, both the stem and root diameters of 'Scoresby Dwarf' were 17% and 13% wider than 'Best Boy Bush'.

Table 3.4.1.b Summary of percentage changes of the main effects and interactions with water treatment for plant growth and morphology

Traits	Water	Cultivar	Devel. stage	Water x Devel. stage	
Height (cm)	Dr: -24% WL: -9%	SBD: -23%	H2: 72% H3: 2.4x	Dr H1: -46% Dr H2: -33%	WL H2: -12% WL H3: -8%
Leaf number	Dr: -35% WL: -15%	SBD: 42%	H2: 2.3x H3: 3.8x	Dr H1: -50% Dr H2: -53% Dr H3: -16%	WL H2: -19% WL H3: -32%
Leaf length	Dr: -20% WL: -8%	SBD: -9%	H3: 9%	Dr H2: -36% Dr H3: -18%	WL H2: -13% WL H3: -12%
Branch number	Dr: -39% WL: -13%	SBD: 28%	H2: 84% H3: 80%	Dr H1: -83% Dr H2: -30% Dr H3: -14%	WL H2: -15% WL H3: -13%
Stem Diameter (mm)	Dr: -15% WL: 9%	SBD: 17%	H2: 16% H3: 23%	Dr H1: -28% Dr H2: -8% Dr H3: -11%	WL H1: 19% WL H2: 9%
Root length	Dr: -9% WL: -8%	ns	H2: 15% H3: 18%	ns	
Root diameter (mm)	Dr: -18%	SBD: 13%	H2: 22% H3: 20%	Dr H1: -34% Dr H2: -15%	WL H2: 17% WL H3: -10%
Leaf area (mm^{-2})	WL: -4%	SBD: -7%	H2: -5%	Dr H2: -9%	WL H1: -6% WL H3: -4%
SLA (mm^{-2} mg^{-1})	Dr: -5% WL: -5%	SBD: 7%	H2: 5% H3: 13%	Dr H2: -15%	WL H2: -11%
% Leaf damage	Dr: 6.2x WL: 7.9x	ns	H2: 73% H3: 2x	Dr H1: 4.5x Dr H2: 8.2x Dr H3: 6.9x	WL H2: 10.2x WL H3: 16.7x
Senesced leaves	Dr: 3.1x WL: 3.2x	ns	H2: 2.4x H3: 86%	Dr H1: 3.5x Dr H2: 4.4x Dr H3: 2x	WL H1: 2.5x WL H2: 5.1x WL H3: 2.x

Drought (Dr), waterlogging (WL), 'Scoresby Dwarf' (SBD), 'Best Boy Bush' (BBB), developmental stage (H, harvest 1: vegetative, 2: flowering, 3: fruiting), non- significant (ns), fold (x)

Relative to vegetative stage, attributes that related to plant size all increased at flowering, (from 5% for SLA to 2.3-fold for leaf number) and further increased at the fruiting stage (from 9% for leaf length to 3.8-fold for leaf number) (Table 3.4.1.a, b, Figure 3.4.1.a, b, c, d and Appendix

C). Plant damage and number of senesced leaves were also increased as plants moved from the vegetative stage to flowering (73%-2.4-fold) and from flowering to the fruiting (86%-twofold) stage of growth.

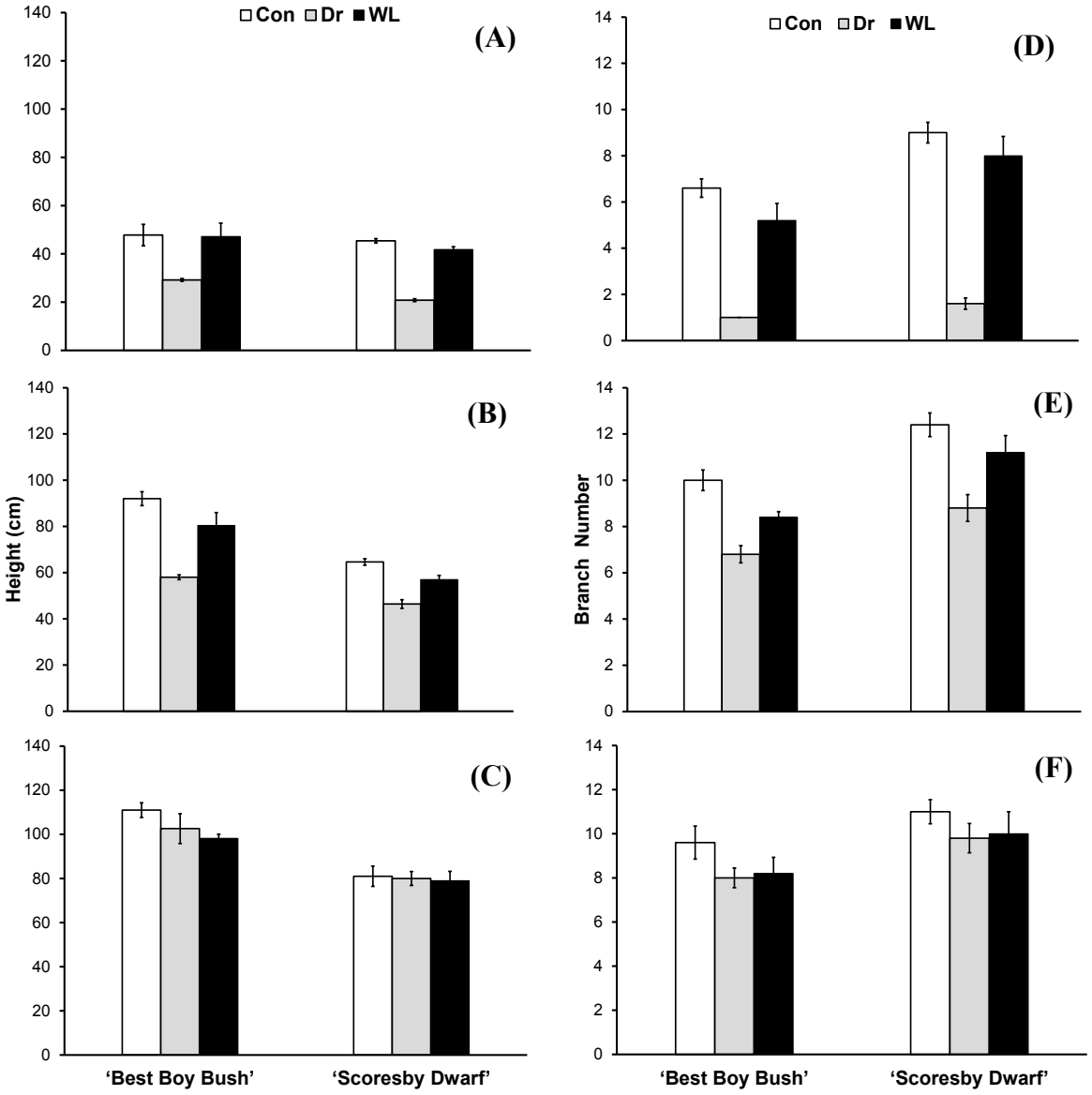


Figure 3.4.1.a Plant height at the vegetative (A), flowering (B) and fruiting (C) stages, and branch number at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

3.4.1.2 Interaction effects

Water x Cultivar

Whilst overall no significant water x cultivar interactions were observed in Anova (Table 3.4.1.a, b, Figure 3.4.1.a, b, c, d and Appendix C), Tukey's 95% confidence intervals showed that the lamina area of 'Best Boy Bush' was 4% lower for plants subjected to waterlogging and the SLA of this cultivar was also 7% smaller for plants subjected to water deficit.

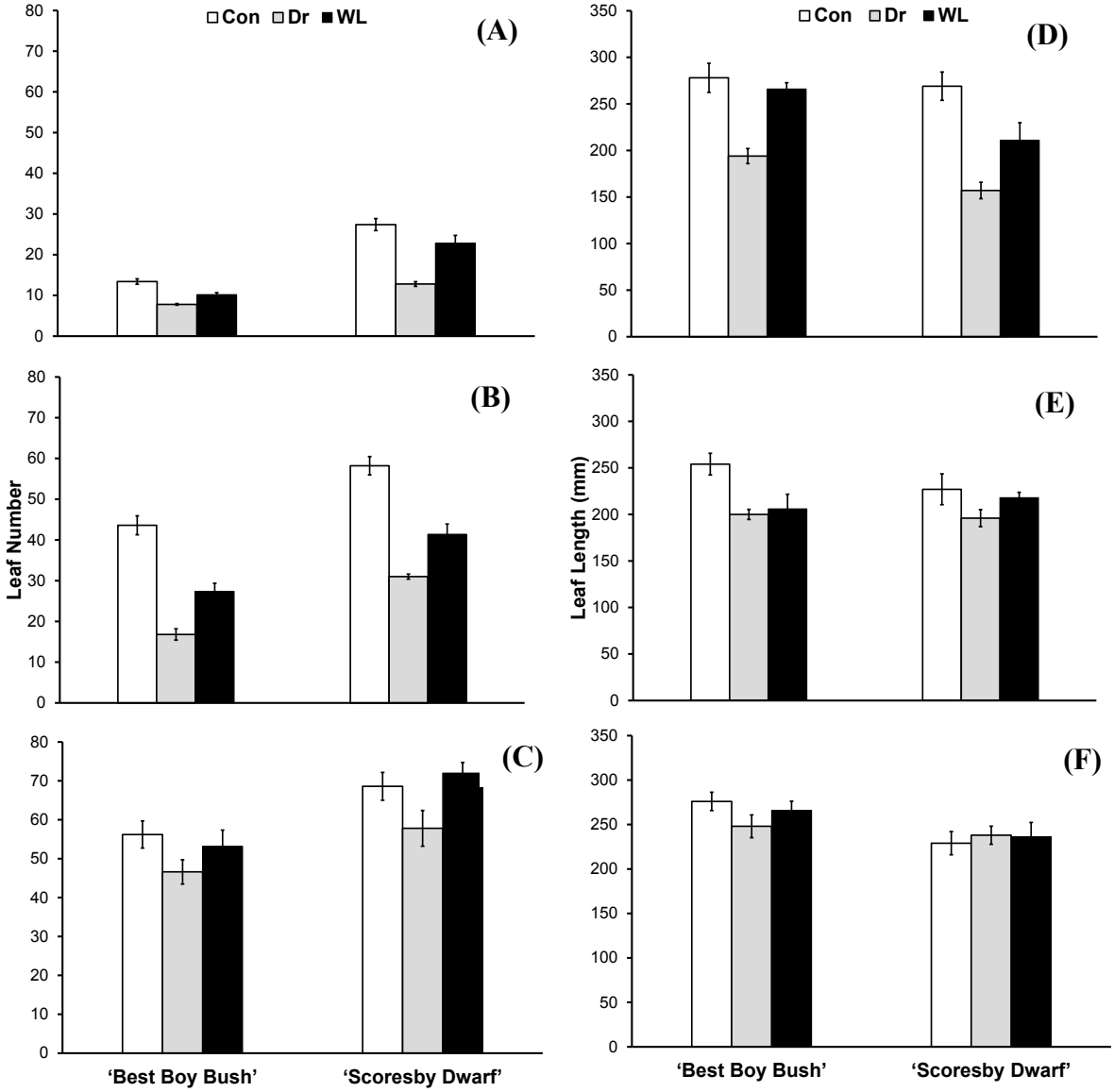


Figure 3.4.1.b Leaf number at the vegetative (A), flowering (B), fruiting (C) stages and leaf length at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

Water x Developmental stage

Averaged across cultivars, drought in the vegetative stage caused reductions in most growth attributes of the tomato plants (plant height, leaf number, branch number, stem diameter and root diameter) by 28-83% (Table 3.4.1.a, b, Figure 3.4.1.a, b, c, d and Appendix C). Waterlogging on the other hand, only caused a small reduction in LA (-6%) and increased the stem diameter by 19%. Similar results were found at the flowering stage of growth: drought decreased all of the plant growth attributes between 8%-53%. Plants subjected to waterlogging at the flowering stage decreased plant height, leaf number, leaf length and branch number by 12-19% but increased diameters of the stem (9%) and root (17%). At the fruiting stage, only leaf number, branch number and stem diameter were reduced (11-16%) under drought stress. At the fruiting stage waterlogging decreased (4-32%) several growth traits including plant height, leaf length, number of leaves and branches, LA and root diameter. At the three developmental stages, plants subjected to water deficit exhibited strong increases in leaf damage (4.5-8.2-fold) and number of senesced leaves (2- 4.4-fold) relative to control plants. Similar increases in the number of senesced leaves (2- to 5.1-fold) were found in hypoxic plants at the three developmental harvests, whilst increases in leaf damage were most pronounced under waterlogging at the flowering and fruiting stages of plant development (10.2- to 16.7-fold).

3.4.1.3 Summary of the key findings

- Extremes of water stress caused reductions in most growth and morphological traits.
- These effects were most pronounced under drought and within that treatment particularly so at the earlier developmental stages for plant height, leaf and branch numbers, stem and root diameter.
- Water stress strongly induced leaf senescence and leaf damage, and even more so under waterlogging at the later developmental stages.

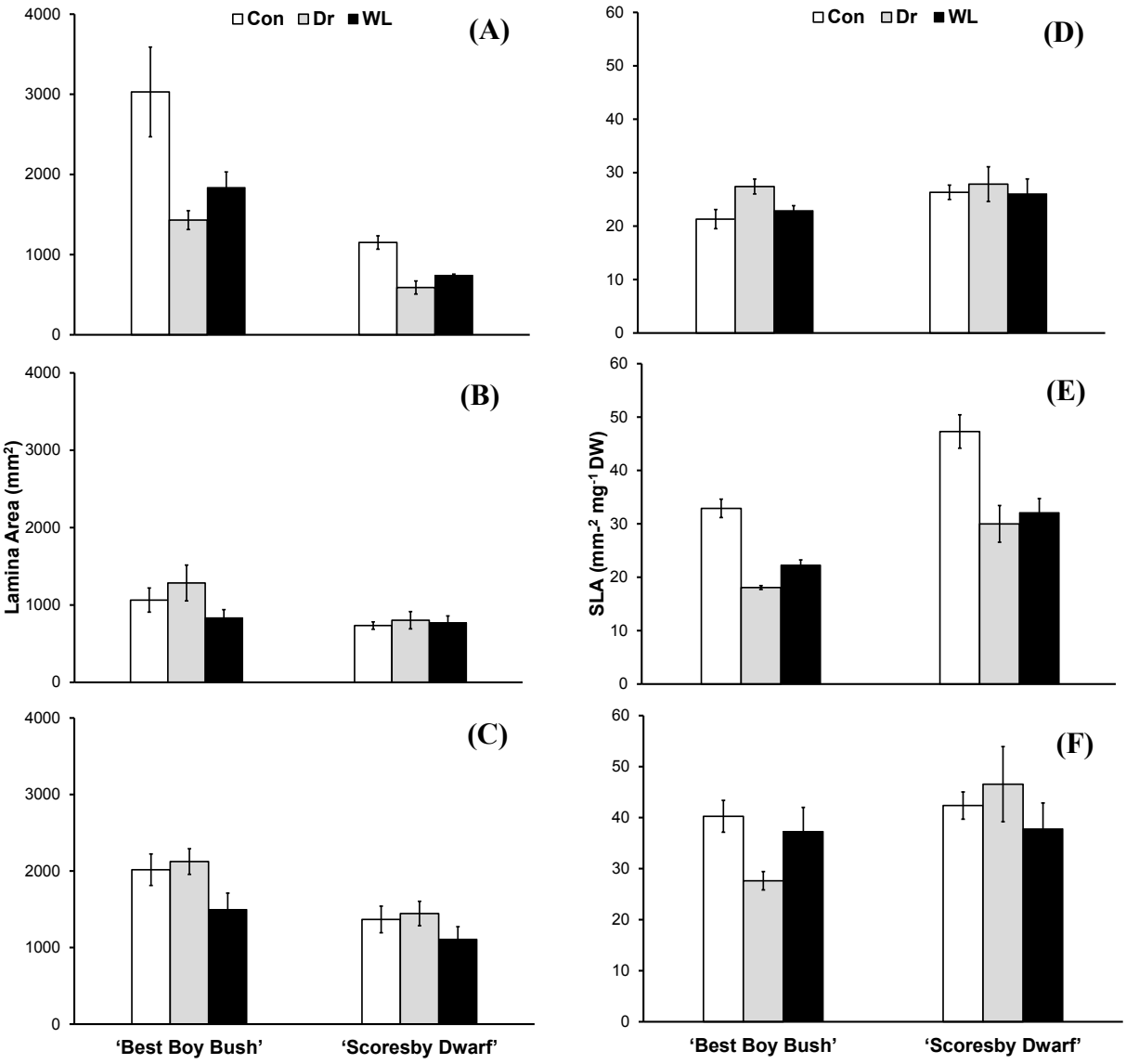


Figure 3.4.1.c Lamina area (LA) at the vegetative (A), flowering (B), fruiting (C) stages and specific lamina area (SLA) at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

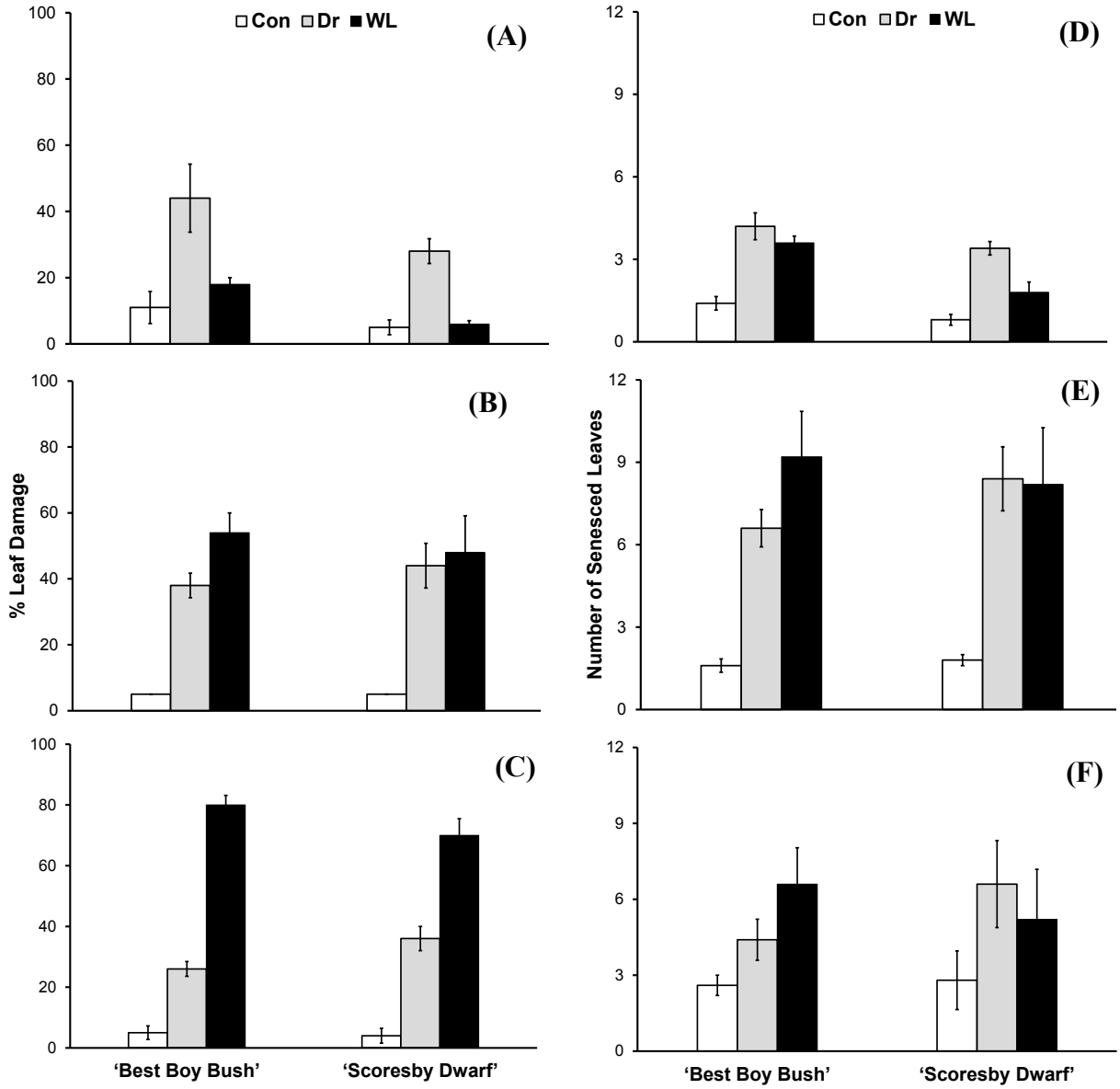


Figure 3.4.1.d % Leaf Damage at the vegetative (A), flowering (B), fruiting (C) stages and number of senesced leaves at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

3.4.1.4 Development of adventitious roots

A few days after exposure to waterlogging, tomato plants at the vegetative growth stage had developed secondary roots at the stem base (Figure 3.4.1.e). These adventitious aerial roots were clearly visible above the surface of water and became bigger and longer over time during the vegetative stage. There was little formation of such roots at the flowering and fruiting stages.

Figure 3.4.1.e Adventitious root formation in plants subjected to waterlogging at the vegetative (A) and fruiting (B) stages.

3.4.2 Plant dry matter traits

3.4.2.1 Main effects

Averaged across cultivar and developmental stages, water stress decreased nearly all the components of plant dry matter (DM of leaves, stems, roots and of the whole plant) by 13-33% under drought stress and by 6-33% under waterlogging (except total plant DM) (Table 3.4.2.a, b, Figure 3.4.2.a, b, c and Appendix C). The root: shoot ratio of well-watered control plants was 2.4 times that of drought stressed plants, and 26% higher than that of the waterlogged plants. The percentage of dry matter in the plant and in various plant organs, on the other hand, were all observed to increase under both water deficit (22-89%) and waterlogging (17-89%).

Table 3.4.2.a Summary of P values of the main effects and interactions with water treatment for plant dry matter traits and % dry matter traits

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage	Water x Cultivar x Devel. stage
Leaf DM (g plant^{-1})	<.001	0.023	<.001	0.011	<.001	0.002
Stem DM (g plant^{-1})	<.001	<.001	<.001	0.107	<.001	0.092
Root DM (g plant^{-1})	<.001	0.049	<.001	0.214	0.014	0.702
Plant DM (g plant^{-1})	<.001	0.302	<.001	0.125	<.001	0.393
Root: shoot	<.001	<.001	<.001	0.017	<.001	0.521
Leaf PDM	<.001	<.001	<.001	0.103	<.001	0.352
Stem PDM	<.001	<.001	0.002	0.448	<.001	0.206
Root PDM	<.001	0.003	<.001	0.613	0.064	0.431
Plant PDM	<.001	<.001	<.001	0.182	<.001	0.061

Raw data are shown in Appendix A

Average across water treatment and developmental stages, plant DM and PDM attributes were all lower (6-15%) in 'Scoresby Dwarf' relative to 'Best Boy Bush' (Table 3.4.2.a, b, Figure 3.4.2.a, b, c and Appendix C).

Table 3.4.2.b Summary of percentage change of the main effects and interactions with water treatment for plant dry matter traits

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage		Water x Cultivar x Devel. stage
Leaf DM (g plant-1)	Dr: -33% WL: -25%	SBD: -6%	H2: 2.1x H3: 4.8x	Dr BBB: -34% Dr SBD: -33% WL BBB: -31% WL SBD: -19%	Dr H1: -82% Dr H2: -55%	WL H1: -34% WL H2: -35% WL H3: -16%	**Dr BBB** H1: -82%, H2: -60% **Dr SBD** H1: -82%, H2: -49% **WL BBB** H1: -35%, H2: -37%, H3: -26% **WL SBD** H1: -33%, H2: -33%
Stem DM (g plant-1)	Dr: -28% WL: -6%	SBD: -10%	H2: 91% H3: 2.4x	ns	Dr H1: -78% Dr H2: -25%	WL H2: -8%	ns
Root DM (g plant-1)	Dr: -13% WL: -33%	SBD: -11%	H3: 2.4x	ns	Dr H1: -45%	WL H1: -47% WL H2: -53%	ns
Plant DM (g plant-1)	Dr: -17%	ns	H2: 34% H3: 87%	ns	Dr H1: -39% Dr H2: -19%	WL H1: -11% WL H2: -10%	ns
Root: shoot	Dr: 2.4x WL: -26%	SBD: -13%	H2: -66% H3: -85%	Dr BBB: 2.4x Dr SBD: 2.3x WL BBB: -29% WL SBD: -24%	Dr H1: 2.8x Dr H2: 2x	WL H1: -28% WL H2: -32%	ns
Leaf PDM	Dr: 89% WL: 89%	SBD: -12%	H2: 18% H3: 31%	ns	Dr H1: 2.1x Dr H2: 2x Dr H3: 58%	WL H2: 89% WL H3: 2.7x	ns
Stem PDM	Dr: 34% WL: 24%	SBD: -9%	H3: 12%	ns	Dr H1: 45% Dr H2: 31% Dr H3: 27%	WL H1: 15% WL H2: 17% WL H3: 39%	ns
Root PDM	Dr: 22% WL: 17%	SBD: -9%	H3: 21%	ns	ns		ns
Plant PDM	Dr: 28% WL: 22%	SBD: -15%	H2: 20%	ns	Dr H1: 18% Dr H2: 48% Dr H3: 17%	WL H2: 45% WL H3: 14%	ns

Drought (Dr), waterlogging (WL), 'Scoresby Dwarf' (SBD), 'Best Boy Bush' (BBB), developmental stage (H, harvest 1: vegetative, 2: flowering, 3: fruiting), non- significant (ns), fold (x)

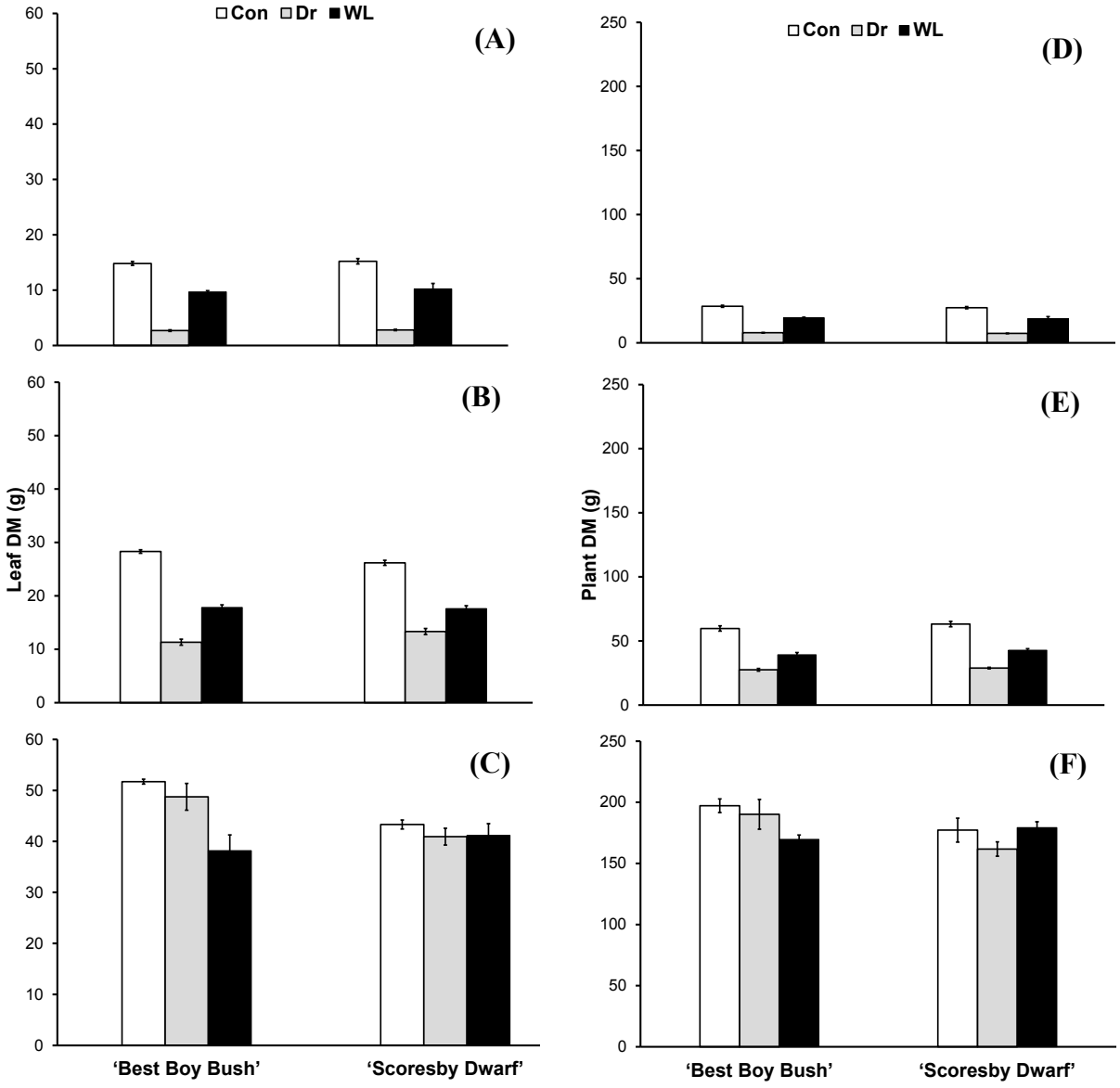


Figure 3.4.2.a Leaf dry matter at the vegetative (A), flowering (B), fruiting (C) stages and total plant dry matter at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

As plants aged and moved from the vegetative stage of growth to the flowering and fruiting stages, dry matter attributes and percentage of dry matter (PDM) attributes all increased except for the root: shoot ratio. For example, dry matter traits increased by 34%-2.1-fold at the flowering stage and a greater increase was observed at the fruiting stage (87%-4.8-fold) (Table 3.4.2.a, b, Figure 3.4.2.a, b, c and Appendix C). A similar picture was measured for PDM attributes which increased by 9-20% at the flowering stage and by 6-31% at the fruiting stage. Root:shoot ratio, on the other hand, decreased at later developmental stages by 66% to 85%.

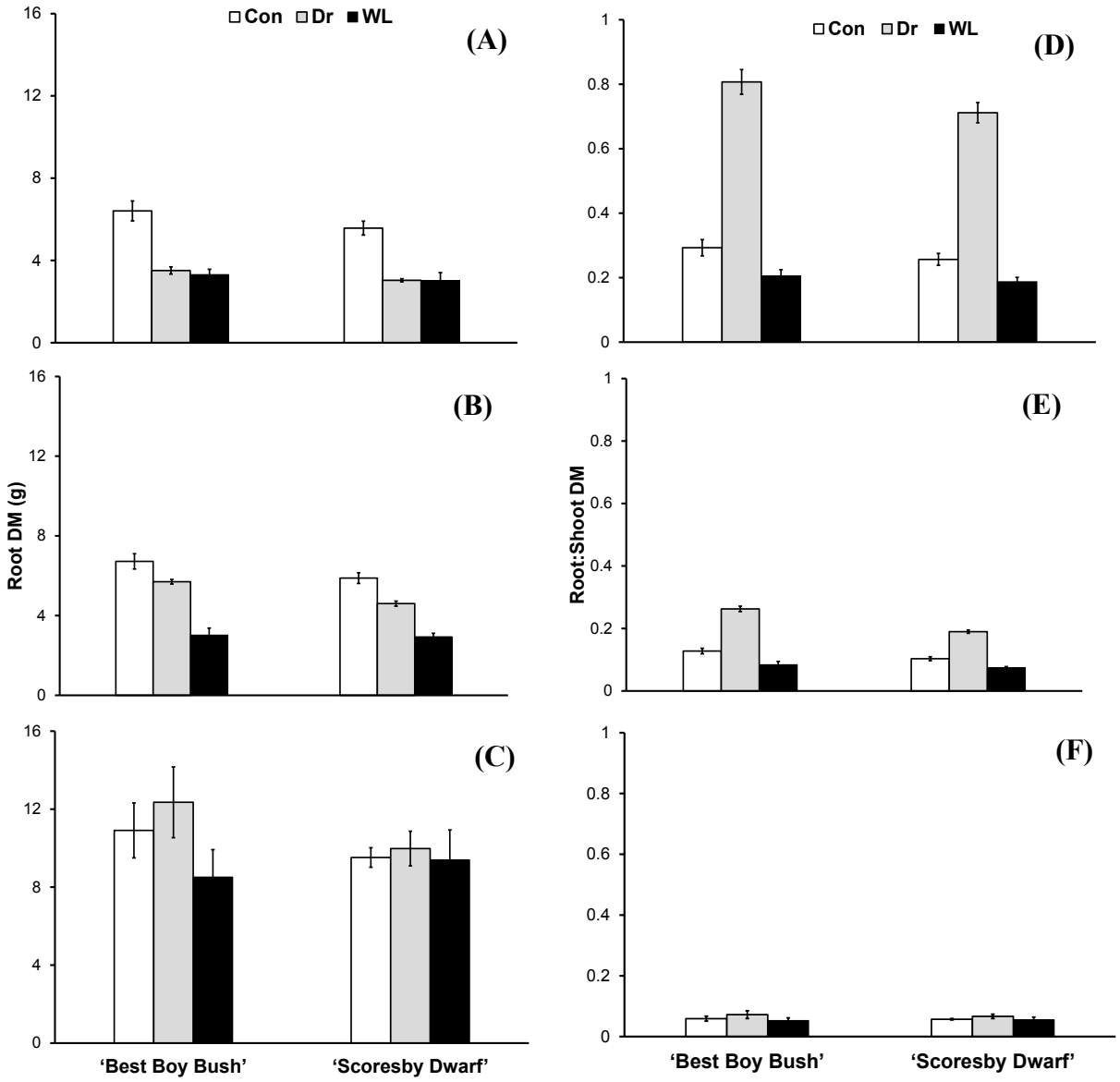


Figure 3.4.2.b Root dry matter at the vegetative (A), flowering (B), fruiting (C) stages and root:shoot ratio at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

3.4.2.2 Interaction effects

Water x Cultivar

Averaged across developmental stages, leaf DM was reduced to a similar degree (-31 to -34%) both cultivars under drought and in 'Best Boy Bush' under waterlogging, whilst in 'Scoresby Dwarf' it was only reduced by 19% in the latter treatment (Table 3.4.2.a, b, Figure 3.4.2.a, b, c and Appendix C). Stem DM decreased by 28% for each cultivar under water deficit, whilst under waterlogging a reduction was only observed in 'Best Boy Bush' (-9%). The root: shoot ratio on the other hand, increased in both cultivars (2.3- 2.4-fold) under water deficit, whereas

plants under waterlogging it decreased by nearly 30% in 'Best Boy Bush' and by 24% in 'Scoresby Dwarf'.

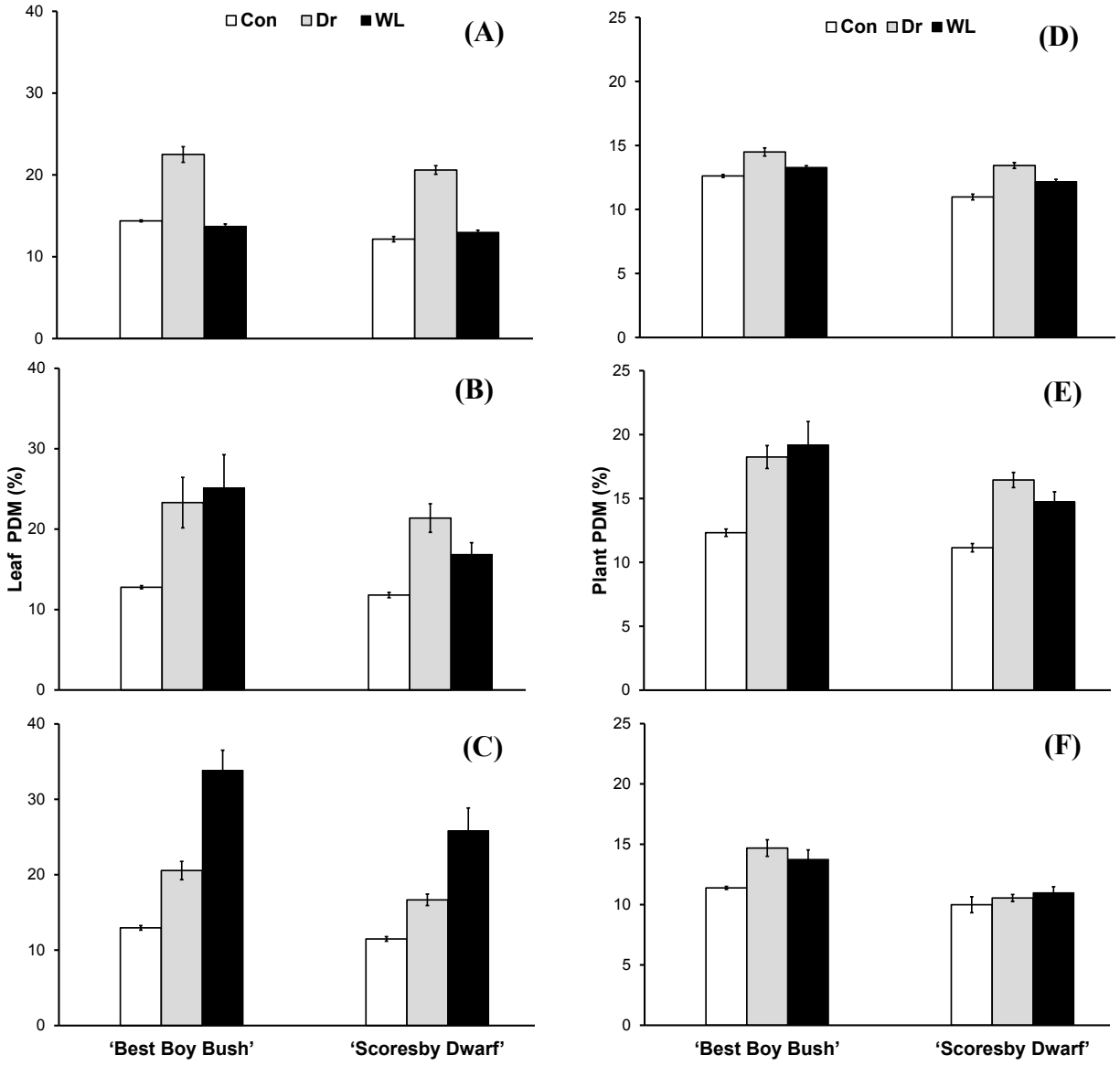


Figure 3.4.2.c Leaf PDM at the vegetative (A), flowering (B), fruiting (C) stages and Plant PDM at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

Water x Developmental stage

Averaged across cultivars, plants in the vegetative growth stage had reduced DM attributes under drought (-39 to -82%), compared to reductions of 11-47% under hypoxia (Table 3.4.2.a, b, Figure 3.4.2.a, b, c and Appendix C). At flowering, DM attributes were lowered both water deficit (-19 to -55%) and by waterlogging (-8 to -53%). At fruiting, only leaf DM was lowered by 6% and 16% when the plants had been drought stressed and waterlogged respectively. The root: shoot DM, on the other hand, was between twofold (flowering) to 2.8-fold (vegetative) higher for plants subjected to drought but about 30% lower for plants subjected to hypoxia in the earlier growth stages. Relative to the well-watered plants, all the PDM components except root PDM increased (17%- twofold) at all developmental stages of the water deficit plants. PDM of hypoxic plants, on the other hand was mostly increased (14% to 2.7-fold) at later development stages (flowering and fruiting).

Water x Cultivar x Developmental stage

The only three-way interaction was observed for leaf DM, which decreased by 26% in waterlogged 'Best Boy Bush' plants at fruiting, with no change for 'Scoresby Dwarf' at that developmental stage (Table 3.4.2.a, b, Figure 3.4.2.a, b, c and Appendix C).

3.4.2.3 Summary of the key findings

- Water stress decreased all the components of dry matter but the reductions were more pronounced in plants subjected to drought stress, except for root DM. These dry matter components increased with developmental stages (higher at flowering and fruiting stages).
- Root: shoot ratios increased in plants subjected to drought stress but decreased in plants subjected to waterlogging and with developmental stages.
- Both extremes in water stress increased dry matter percentages but these increases were slightly less pronounced in waterlogged plants.
- 'Scoresby Dwarf' had less dry matter and percentage of dry matter relative to 'Best Boy Bush'.
- There were some indications of higher stress resistance in 'Scoresby Dwarf', with maintenance of leaf DM under waterlogging at the fruiting stage.

3.4.3 Reproductive components

Table 3.4.3.a Summary of P values of the main effects and interactions with water treatment for plant reproductive components

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage	Water x Cultivar x Devel. stage
Flower number	<.001	<.001	<.001	0.445	<.001	0.691
Fruit number	0.002	<.001	<.001	0.341	0.002	0.194
Flower and Fruit DM (g plant^{-1})	<.001	<.001	<.001	0.289	<.001	0.425
Flower and Fruit PDM	<.001	<.001	<.001	0.490	<.001	0.270

Raw data are shown in appendix A

3.4.3.1 Main effects

Averaged across cultivars and developmental stages, plants subjected to water deficit had 19%-35% lower values for plant reproductive attributes including the numbers of flowers and of fruits, as well as flower and fruit DM, (Table 3.4.3.a, b, Figure 3.4.3a, b and Appendix C). Waterlogging, on the other hand, only reduced the number of flowers (-20%) and both water stress extremes increased flower and fruit PDM by 8%-13%.

Table 3.4.3.b Summary of percentage change of the main effects and interactions with water treatment for plant reproductive components

Traits	Water	Cultivar	Devel. stage	Water x Devel. stage	
Flower number	Dr: -35% WL: -20%	SBD: 58%	H3: -59%	Dr H3: -45%	WL H3: -18%
Fruit number	Dr: -19%	SBD: 44%	H3: 3x	Dr H3: -59%	WL H3: -25%
Flower and Fruit DM (g plant-1)	Dr: -29%	SBD: 55%	H3: 7x	Dr H3: -2.2x	WL H3: -37%
Flower and Fruit PDM	Dr: 13% WL: 8%	SBD: -13%	H3: -17%	Dr H3: 23%	WL H3: 16%

Drought (Dr), waterlogging (WL), 'Scoresby Dwarf' (SBD), 'Best Boy Bush' (BBB), developmental stage (H, harvest 1: vegetative, 2: flowering, 3: fruiting), non- significant (ns), fold (x)

In the averaged values across both water treatments and developmental stages, 'Scoresby Dwarf' had more 44%-58% more flowers, fruits and dry matter of flowers and fruits (55%), but less PDM than 'Best Boy Bush' (Table 3.4.3.a, b, Figure 3.4.3.a, b and Appendix C).

Relative to the flowering stage, plants at fruiting carried a lower number of flowers (-59%) but much higher number of fruits (threefold) and therefore increased flower and fruit DM by seven-

fold but slightly decreased flower and fruit PDM (-17%) (Table 3.4.3.a, b, Figure 3.4.3.a, b and Appendix C).

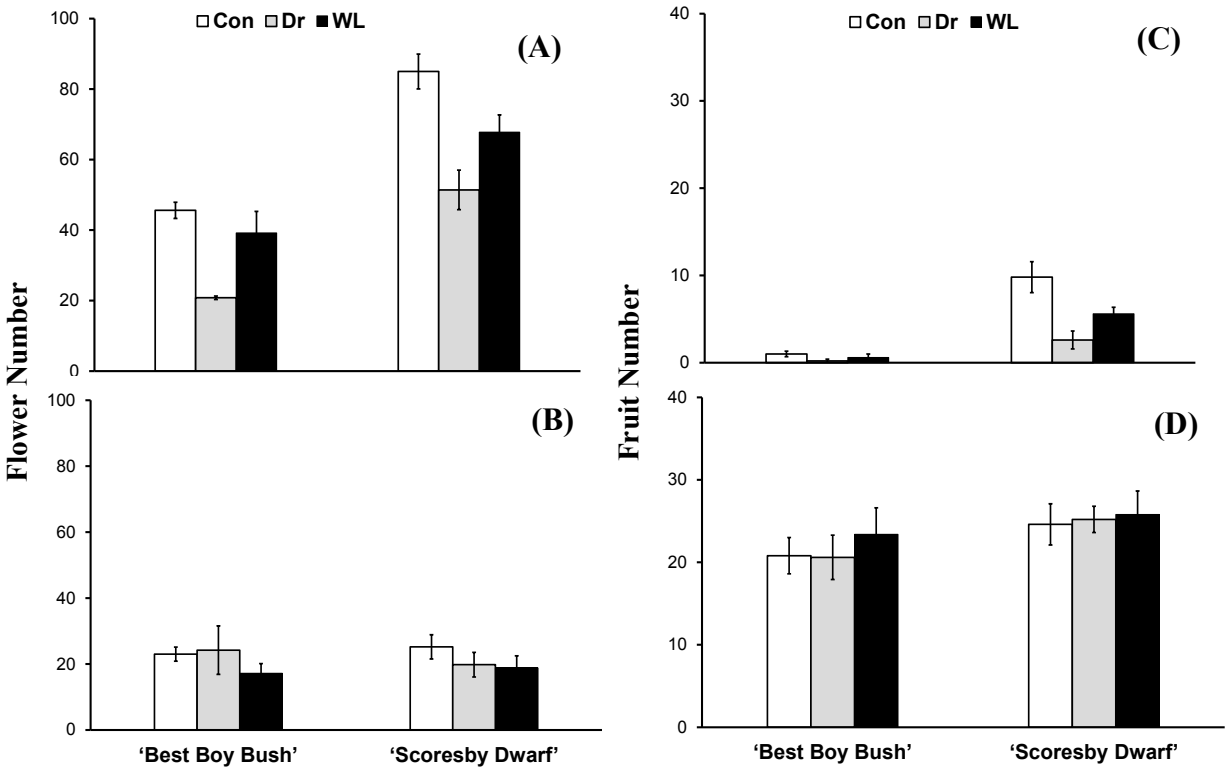


Figure 3.4.3.a Numbers of flowers at the flowering (A), fruiting (B) stages and number of fruits at the flowering (C) and fruiting (D) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

3.4.3.2 Interaction effects

Water x Developmental stage

Averaged across cultivars, reproductive components (flower number, fruit number, flower and fruit DM) at fruiting were reduced under both treatments, water deficit (45%-2.2-fold) and waterlogging (18%-37%) (Table 3.4.3.a, b, Figure 3.4.3.a, b and Appendix C). Flower and fruit PDM, on the other hand, was higher for plants that were subject to either water stress treatment, water deficit (23%) or excessive water (16%).

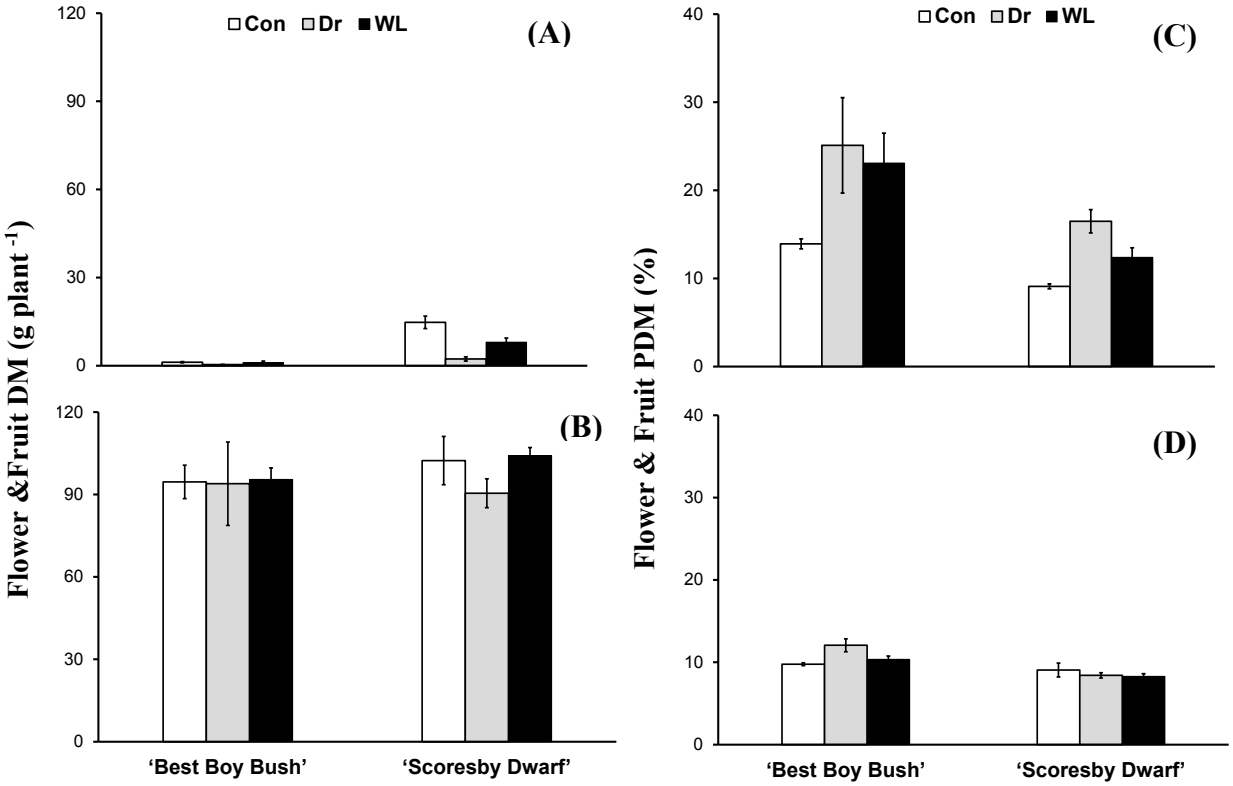


Figure 3.4.3.b Flower and fruit DM at the flowering (A), fruiting (B) stages and flower and fruit PDM at the flowering (C) and fruiting (D) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

3.4.3.3 Summary of the key findings

- Water deficit had a stronger effect than waterlogging on decreasing the number of flowers, number of fruits and dry matter of flowers and fruits and on increasing the fruit and flower dry matter percentages.
- 'Scoresby Dwarf' had more flowers and fruits and higher dry matter of flowers and fruits but less PDM relative to 'Best Boy Bush'.

3.5 Results of the field studies

3.5.1 Plant growth and morphology

3.5.1.1 Main effects

Averaged across cultivars, water deficit caused a reduction in the number of leaves by 17%, whilst waterlogging decreased leaf number, root length and SLA by 10%-29% (Table 3.5.1.a, b and Figure 3.5.1.a, b and Appendix C). Water stress increased leaf damage and senescence nearly 4-fold, whereas under waterlogging, leaf damage increased by 69%. Averaged across water treatments, 'Scoresby Dwarf' was 32% shorter but had 34% more leaves and 43% lower lamina area.

Table 3.5.1.a Summary of P values of the main effects and interactions with water treatment for plant and morphology

Traits	Water	Cultivar	Water x Cultivar
Plant height (cm)	0.678	<.001	0.019
Leaf number	0.006	<.001	0.711
Node number	0.586	0.431	0.777
Stem diameter (mm)	0.104	0.040	0.422
Root diameter (mm)	0.071	0.306	0.441
Root length	0.041	0.742	0.779
LA (mm^{-2})	0.053	<.001	0.615
SLA (mm^{-2} mg^{-1})	0.042	0.103	0.278
% Leaf Damage	<.001	0.083	0.220
Number of senesced leaves	<.001	0.057	0.437

Raw data are shown in Appendix B

3.5.1.2 Interaction effects

There no water treatment x cultivar interactions, except for a small 9% decrease in plant height in 'Scoresby Dwarf' compared to 'Best Boy Bush' (Table 3.5.1.a, b and Figure 3.5.1.a, b and Appendix C)

Table 3.5.1.b Summary of percentage change of the main effects and interactions with water treatment for plant growth and morphology.

Traits	Water	Cultivar	Water x Cultivar
Plant height (cm)	ns	SBD: -32%	Dr SBD: -9%
Leaf number	Dr: -17% WL: -11%	SBD: 34%	ns
Node number	ns	ns	ns
Stem diameter (mm)	ns	SBD: 8%	ns
Root diameter	ns	ns	ns
Root length	WL: -10%	ns	ns
LA (mm^{-2})	ns	SBD: -43%	ns
SLA (mm^{-2} mg^{-1})	WL: -29%	ns	ns
% Leaf Damage	Dr: 3.9x WL: 69%	ns	ns
Number of senesced leaves	Dr: 3.9 x	ns	ns

Drought (Dr), waterlogging (WL), 'Scoresby Dwarf' (SBD), 'Best Boy Bush' (BBB), non-significant (ns), fold (x)

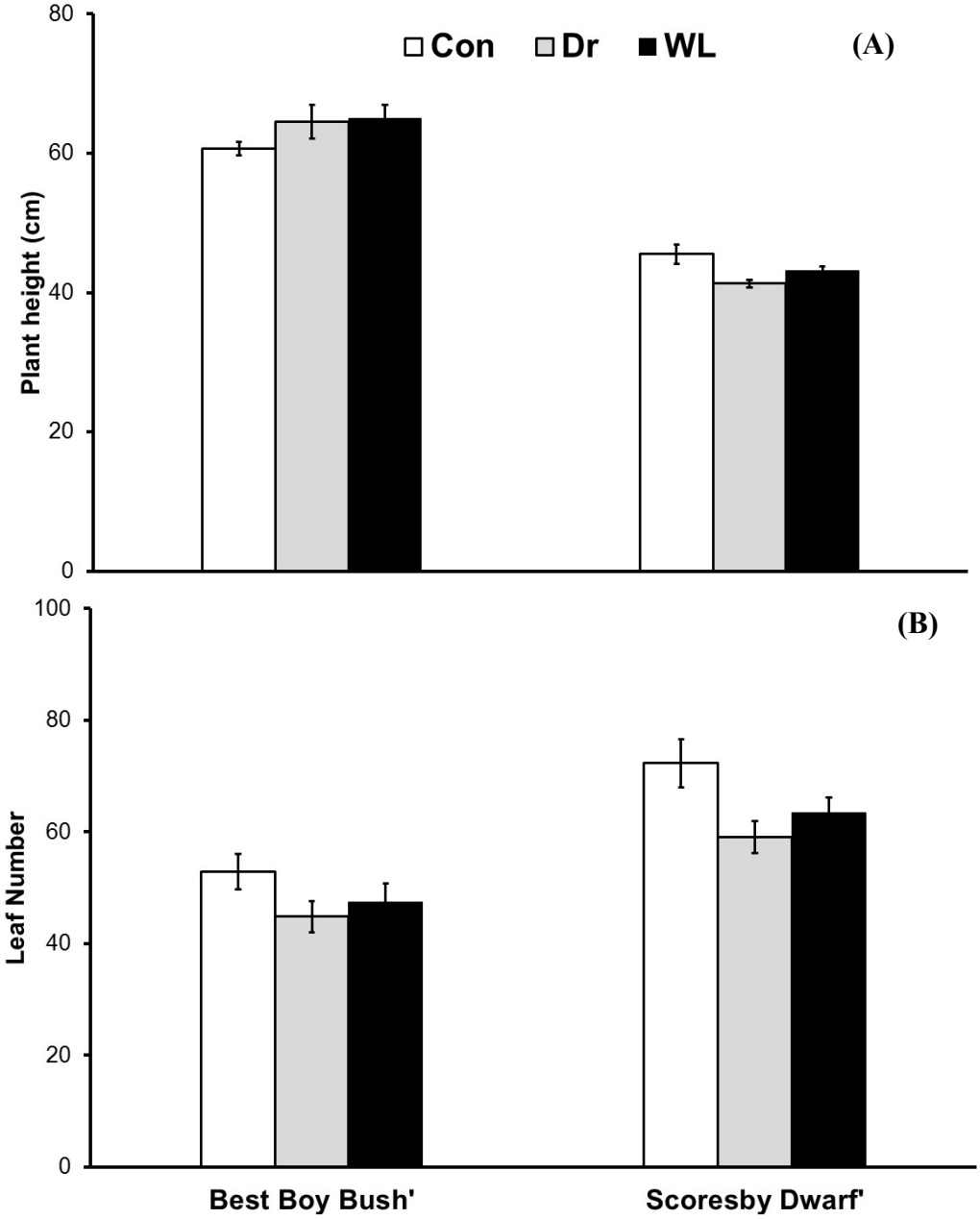


Figure 3.5.1.a Plant height (A) and number of leaves (B) of tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' after eight days of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

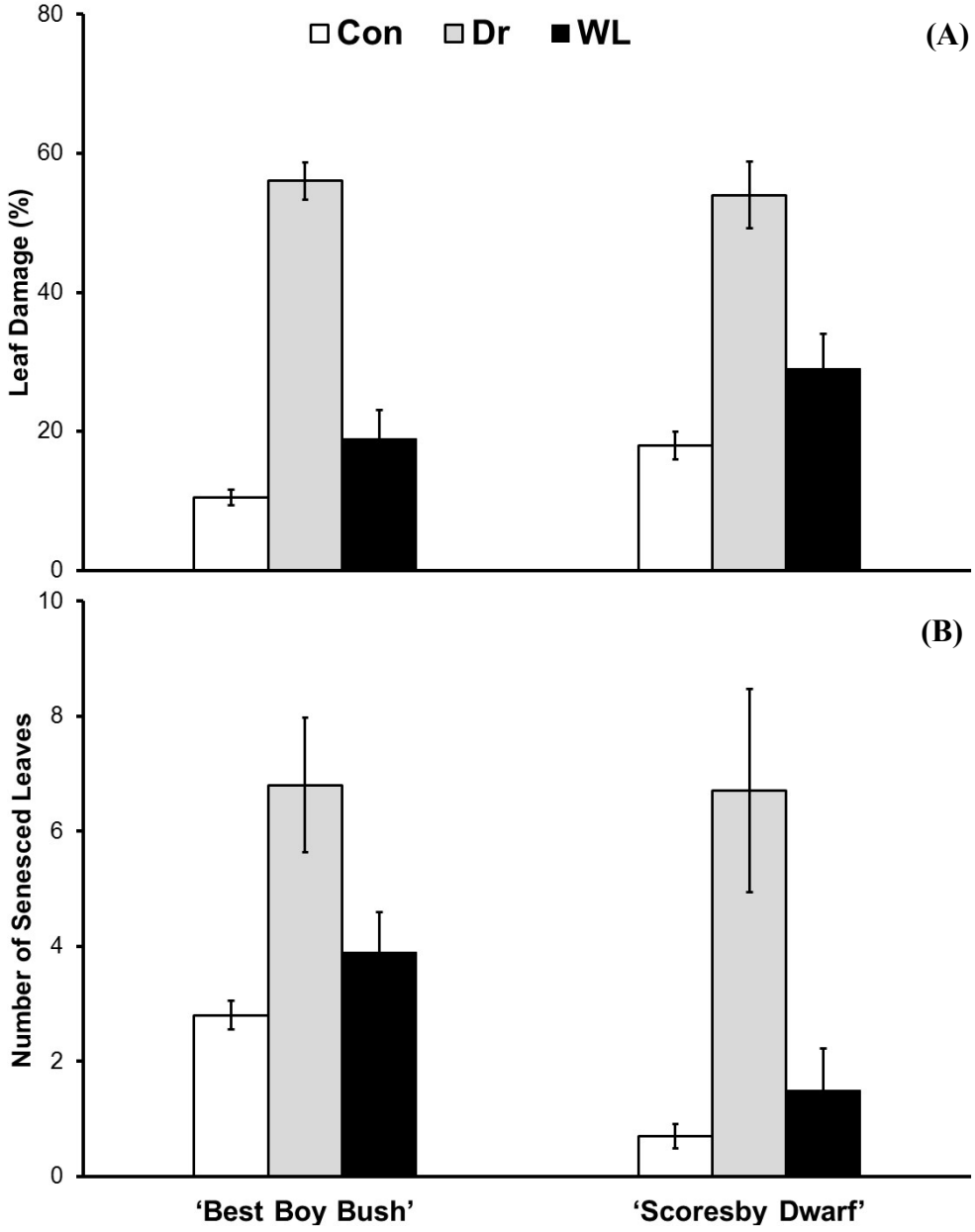


Figure 3.5.1.b % Leaf Damage (A) and senesced leaf number (B) of tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' after eight days of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

3.5.1.3 Summary of the key findings

- ❖ Drought did not have significant effects on plant growth and morphological traits at the fruiting stage, apart from a reduction in the number of leaves, which was also observed under waterlogging, together with reductions in root length and SLA.
- ❖ Drought strongly increased leaf senescence and leaf damage, which was more pronounced than under waterlogging.

3.5.2 Plant dry matter traits

3.5.2.1 Main effects

Averaged across cultivars, drought reduced leaf and root DM as well as RSR by 20%-27%. Waterlogging reduced the latter two parameters by 32%-33%. (Table 3.5.2.a, b, Figure 3.5.2.a, b and Appendix C). The percentage of dry matter, on the other hand, increased in aboveground plant organs by 21% - 2.4-fold under drought and by 9% - 40% under waterlogging. Averaged across water treatments, 'Scoresby Dwarf' had 18% - 39% lower DM and 12% - 16% lower PDM values than 'Best Boy Bush'.

Table 3.5.2.a Summary of P values of the main effects and interactions with water treatment for plant dry matter traits and % dry matter traits

Traits	Water	Cultivar	Water x Cultivar
Leaf DM	0.001	<.001	0.229
Stem DM	0.136	<.001	0.111
Above ground DM	0.189	<.001	0.049
Root DM	0.014	<.001	0.059
Plant DM	0.308	<.001	0.054
Root:shoot ratio	<.001	0.003	0.018
Leaf PDM	<.001	<.001	0.993
Stem PDM	<.001	<.001	0.682
Above ground PDM	<.001	0.014	0.008
Root PDM	0.519	0.071	0.353
Plant PDM	<.001	0.010	0.010

Raw data are shown in Appendix B

3.5.2.2 Interaction effects

Under waterlogging, aboveground DM increased in 'Scoresby Dwarf' whilst its RSR was reduced, similar to 'Best Boy Bush', where RSR decreased by 35%. Aboveground PDM and plant PDM only increased in 'Best Boy Bush' under waterlogging (by about 20%), whilst under drought it increased more than double compared to 'Scoresby Dwarf' (Table 3.5.2.a, b, Figure 3.5.2.a, b and Appendix C).

Table 3.5.2.b Summary of percentage changes of the main effects and interactions with water treatment for plant dry matter traits

Traits	Water	Cultivar	Water x Cultivar
Leaf DM	Dr: -20%	SBD: -18%	ns
Stem DM	ns	SBD: -34%	ns
Above ground DM	ns	SBD: -20%	WL SBD: 26%
Root DM	Dr: -27% WL: -32%	SBD: -39%	ns
Plant DM	ns	SBD: -21%	ns
Root:shoot ratio	Dr: -22% WL: -33%	SBD: -21%	Dr BBB: -37% WL BBB: -35% WL SBD: -29%
Leaf PDM	Dr: 2.4x WL: 40%	SBD: -16%	ns
Stem PDM	Dr: 21% WL: 9%	SBD: -13%	ns
Above ground PDM	Dr: 47% WL: 20%	SBD: -12%	Dr BBB: 69% Dr SBD: 25% WL BBB: 23%
Root PDM	ns	ns	ns
Plant PDM	Dr: 45% WL: 19%	SBD: -12%	Dr BBB: 65% Dr SBD: 25% WL BBB: 21%

Drought (Dr), waterlogging (WL), 'Scoresby Dwarf' (SBD), 'Best Boy Bush' (BBB), non-significant (ns), fold (x)

3.5.2.3 Summary of the key findings

- Water stress reduced leaf and root DM, as well as RSR.
- Aboveground percentage of dry matter increased more under drought than under waterlogging.
- 'Scoresby Dwarf' showed tolerance in aboveground DM under waterlogging and its RSR was unchanged under drought, in contrast to 'Best Boy Bush', where RSR decreased.
- Aboveground PDM only increased in 'Best Boy Bush' under waterlogging, and more than double under drought when compared to 'Scoresby Dwarf'.

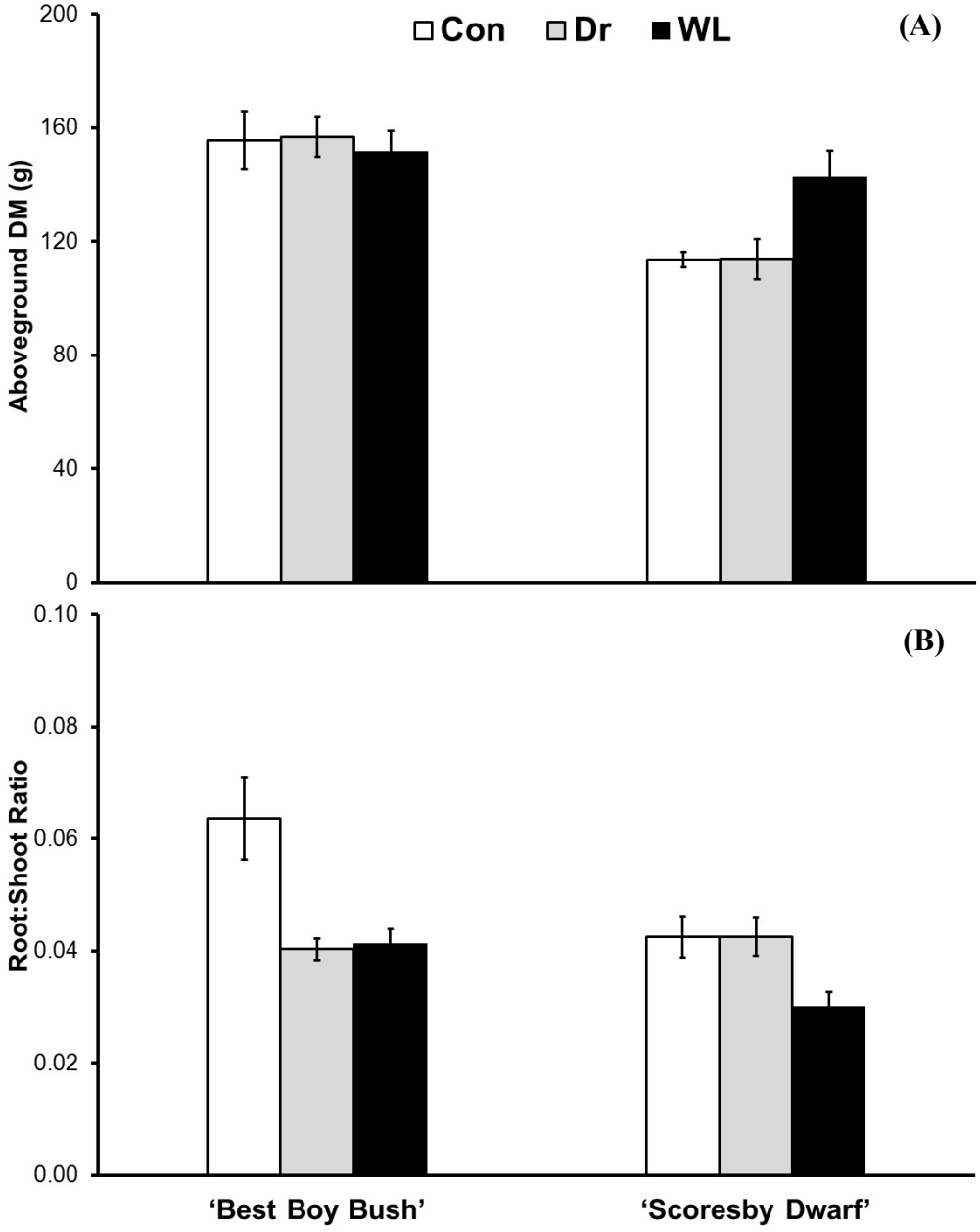


Figure 3.5.2.a Aboveground DM (A) and root:shoot ratio (B) of tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' after eight days of exposure to control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

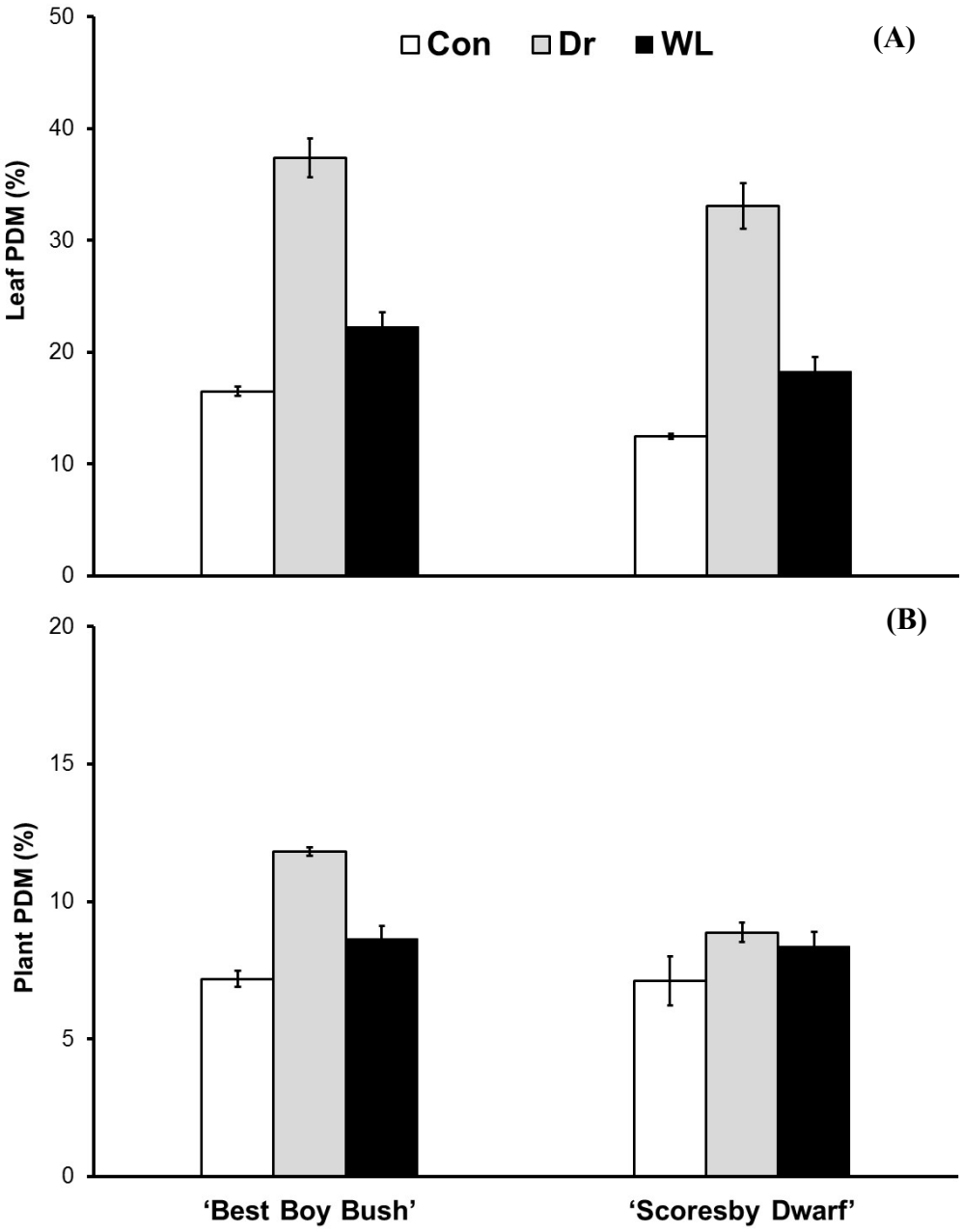


Figure 3.5.2.b Leaf PDM (A) and plant PDM (B) of tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' after eight days of exposure to control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

3.5.3 Reproductive components

3.5.3.1 Main effects

Averaged across cultivars, flower and fruit PDM increased in plants subjected to water deficit by 44% (Table 3.5.3.a, b, Figure 3.5.3.a and Appendix C). Across water treatments, 'Scoresby Dwarf' had 18% lower flower and fruit DM than 'Best Boy Bush'.

Table 3.5.3.a Summary of P values of the main effects and interactions with water treatment for reproductive components

Traits	Water	Cultivar	Water x Cultivar
Flower number	0.318	0.990	0.344
Fruit number	0.489	0.205	0.223
Flower and fruit DM	0.136	0.002	0.082
Flower and fruit PDM	0.001	0.367	0.044

Raw data are shown in Appendix B

3.5.3.2 Interaction effects

The two way interaction revealed a drought-induced increase in flower and fruit PDM by 75% in 'Best Boy Bush' (Table 3.5.3.a, b, Figure 3.5.3.a and Appendix C).

Table 3.5.3.b Summary of percentage change of the main effects and interactions with water treatment for reproductive components

Traits	Water	Cultivar	Water x Cultivar
Flower number	ns	ns	ns
Fruit number	ns	ns	ns
Flower and fruit DM	ns	SBD: -18%	ns
Flower and fruit PDM	Dr: 44%	ns	Dr BBB: 75%

Drought (Dr), waterlogging (WL), 'Scoresby Dwarf' (SBD), 'Best Boy Bush' (BBB), non-significant (ns), fold (x)

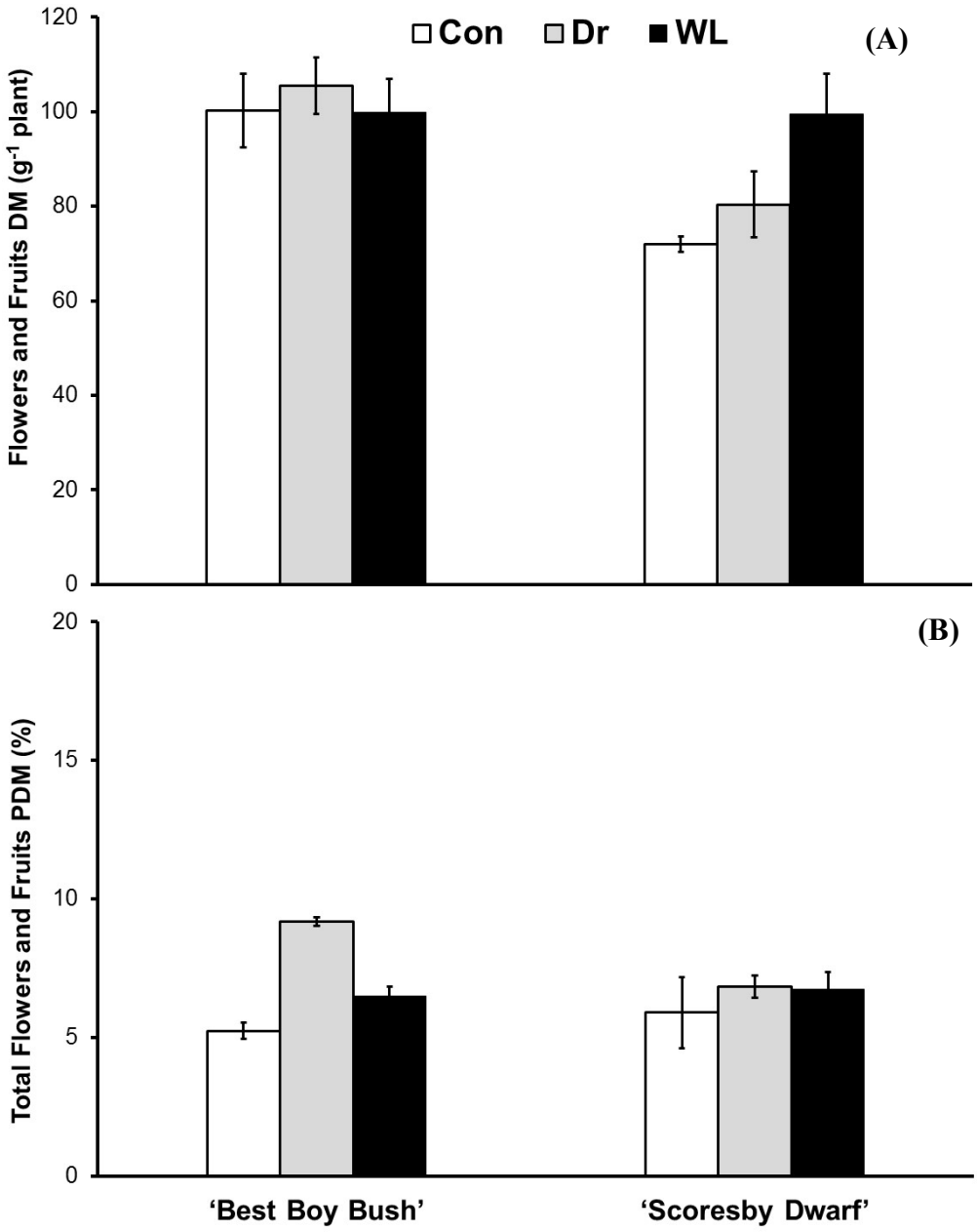


Figure 3.5.3.a Flower and fruit DM (A) and flower and fruit PDM (B) of tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' after eight days of exposure to control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

3.5.3.3 Summary of the key findings

- Extreme water stress had no significant effect on reproductive components except an increase in PDM of flowers and fruits in 'Best Boy Bush'.

3.6 Multivariate traits response to the principal component analysis (PCA)

3.6.1 Plant trait responses to the effects of water treatment in the glasshouse

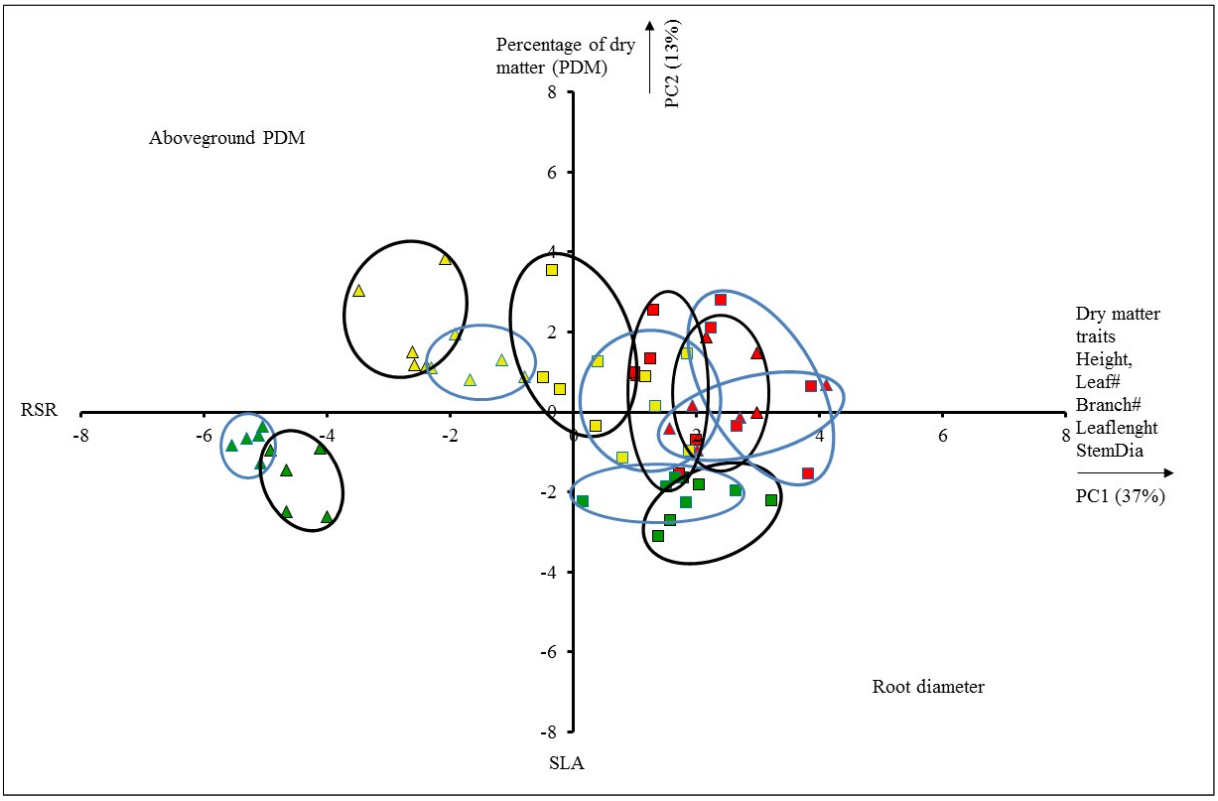


Figure 3.6.1.a Biplot of treatment responses in two tomato cultivars at different developmental stages. 'Best Boy Bush' responses are circled in black and 'Scoresby Dwarf' responses are circled in blue. Triangles represent drought responses at vegetative (▲), flowering (△), fruiting (▲) stages. Squares represent waterlogging responses at the vegetative (■), flowering (□), fruiting (■) stages.

3.6.1.1 Plant trait responses to water stress in the first principal component

The first principal component (PC1) explained 37% of the variance in the dataset, nearly three times that of the next principal component. Growth traits were located on the positive end of PC1 (Figure 3.6.1.a), with high scores for all the dry matter traits, including leaf, stem, above ground and total plant DM. These were followed on PC1 by plant height, leaf number, branch number, leaf length, stem diameter and root diameter. All of these traits were inversely associated with root: shoot, which was located on the opposite end of the PC1 axis.

3.6.1.2 Developmental stages and cultivar responses to water stress in the first principal component

Waterlogging responses generally located towards the positive part of the PC1 axis, whilst drought responses mostly had negative PC1 scores. The drought responses were further characterised in PC1 by distinctive developmental patterns, with positive scores at fruiting, negative scores for flowering and the most negative scores for the vegetative stage (Figure 3.6.1.a). There were no distinctive cultivar response patterns, although the PC1 scores in 'Scoresby Dwarf' were generally higher at the flowering and fruiting stages than in 'Best Boy Bush' under both stress factors.

3.6.1.3 Plant trait responses to water stress in the second principal component

The second principal component (PC2) accounted for 13% of the variance in the dataset (Figure 3.6.1.a). The percentage of dry matter of the leaves, stems, above ground and total plant material located at the positive end of the PC2 axis, whereas the specific lamina area score was at the opposite end of PC2.

3.6.1.4 Developmental stages and cultivar responses to water stress in the second principal component

PC2 separated the responses to both stress factors at the vegetative stages from the other developmental stages. There were no clear cultivar response patterns in PC2, except for positive correlations of 'Best Boy Bush' plants with PC2 at the flowering stage.

3.6.2 Plant trait responses to the effect of water treatments in the field

3.6.2.1 Plant trait responses to water stress in the first principal component

PC1 accounted for 22% of the variance in the dataset. The positive end of PC1 was characterised by high scores for the stress responses of all DM attributes (leaf, stem and total plant), the number of leaves as well the vegetative percentage dry matter content (Figure 3.6.2.a). The negative end of PC1 was associated with changes in SLA.

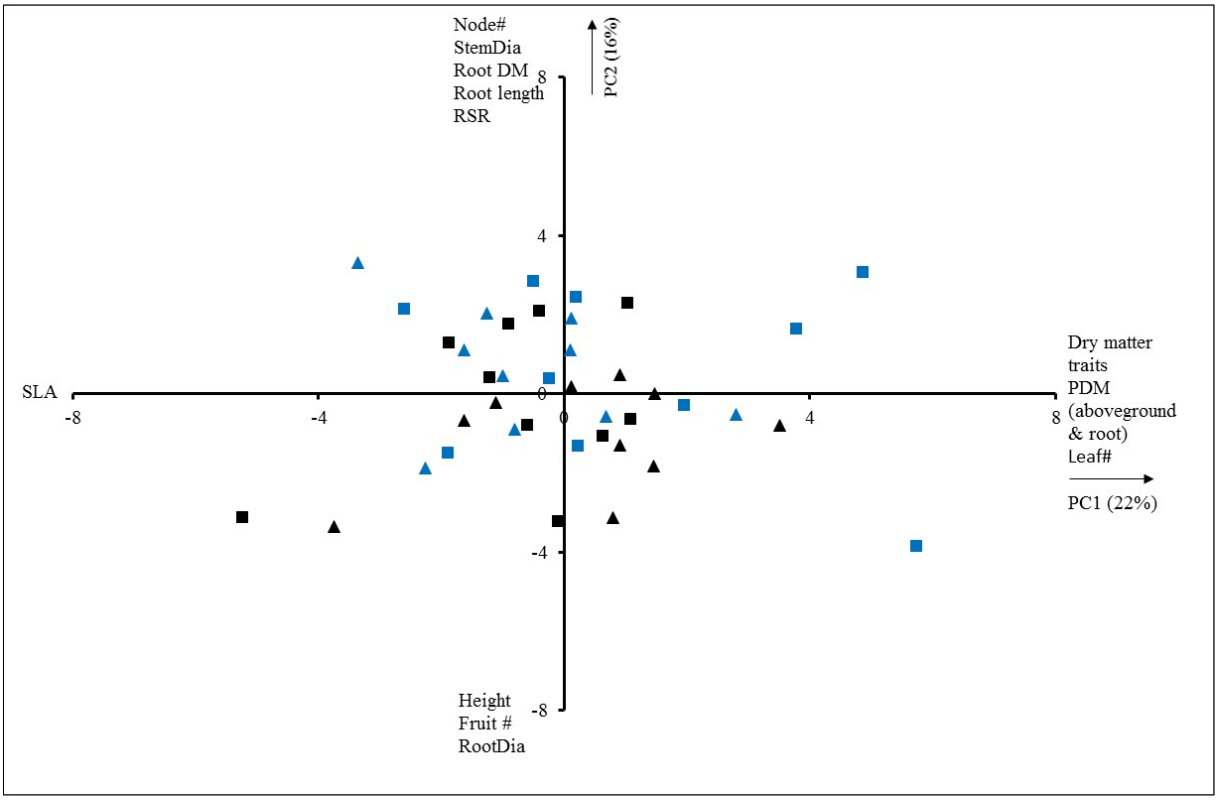


Figure 3.6.2.a Biplot of treatment responses (ratios of treatment/control) of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf' measured after eight days of exposure to drought and waterlogging. Symbols: 'Best Boy Bush'-drought (▲) and waterlogging (■). 'Scoresby Dwarf'-drought (▲) and waterlogging (■).

3.6.2.2 Plant trait responses to water stress in the second principal component

The second principal component (PC2) explained 16% of the variance in the dataset (Figure 3.6.2.a). The positive end of the axis had high scores for stress-induced changes in root characteristics, as well as node number and stem diameter. The negative end of this axis associated with changes in fruit number plant height and root diameter.

3.7 Discussion

Drought and waterlogging studies in plants have, historically, been conducted separately but, to the best of the author's knowledge, there has been no study that has directly compared these two stress factors. In the findings presented here, water stress has, in general, modified the growth of plants so that, under both drought and waterlogging, plants were smaller than the untreated plants. This is consistent with previous studies that have shown that drought or waterlogging reduces plant growth (Liu et al., 2010; Lopez & Rosario, 1983; Rezaei et al., 2012; Selim & El-Nady, 2011). This reduction in growth was more apparent in the glasshouse in comparison to the field grown plants. However, the field observations were conducted on field grown plants at the fruiting stage. In the glasshouse studies that stage was the least drought-sensitive developmental stage which also overlapped with the waterlogging responses (Figure 3.6.1.a).

3.7.1 Plant growth and morphological traits

Growth cessation and leaf structure alterations have also been reported previously in tomato plants subjected to drought stress or waterlogging (Aloni & Rosenshtein, 1982; Selim & El-Nady, 2011). Plant height and numbers of leaves were reported to decrease in tomato plants under drought stress (Liu et al., 2010; Torreceillas et al., 1995). The authors reported that the drought-induced reduction in these traits was more pronounced in the early, vegetative stage of plant growth, similar to the findings in the present study. The PCA clearly identified a morphological stress response axis on the basis of changes in traits related to plant growth (Figure 3.6.1.a). Thus, the most pronounced decreases in plant growth (negative PC1 scores) occurred under drought at the vegetative stage, followed by growth decreases at flowering and least pronounced plant growth changes at fruiting. There are two possible explanations for this observation. The first one is lowered resource availability for plant growth. Plants can adjust to a water deficit by developing a modified leaf structure, increasing carbohydrate allocation to the roots, reducing the plant growth rate and the rate of organ turnover (Chaves et al., 2003). When the water supply to the root system is low, there is a reduction in nutrient accessibility for plants (Chaves et al., 2003). Coupled with a lower nutrient uptake is a reduction in net photosynthesis and photosynthetic products (Horchani et al., 2008; Selim & El-Nady, 2011). Therefore, plants needed to adjust themselves to lower resource availability. Thus there is less carbon investment in the initiation of new leaves and an inhibition in shoot growth follows (Chaves et al., 2003; Selim & El-Nady 2011). Secondly, it has been suggested that the meristems are the first target of water stress (Irfan et al., 2010; Selim & El-Nady, 2011), causing

a reduction in the number of cells produced by the apical meristems. Lowered cell division is accompanied by a reduction in cell expansion and, as a consequence, reduced overall leaf growth (Selim & El-Nady, 2011). Reductions in cell expansion and cell division affect the number, length and width of leaves and internodes and thus affect plant growth. It is likely that these two mechanisms both contributed to the pronounced reductions in vegetative plant growth attributes, as vegetative growth is the main focus during that the early plant developmental stage. Aloni and Rosenshtein (1982) suggested that waterlogging induced a water deficit in the root zone of tomato plants. The PCA revealed a general pattern of higher sensitivity of plant growth parameters to waterlogging with generally lower PC1 scores for 'Best Boy Bush' at the later developmental stages, compared to 'Scoresby Dwarf', which was also highlighted in the latter cultivar by the maintenance of leaf DM under waterlogging at fruiting (Figure 3.4.2.a).

Stem diameters were slightly reduced for plants subjected to water deficit treatments in both the glasshouse and under field conditions. A reduction in stem diameter might be caused by a reduction in cortex thickness and in vascular tissues resulting from a lessening of cell division and cell expansion in the lateral meristems (Selim & El-Nady, 2011). However, the stem diameter increased under waterlogging and this was particularly pronounced at the early stages of plant growth. This increase in stem and root diameter in waterlogged plants was caused by the formation of adventitious roots at the stem base of young tomato plants, prior to reaching the reproductive stage of growth. The formation of adventitious aerial roots (Figure 3.4.1.e) is likely to help plants survive under waterlogging due to increased access to oxygen.

Plants cope with water deficit by minimising water loss and maximising water uptake (Chaves et al., 2003). Typical plant strategies to avoid drought stress and maintain tissue water potential is through stomatal closure, development of smaller leaf area (via reductions in leaf size and/or leaf number) and a strengthened root system, frequently increasing the root: shoot ratio (Reddy et al., 2004). Whilst a clear symptom of stress damage, the increased leaf senescence under drought and waterlogging (Figure 3.4.1.d and Figure 3.5.1.b) also serves as a water saving method due the functional reduction of transpiring leaf area. Furthermore, plants can reallocate nutrients stored in senescing leaves to younger leaves. Chaves et al. (2003) suggested that this reallocation of nutrients might occur more in susceptible plants than in tolerant plants. Leaf senescence in plants subjected to waterlogging is thought to be induced by a reduction in chlorophyll content, causing leaf chlorosis and, ultimately, leaf death (Ahsan et al., 2007).

3.7.2 Plant dry matter traits

Selim and El-Nady (2011) and Calcagno et al. (2011) reported that tomato plants subjected to water deficit showed a decrease in plant dry matter, generally, and, specifically, decreases in dry matter of the leaves, stems and roots. This is in accordance with the results of this present study (both in the glasshouse and the field experiment). PC1 in the PCA of the field results identified decreases particularly in aboveground DM attributes as the main multivariate dimension of sensitivity under both water stress extremes and in both cultivars (Figure 3.6.2.a). It showed that these biomass decreases were closely aligned with stress-induced decreases in leaf numbers. The second-most important trait combination characterising responsiveness to both water stress factors and in both cultivars were changes in root characteristics. PC2 showed that water stress-induced decreases in root DM were related to decreases in root length, not root diameter.

The results from the glasshouse studies here are supported by other findings showing the promotion of root growth characteristics under water deficit as a morphological response to increase water uptake (Shao et al., 2008; Sharp et al., 2004). Accordingly, in PC1 of the glasshouse PCA, the juxtaposition of stress-induced changes in morphological and DM attributes (positive PC1 end) and in the root:shoot ratio (negative PC1 end) identified the latter trait as the main stress-protective morphological response to decreases in plant growth (Figure 3.6.1.a). Water gain, through optimised water uptake can be sustained by enhancing root growth (Chaves et al., 2003).

In contrast to the findings under drought, RSR decreased under waterlogging, due to pronounced decreases in root DM, compared to shoot DM (Figure 3.4.2.b). This is likely to be due to an alteration of carbohydrate allocation away from root tissues and organs (Horchani et al., 2010b). A reduction in root DM can also be attributed to cellular deterioration in root if root hypoxia is prolonged (Aloni & Rosenshtein, 1982; Horchani et al., 2008). Waterlogging can also modify leaf structure, with a smaller leaf area but increased leaf thickness (Calcagno et al., 2011; Horchani et al., 2010a). Whilst decreases in RSR were also observed in the field studies under waterlogging in both cultivars, the decrease in RSR under drought in ‘Best Boy Bush’ was unexpected and suggests particularly strong drought sensitivity in this cultivar (Figure 3.5.2.a). It was of further interest to note increases in aboveground DM in field-grown ‘Scoresby Dwarf’ plants under waterlogging (Figure 3.5.2.a), suggesting that this cultivar was tolerant enough under waterlogging to utilise the excess water for turgor-driven growth.

The percentage of dry matter attributes increased with water stress, similar to other findings (Dresboll et al., 2013). The PCA also identified PDM increases as the main form of stress sensitivity in PC2 in both cultivars and under both stress factors, particularly at the reproductive developmental stages and that this came at the cost of SLA formation (Figure 3.6.2.a). Thus, plants that became more dry (increasing their PDM) under stress also reduced their leaf area compared to their leaf dry weight (i.e. decreasing SLA). This juxtaposition of PDM and SLA responses is in line with ecological theory, considering SLA as a plant productivity trait and PDM as a measure of plant stress (Wilson et al., 1999). This suggests higher stress sensitivity for 'Best Boy Bush', which increased its aboveground PDM under waterlogging in contrast to 'Scoresby Dwarf' (no change in this attribute), combined with significantly higher aboveground PDM increases under drought in 'Best Boy Bush' than in 'Scoresby Dwarf'.

3.7.3 Reproductive components

Water deficit had a stronger effect than waterlogging on plant reproductive characteristics. Reductions in the numbers of tomato flowers and fruits is a well-known plant response to drought (Rezaei et al., 2012; Horchani et al., 2008; Lopez & Rosario, 1983) and can be attributed to the abscission and dying of flowers and premature fruits. Horchani et al. (2008) suggested that there were two possible reasons for tomato flower and fruit abscission. First, there might be stress-induced ethylene accumulation in the aboveground organs. Secondly, the carbohydrate supply to the flowers and fruits might be restricted because of a limitation in photosynthetic activity. According to Lopez and Rosario (1983), flowering is the most sensitive morphogenetic stage when plants experience waterlogging, whilst at the fruiting stage plants are less affected since they have already set fruits (Lopez & Rosario, 1983). As mentioned above, this was observed for maintenance of leaf DM in 'Scoresby Dwarf', but not in 'Best Boy Bush' under waterlogging in the glasshouse studies.

It is possible that these studies could have discerned further treatment-induced morphological differences and differential in cultivar responses under longer duration of stress exposure. This timeframe was determined by the concurrent exposure to water stress extremes which themselves were governed by the fact that once plants were exposed to waterlogging they approached severe stress, indicated by wilting, within 1-2 weeks. At that point, all plants had to be harvested to allow direct stress comparisons, but this also meant that plants had limited

time to express differences in morphology as a response to stress. The physiological and biochemical aspects of these responses will be discussed in the next chapters.

3.7.4 Conclusions

The results from the field study, which was conducted at the fruiting stage were in general agreement with the results observed for plants examined at the same growth stage in the glasshouse experiment. Drought and waterlogging, both caused reductions in attributes contributing to plant growth, with particularly strong responses under drought at the early developmental stage. Both water stress factors and particularly drought affected reproductive features. Increased plant damage characteristics were also observed under both stress factors, and particularly at the later developmental stages under waterlogging. There were several indications of intraspecific differences in morphological stress responsiveness in the glasshouse and in the field studies, suggesting higher water stress tolerance for 'Scoresby Dwarf' and sensitivity for 'Best Boy Bush'.

Chapter 4

Effects of water stress extremes on the physiology of two tomato cultivars

Abstract

This chapter examined physiological responses of two tomato cultivars at various developmental stages under two water treatment extremes, water deficit and waterlogging, in two environments, the glasshouse and field. Plant water status attributes decreased, as measured by leaf water potential (with reductions up to 3.6-fold), leaf relative water content (about -20%), and leaf osmotic potential (-13% to -45%) by either stress, except at the vegetative stage in plants subjected to waterlogging in the glasshouse experiment. These effects were most pronounced under drought. Osmotic adjustment occurred only in drought-treated plants, via reduction in the adjusted osmotic potential by 29%. This was accompanied with increases in the production of proline (32-76%) in drought treated plants. In contrast, proline levels decreased by 34% to 58% in the waterlogged plants. Water stress (drought and waterlogging) suppressed leaf gas exchange in the tomato plants under both glasshouse and field conditions, via reductions in photosynthetic rates (-33% to -84%), stomatal conductance (-51% to -91%) and transpiration rates (-27% to -85%). Intraspecific differences in treatment responses were observed between 'Best Boy Bush' and 'Scoresby Dwarf' in the glasshouse study, where the latter cultivar showed higher drought-induced increases in proline levels and was able to maintain its osmotic adjustment under waterlogging. In conclusion, drought and waterlogging cause similar stress responses in gas exchange and plant water status attributes, some of which are cultivar-specific.

4.1 Introduction

Despite complete root submergence, waterlogging induces a water deficit in the rhizosphere (Aloni & Rosenshtein, 1982). Therefore, the water supply to the above ground plant organs is limited under waterlogged conditions. This restriction of plant water supply may be caused by oxygen deprivation in the root zone (hypoxia) that can lead to a disruption of the metabolic activity of roots (Aloni & Rosenshtein, 1982). It has been suggested that shoot responses resulting from root exposure to a scarcity of water were dependent on hydraulic signalling (Lipiec et al., 2013). This signalling begins with a reduction in root water uptake and is followed by a reduction in leaf water potential and leaf turgor potential. Stomatal closure then occurs in order to minimise water loss through leaf transpiration (Aloni & Rosenshtein, 1982; Lipiec et al., 2013). The closure of stomata is a key step in the response of the plant to maintain cell turgor and plant metabolic processes (Lipiec et al., 2013). A reduction in leaf photosynthetic rates usually occurs in parallel to the reduction of leaf water potential and leaf relative water content after the closure of the stomata (Reddy et al., 2004).

Lipiec et al. (2013) suggested that hydraulic signalling also triggers osmotic adjustment, one of the most well-documented drought survival mechanisms in plants. In contrast to stomatal closure, osmotic adjustment is a slow process and involves the accumulation of numerous osmotically active compounds including organic solutes, amino acids and carbohydrates (Mahajan & Tuteja, 2005). During osmotic adjustment these solutes attract water into the cell and stabilise proteins and membranes (Chaves et al., 2003). The metabolic process of osmotic adjustment is well documented under drought stress, but less so under waterlogging. Jackson et al. (2003) reported measuring osmolality increases (production of solutes) in the tissue of tomato plants suffering from flooding. Proline levels were also examined in root tissues of tomato plants subjected to waterlogging treatment (Lopez & Rosario, 1983). The studies of osmotic adjustment were observed mainly by examining the production of particular organic solutes such as proline in the tissues, but not the osmotic potential itself. It was reported that free proline levels increased in plant cells and leaf tissues under water stress, particularly in drought tolerant species (Handa et al., 1986; Sanchez-Rodriguez et al., 2010a).

Research has shown that both chlorophyll content and chlorophyll fluorescence are degraded under both water deficit and waterlogging conditions (Lopez & Rosario, 1983; Zgallai et al., 2005). However, others suggested that a reduction in chlorophyll content was observed only in sensitive tomato cultivars (Sanchez-Rodriquez et al., 2012). Chlorophyll degradation is likely a drought response mechanism to limit light harvesting by chloroplasts. The degradation of chlorophyll becomes more pronounced with the imposition of prolonged waterlogging and chlorosis and leaf senescence were ultimately observed under severe waterlogging conditions (Ahsan et al., 2007; Lin et al., 2004). Several studies have investigated the physiological responses of tomato plants under drought stress or stress induced by waterlogging. However, there has been no study comparing these two stress factors.

The general objective of this chapter was therefore to compare the physiological responses of tomato plants under two water stress extremes. This chapter specifically investigated how water deficit and waterlogging affected plant water status attributes, the occurrence of osmotic adjustment and proline levels. An additional aim of this chapter was to measure parameters related to photosynthesis and leaf gas exchange, such as photosynthesis rates, stomatal conductance and transpiration rates, as well as chlorophyll fluorescence. It was hypothesised that these attributes will be affected differently by the two water stress extremes under the three plant developmental growing stages. In addition, we expected cultivar differences in water

stress responses between the cultivars 'Best Boy Bush' and 'Scoresby Dwarf', as well as differences between glasshouse- and field-grown tomatoes.

Plate 2. Field tomato at the vegetative stage before the imposition of water stress

4.2 Material and methods

Plant growth conditions, experimental design and statistical analysis have been outlined earlier, in Chapter 3. In addition, details of the analyses of plant physiological changes are described below:

4.2.1 Water status, osmotic adjustment and proline levels

4.2.1.1 Time domain reflectometry (TDR)

The percentage of substrate moisture for each plant pot was determined using TDR (Hydrosense^{TM}, Campbell Scientific, Inc) and recorded as volumetric water content (%) as a measure of soil water status.

4.2.1.2 Leaf relative water content

Two laminae from the second fully developed leaf were used to calculate the relative leaf water content (LRWC) using the following formula, as described by Yuan et al. (2010):

LRWC = FW-DW/ (TW-DW) x 100

The fresh weight (FW) was determined using a balance. The turgid weight (TW) was determined by weighing the laminae after rehydration for 20–24 h at 4°C (laminae were placed in a Petri dish with water and covered with filter paper). The dry weight (DW) was determined by weighing the laminae after drying at 70°C for 24 h in an oven.

4.2.1.3 Leaf water potential

A pressure chamber technique was used to determine leaf water potential following the procedure used by Hofmann et al. (2003) with minor modifications. First, a leaflet at the end of the third fully developed leaf from the top of the plant was enclosed in the pressure chamber and then excised at the leaf collar. The equilibrium pressure required to bring water to the cut leaf collar cross section was monitored and recorded as leaf water potential (MPa).

4.2.1.4 Osmotic potential

Laminae from the third fully developed leaf were put into Eppendorf tubes equipped with a sieve assembly and the tubes were then submerged in liquid nitrogen, before storing at -35°C for analysis. The samples were divided into small batches and each batch was taken out of the freezer and left to thaw at room temperature, then centrifuged (Eppendorf – Centrifuge 5415D) for three minutes at 12 000 *g*. The osmolality was measured using a protocol described by Thomas and James (1993) with WESCOR VAPRO 5520 vapor pressure osmometer–WESCOR. Once the osmolality (mol.kg $^{-1}$) was measured, the osmotic potential was calculated using the Van't Hoff equation -

$$\psi_\pi = -RTcj$$

where RT = 0.002437 m^3 $MPa.Mol^{-1}$ at 20°C and cj is the total solute concentration or osmolality ($mol.kg^{-1}$).

The adjusted osmostic potential was then calculated using the following equation described by Butler (2008):

$$\Psi_\pi (100) = \frac{\Psi_\pi (LRWC\text{-}RWCa)}{1\text{-}RWCa}$$

where RWCa is the correction factor for dilution by apoplastic water. This is an estimated value and is thought to be approximately 0.1 for most plants under most situations. The difference, $\Psi\pi(100)$, between the control and stressed plants is a determination of whether osmotic adjustment has occurred: osmotic adjustment occurred when the value for adjusted osmotic potential is lower (i.e. more negative) under stress than under control conditions.

4.2.1.5 Proline determination

The proline content was measured in both leaf and root tissue samples taken from the tomato plants. Laminae from the second fully developed leaf (approx 300 mg) were excised from the plant and immediately frozen in liquid nitrogen. The freshly frozen tissues were stored at -80°C for later analysis. Approximately 300 mg of roots were taken from the root system after gently washing the roots with a water gun. Roots were added to 12.5 mL microtubes and immediately frozen in liquid nitrogen. The frozen fresh tissue was stored at -80°C for later analysis. The extraction was prepared according to Ringel et al. (2003) with minor changes by Burritt (2012). The plant tissue was ground to a fine powder in liquid nitrogen. Fifty mg of homogenate powdered tissue was added to 1.5 mL of 3% (w/v) aqueous sulfo-salicylic acid solution (Ringel et al., 2003). The homogenate was then processed using a 1 min vortex followed by 20 min (1400/min) in a shaking device (Ika-Vibrax, with an Eppendorf upper part) (Ringel et al., 2003). The resulting homogenate was centrifuged at 12 000 *g* for 10 minutes at room temperature (Burritt, 2012). The extraction was assayed using a Perkin Elmer (Wallac) 1420 multilabel counter (Perkin Elmer, San Jose, CA, USA), controlled by a PC with a temperature control cell and an auto dispenser (Lister et al., 2010). Data were obtained and processed by the WorkOut 2.0 software package (Perkin Elmer) (Schweikert & Burritt, 2012).

4.2.2 Leaf gas exchange, SPAD value and chlorophyll fluorescence

Photosynthetic rates, stomatal conductance, intercellular CO_2 concentration and transpiration rates were all measured using a LI-COR model 6400 infrared gas analyser (IRGA) (Li-COR Biosciences, Lincoln, Nebraska) at the end of the water stress period. The readings were taken

using the second fully unfolded leaf from each plant. The conditions in the IRGA chamber were adjusted to ambient conditions to allow for rapid gas exchange measurements.

4.2.2.1 SPAD values and chlorophyll fluorescence

Three leaflets of the second fully developed leaf were measured for relative chlorophyll content, T using a SPAD 502 Plus meter (Konica Minolta Sensing Inc. Osaka, Japan). The SPAD value is the optical density difference at two wavelengths - 650 nm and 940 nm) (Ballizany et al., 2012). The same laminae were dark adapted (using dark leaf clips) for about 30 minutes, before analysis of chlorophyll fluorescence, using a MINI-PAM Photosynthesis Yield Analyser (WALZ, Effeltrich) for the determination of F_v/F_m (Hofmann et al., 2003).

4.3 Statistical analyses

Statistical analyses and data presentation followed the procedures outlined in Chapter 3.
The supplementary information can be found in Appendix D, whereas the raw data are shown in Appendix A and B.

4.4 Results of the glasshouse studies

4.4.1 Water status, osmotic adjustment and proline levels

4.4.1.1 Main effects

Averaged across cultivars and developmental stages, the TDR values were reduced by 70% under drought stress but increased 7.7-fold under waterlogging relative to the well-watered control (Table 4.4.1.a, b and Figure 4.4.1.a, b, c, d). Most physiological attributes, including LRWC, leaf water potential, leaf osmotic potential and adjusted osmotic potential were reduced under both water deficit (-21% to 3.6-fold) and waterlogging (-23% to -75%, except adjusted osmotic potential). Proline concentration, on the other hand increased in both leaf and root tissues (48% - 65%) from plants that experienced water deficit. By way of contrast, waterlogged plants showed reductions in this free amino acid for both the leaf (-34%) and root tissues (-58%).

Table 4.4.1.a Summary of P values of the main effects and interactions with water treatment on soil and plant water status, osmotic adjustment and proline levels

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage	Water x Cultivar x Devel. stage
TDR (%)	<.001	0.021	0.045	0.065	0.001	0.328
LRWC (%)	<.001	0.081	0.011	0.617	<.001	0.030
Water Potential (MPa)	<.001	0.024	<.001	0.189	0.001	0.170
Osmotic potential (MPa)	<.001	0.001	<.001	0.726	<.001	0.547
Adjusted osmotic potential (MPa)	<.001	0.152	<.001	0.144	<.001	0.121
Proline Leaf (µmol gFW^{-1})	<.001	<.001	0.961	<.001	0.303	0.156
Proline Root (µmol gFW^{-1})	<.001	<.001	0.045	<.001	0.735	0.298

Raw data are shown in Appendix A

Table 4.4.1.b Summary of percentage changes of the main effects and interactions with water treatment for soil and plant water status, osmotic adjustment and proline levels

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage		Water x Cultivar x Devel. stage
TDR (%)	Dr: -70% WL: 7.7x	SBD:-3%	ns	ns	Dr H1: -83% Dr H2: -64% Dr H3: -58%	WL H1: 6.4x WL H2: 8.5x WL H3: 8.7x	ns
LRWC (%)	Dr: -21% WL: -23%	ns	H3: -12%	ns	Dr H1: -36% Dr H3: -25%	WL H2: -19% WL H3: -45%	**Dr BBB** H1: -36% **Dr SBD** H1: -37%, H3: -34% **WL BBB** H2: -36%, H3: -50% **WL SBD** H3: -41%
Water Potential (MPa)	Dr: -3.6x WL: -75%	SBD: 20%	H2: -56% H3: -63%	ns	Dr H1: -6.6x Dr H2: -2.3x Dr H3: -3.7x	WL H3: -2.7x	ns
Osmotic potential (MPa)	Dr: -45% WL: -36%	SBD: 12%	H2: -33% H3: -60%	ns	Dr H1: -45% Dr H2: -40% Dr H3: -50%	WL H2: -22% WL H3: -75%	ns
Adjusted osmotic potential (MPa)	Dr: -29%	ns	H2: -32% H3: -61%	ns	Dr H2: -20% Dr H3: -45%	WL H1: 43% WL H3: -16%	ns
Proline Leaf (µmol gFW^{-1})	Dr: 48% WL: -34%	SBD: 13%	ns	Dr BBB: 35% Dr SBD: 59% WL BBB: -31% WL SBD: -36%	ns		ns
Proline Root (µmol gFW^{-1})	Dr: 65% WL: -58%	SBD: 15%	H3: -8%	Dr BBB: 40% Dr SBD: 89% WL BBB: -58% WL SBD: -58%	ns		ns

Drought (Dr), Waterlogging (WL), 'Scoresby Dwarf' (SBD), 'Best Boy Bush' (BBB), developmental stage (H, harvest 1: vegetative, 2: flowering, 3: fruiting), non- significant (ns), fold (x)

Averaged across water treatments and developmental stages, 'Scoresby Dwarf' had about 10% to 20% higher values in most physiological attributes relative to ‘Best Boy Bush (Table 4.4.1.a, b and Figure 4.4.1.a, b, c, d).

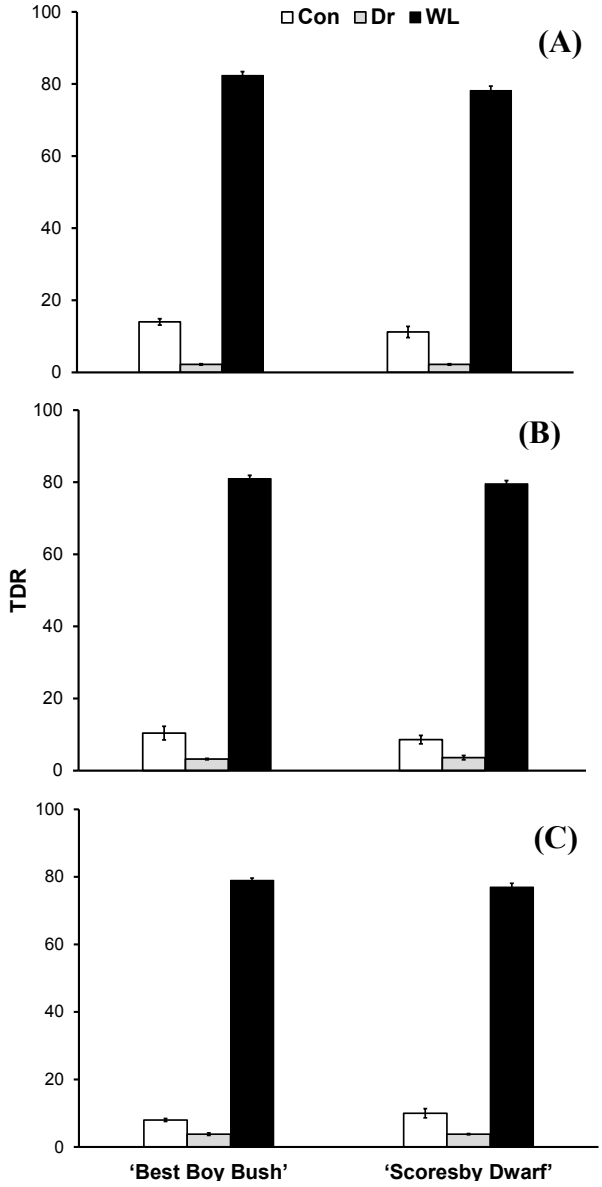


Figure 4.4.1.a TDR at the vegetative (A), flowering (B) and fruiting (C) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

Averaged across water treatments and cultivars and relative to the vegetative stage, leaf water potential and leaf osmotic potential were lower at flowering (33-56%) and fruiting (about 60%) (Table 4.4.1.a, b, Figure 4.4.1.a, b, c, d and Appendix D). Adjusted osmotic potential on the other hand, increased by 32% at the flowering stage and by 61% at fruiting, relative to the vegetative stage.

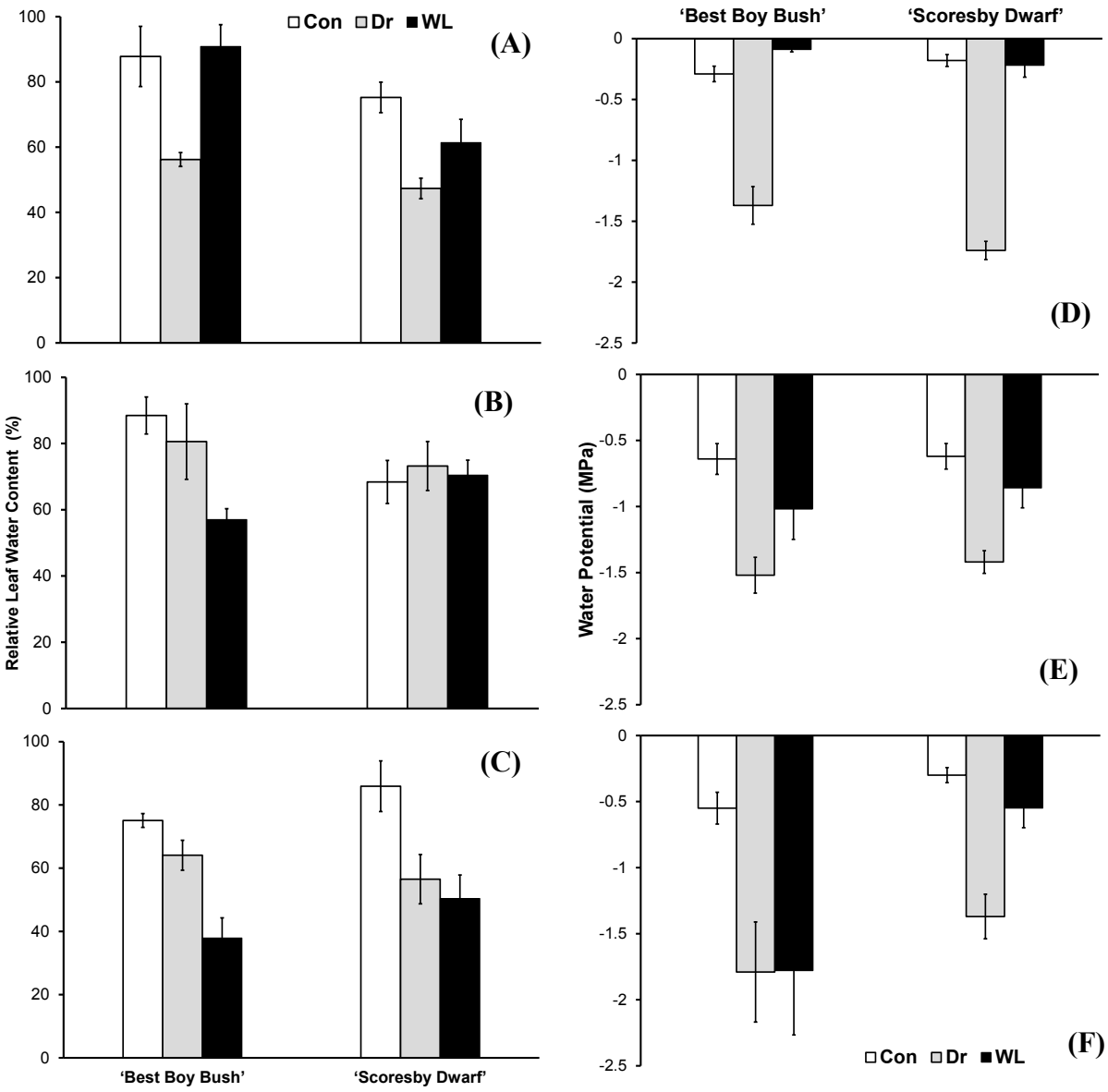


Figure 4.4.1.b Leaf relative water content (LRWC) at the vegetative (A), flowering (B) and fruiting (C) stages, and water potential at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

4.4.1.2 Interaction effects

Water x cultivar

There were significant interactions between the two water regimes and cultivars for proline levels. Drought stressed plants had increased proline levels in both leaf and root tissues of 'Best Boy Bush' (nearly 40%) and greater increases in 'Scoresby Dwarf' (about 60-90%) (Table 4.4.1.a, b, Figure 4.4.1.a, b, c, d and Appendix D). By way of contrast, both cultivars had similar reductions in the levels of this free amino acid in leaves (about 30%) and in roots (nearly 60%) under waterlogging. The water x cultivar interaction LSD also revealed cultivar-specific

differences in the waterlogging response of adjusted osmotic potential levels. Whilst these levels increased with increasing plant maturity in 'Best Boy Bush', they remained stable in 'Scoresby Dwarf' under waterlogging.

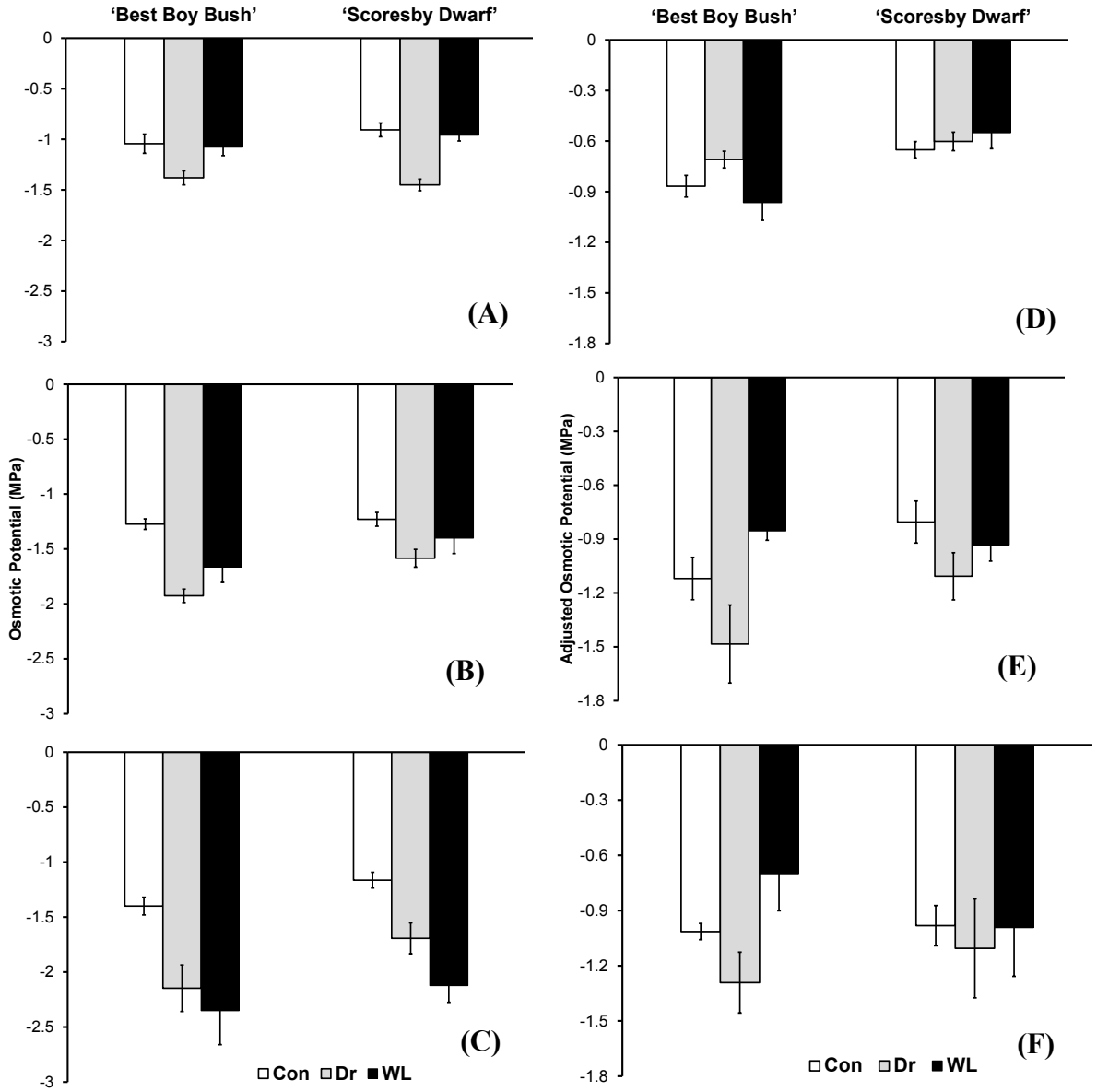


Figure 4.4.1.c Osmotic potential at the vegetative (A), flowering (B) and fruiting (C) stages, and adjusted osmotic potential at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

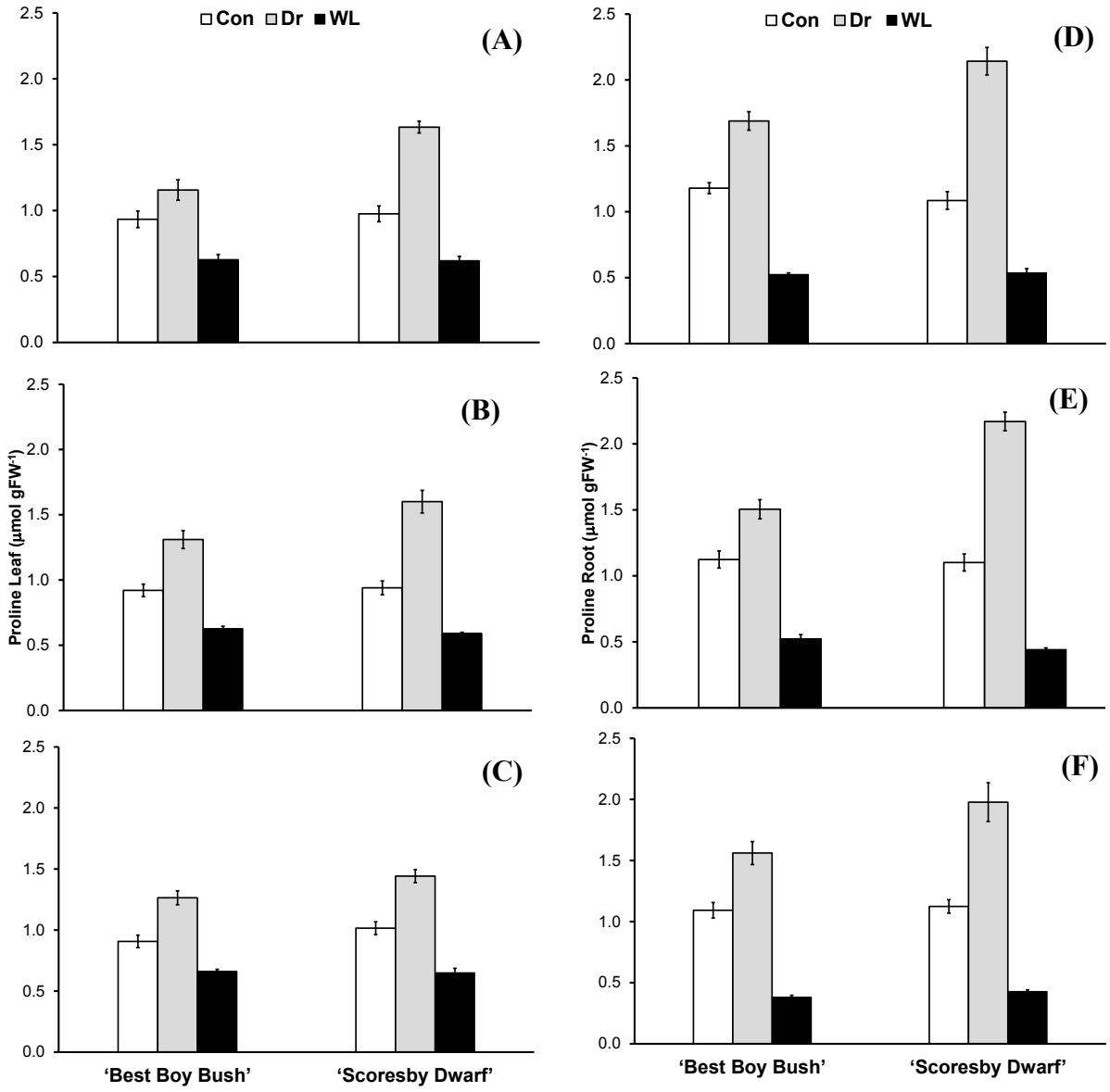


Figure 4.4.1.d Proline levels in leaf at the vegetative (A), flowering (B) and fruiting (C) stages, and proline in root at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

Water x Developmental stage

Averaged across cultivars, drought-treated plants had decreased TDR values (–from -83% at the vegetative stage to -58% at fruiting) at all stages of plant development relative to well-watered plants, whilst waterlogging significantly increased this trait (6.4-8.7-fold) during the three developmental stages (Table 4.4.1.a, b, Figure 4.4.1.a, b, c, d and Appendix D). Drought-induced reductions of LRWC improved from -36% at the vegetative stage to -25% at fruiting, whilst LRWC had decreased by 45% by the latter stage under waterlogging. Similarly, drought-induced leaf water potential reductions recovered under drought from -6.6-fold at the vegetative

stage to -3.7 at fruiting, whilst at that stage waterlogged plants had experienced their first water potential decreases by 2.7-fold. Leaf osmotic potential decreased by 40%-50% during the three developmental stages under drought, whilst it decreased progressively under waterlogging, with 75% reductions at fruiting. Adjusted osmotic potential decreased progressively with plant maturity under both stress factors, achieving reductions by 45 % under drought and by 16% waterlogging at fruiting.

Water x Cultivar x Developmental stage

Water deficit decreased LRWC by 36% in 'Best Boy Bush' at the vegetative stage and by a similar amount in 'Scoresby Dwarf' at the vegetative and fruiting stages (Table 4.4.1.a, b, Figure 4.4.1.a, b, c, d and Appendix D). Hypoxia caused a reduction in LRWC in 'Best Boy Bush' at flowering (-36%) and fruiting (-50%), where it also reduced LRWC in 'Scoresby Dwarf' by 41%.

4.4.1.3 Summary of the key findings

- Water deficit decreased the levels of all water status attributes measured here, whilst decreases in plant water status were less pronounced under waterlogging.
- Drought strongly increased proline levels in both leaf and root tissues, whilst waterlogging decreased these levels.
- 'Scoresby Dwarf' maintained adjusted osmotic potential levels under waterlogging, whilst these levels increased in 'Best Boy Bush'.
- Drought-induced increases in proline accumulation were more pronounced in 'Scoresby Dwarf' than in 'Best Boy Bush'.
- Stress-induced decreases in soil (TDR) and plant water status (LRWC and water potential) became less pronounced under drought with increasing plant maturity, whilst they became more pronounced under waterlogging.
- Decreases in adjusted osmotic potential became more pronounced under drought with increasing plant maturity, whilst they became less pronounced under waterlogging.

4.4.2 Leaf gas exchange, SPAD value and chlorophyll fluorescence

4.4.2.1 Main effects

Averaged across cultivars and developmental stages, all the components of leaf gas exchange including photosynthetic rate, stomatal conductance and transpiration rate decreased under both drought stress (-84 to -91%) and waterlogging (-53% to -81%) (Table 4.4.2.a, b, Figure 4.4.2.a, b and Appendix D).

Averaged across water treatments and developmental stages, photosynthetic and transpiration rates were 26%-28% higher in 'Scoresby Dwarf' relative to 'Best Boy Bush' (Table 4.4.2.a, b, Figure 4.4.2.a ,b and Appendix D).

Averaged across water treatments and cultivars, the gas exchange parameters were all reduced at later developmental stages including flowering (-17% to -76%) and fruiting (-66% to -80%), relative to vegetative stage (Table 4.4.2.a, b, Figure 4.4.2.a ,b and Appendix D). Waterlogging on the other hand, only resulted in a 6% increase of chlorophyll fluorescence at the fruiting stage.

Table 4.4.2.a Summary of P values of the main effects and interactions with water treatment for leaf gas exchange parameters, chlorophyll fluorescence and SPAD values.

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage	Water x Cultivar x Devel. stage
Photosynthesis (µmol CO_2 m^{-2} s^{-1})	<.001	<.001	<.001	0.043	<.001	0.210
Conductance (mol H_2O m^{-2} s^{-1})	<.001	0.152	<.001	0.144	<.001	0.121
Transpiration (mmol H_2O m^{-2} s^{-1})	<.001	0.007	<.001	0.034	<.001	0.202
SPAD	0.847	0.058	0.847	0.124	<.001	0.615
Chlorophyll fluorescence	0.004	0.722	<.001	0.715	0.138	0.335

Raw data are shown in Appendix A

Table 4.4.2.b Summary of percentage change of the main effects and interactions with water treatment for leaf gas exchange parameters, chlorophyll fluorescence and SPAD values.

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage	
Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Dr: -84% WL: -53%	SBD: 28%	H2: -53% H3: -67%	Dr BBB: -82% Dr SBD: -85% WL BBB: -56% WL SBD: -51%	Dr H1: -81% Dr H2: -84% Dr H3: -87%	WL H2: -87% WL H3: -103%
Conductance (mol H_2O m^{-2} s^{-1})	Dr: -91% WL: -81%	ns	H2: -73% H3: -75%	ns	Dr H1: -95% Dr H2: -88% Dr H3: 88% WL H1: -25%	WL H2: -91% WL H3: -90%
Transpiration (mmol H_2O m^{-2} s^{-1})	Dr: -85% WL: -57%	SBD: 26%	H2: -17% H3: -66%	Dr BBB: -82% Dr SBD: -88% WL BBB: -57% WL SBD: -56%	Dr H1: -87% Dr H2: -83% Dr H3: -86%	WL H2: -86% WL H3: -89%
SPAD	ns	ns	ns	ns	Dr H1: -15%	
Chlorophyll fluorescence	WL: -3%	ns	H3: 6%	ns	ns	

Drought (Dr), Waterlogging (WL), 'Scoresby Dwarf' (SBD), 'Best Boy Bush' (BBB), developmental stage (H, harvest 1: vegetative, 2: flowering, 3: fruiting), non- significant (ns), fold (x)

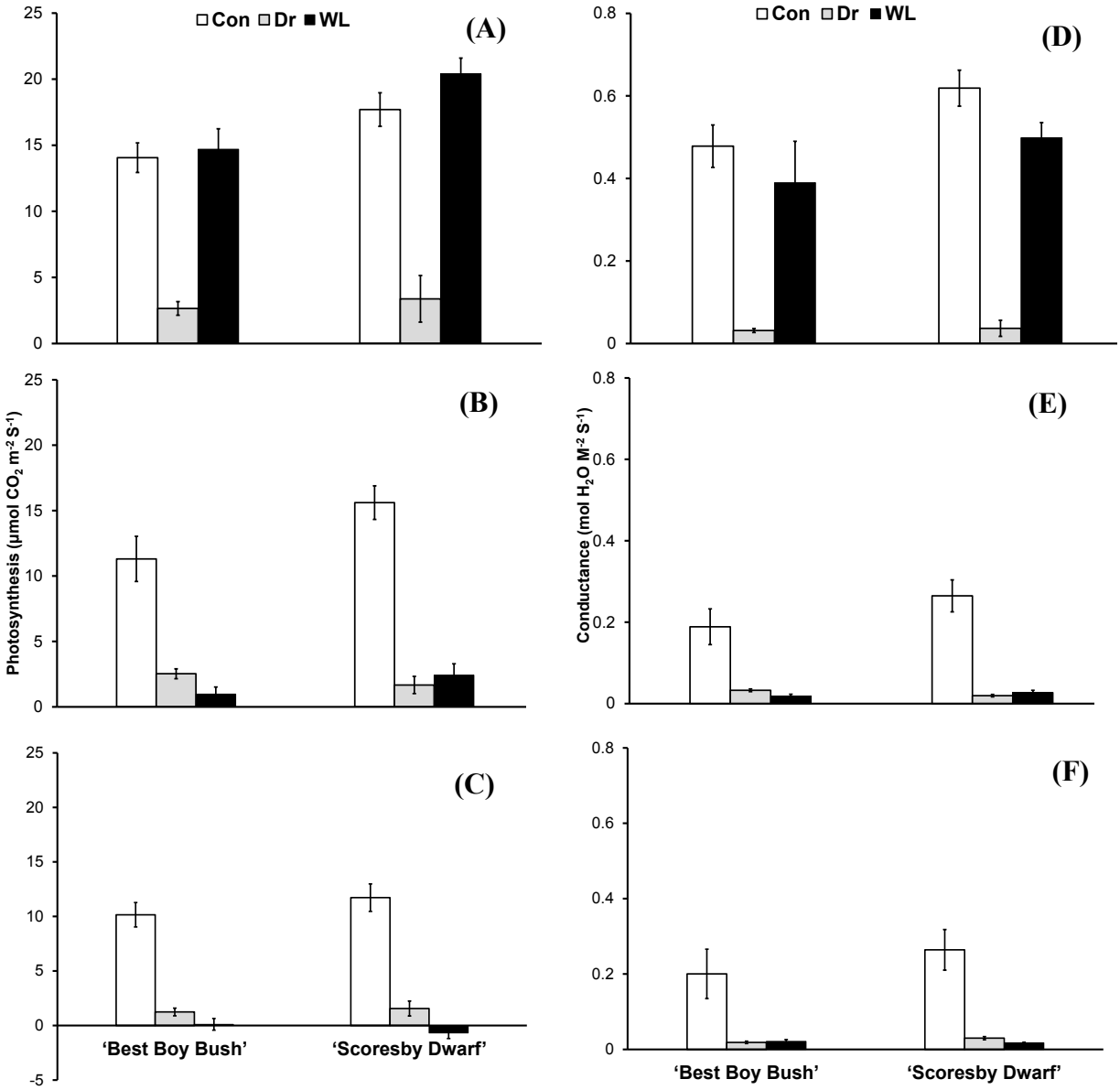


Figure 4.4.2.a Photosynthesis at the vegetative (A), flowering (B) and fruiting (C) stages, and stomatal conductance at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

4.4.2.2 Interaction effects

Water x cultivar

Averaged across developmental stages, waterlogging decreased photosynthetic and transpiration rates by 51% - 56% in 'Scoresby Dwarf' and by 56%-57% in 'Best Boy Bush'. Drought decreased these rates by 82% in the latter cultivar and by 85%-88% in the former. (Table 4.4.2.a, b, Figure 4.4.2.a, b and Appendix D).

Water x Developmental stage

Averaged across cultivars, water deficit caused reduction of 81% to 95% in all the components of leaf gas exchange at all developmental stages (Table 4.4.2.a, b, Figure 4.4.2.a, b and Appendix D). Reductions in these traits were mostly observed at later developmental stages under waterlogging (-86% to -103%, i.e. dropping down to net respiration). SPAD values, on the other hand only decreased by 15% at the vegetative stage under drought stress.

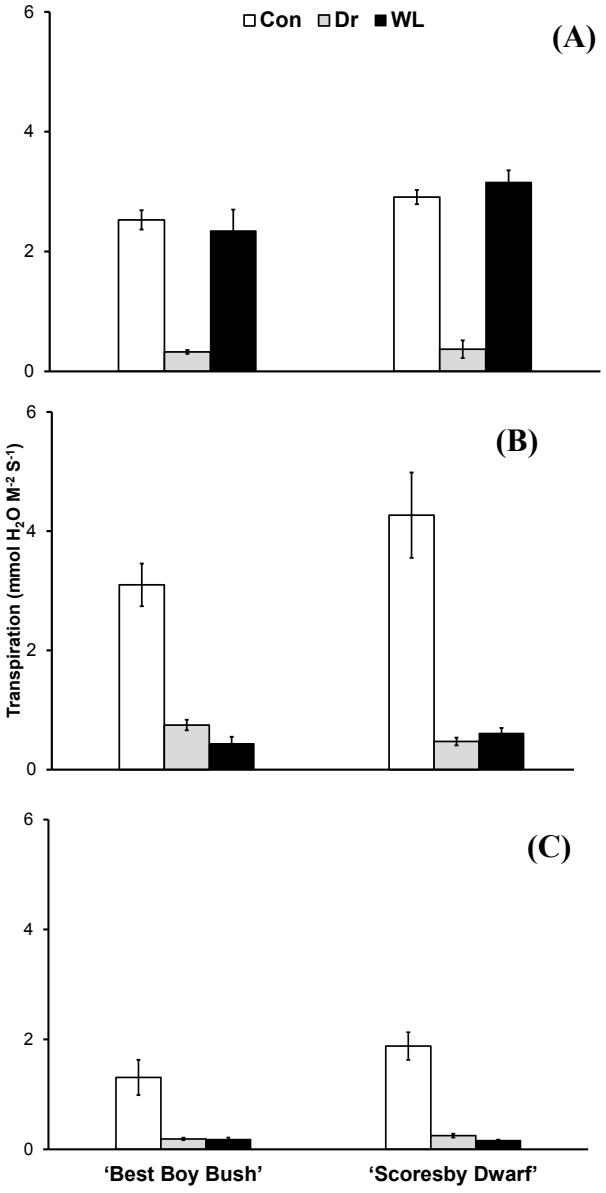


Figure 4.4.2.b Transpiration at the vegetative (A), flowering (B) and fruiting (C) stages of two tomato cultivars grown well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

4.4.2.3 Summary of the key findings

- ❖ Reductions in all the components of leaf gas exchange were observed at all developmental stages under drought and at later developmental stages under waterlogging, with net respiration rates at fruiting.
- ❖ 'Scoresby Dwarf' had higher photosynthetic and transpiration rates relative to 'Best Boy Bush'.

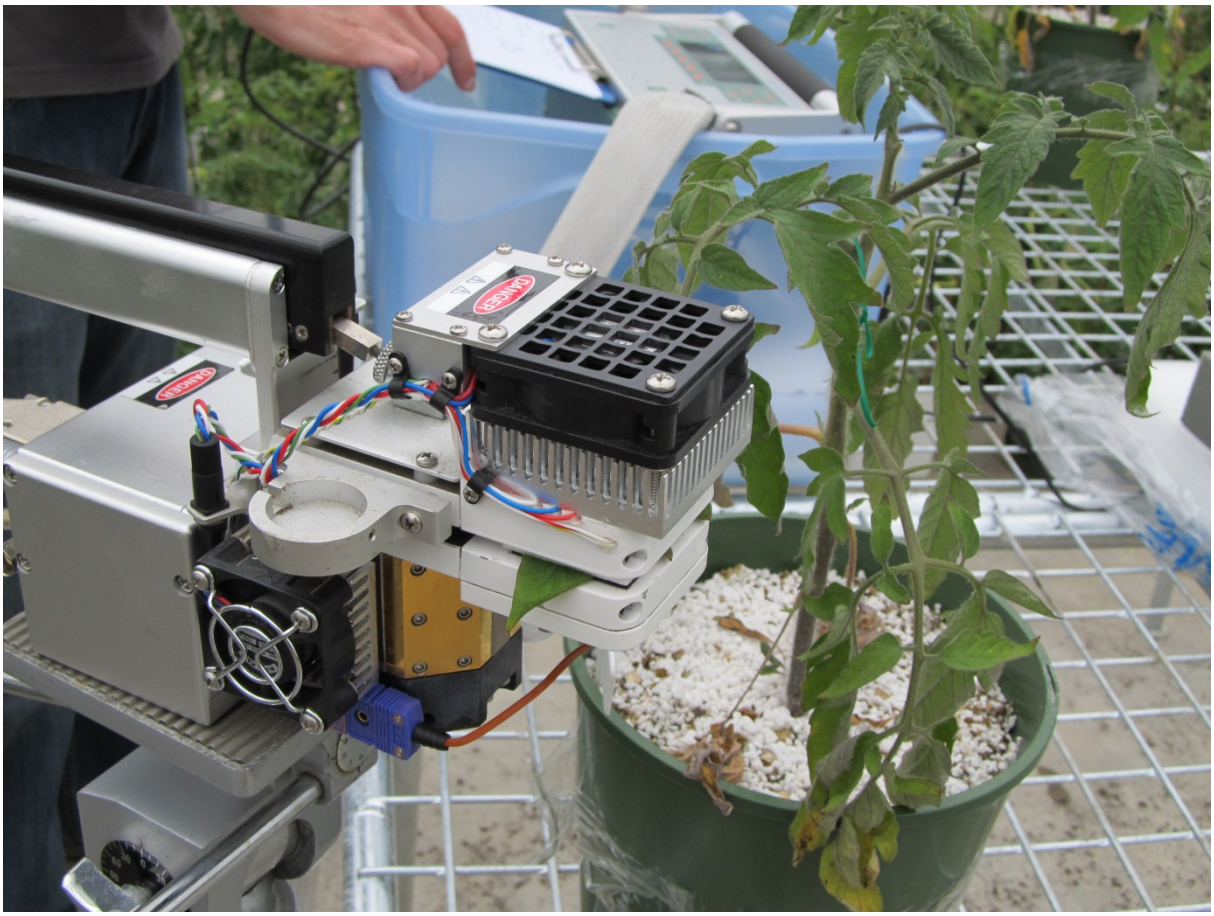


Plate 3. Leaf gas exchange measurement of water stressed tomato plants using LI-COR model 6400 infrared gas analyser (IRGA)

4.5 Results of the field studies

4.5.1 Water status, osmotic adjustment and proline levels

4.5.1.1 Main effects

Averaged across cultivars, water deficit caused reductions in TDR, LRWC and leaf osmotic potential by 44% to 82%, whilst water potential decreased 4.2-fold (Table 4.5.1.a, b, Figure 4.5.1.a, b, c and Appendix D). Waterlogging reduced the levels of these traits to a lesser degree, but increased TDR values by 58%. Proline levels in leaf and root tissues increased by 76% and 32%, respectively, whist they decreased by 34% to 41% under waterlogging.

Averaged across cultivars and water treatments, proline levels were increased from Day 2 to Day8 in leaves (28%-56%) whilst increases by 24%-36% in root tissues were independent of time (Table 4.5.1.a, b, Figure 4.5.1.a, b, c and Appendix D).

Table 4.5.1.a Summary of P values of the main effects and interactions with water treatment for soil and plant water status, osmotic adjustment and proline levels

Traits	Water	Cultivar	Time	Water x Cultivar	Water x Time	Water x Cultivar x Time
TDR (%)	<.001	0.237	-	0.667	-	-
LRWC (%)	<.001	0.154	-	0.383	-	-
Water potential (MPa)	<.001	0.801	-	0.356	-	-
Osmotic Potential (MPa)	<.001	0.824	-	0.598	-	-
Adjusted osmotic potential (Mpa)	0.422	0.382	-	0.581	-	-
Proline leaf (µmol/g FW)	<0.001	0.785	<0.001	0.522	<0.001	0.314
Proline root (µmol/g FW)	<0.001	0.453	<0.001	0.985	<0.001	0.750

Raw data are shown in Appendix B

Table 4.5.1.b Summary of percentage change of the main effects and interactions with water treatment for soil and plant water status, osmotic adjustment and proline levels

Traits	Water	Time	Water x Time	
TDR (%)	Dr: -65% WL: 58%	-	-	
LRWC (%)	Dr: -44% WL: -32%	-	-	
Water potential (MPa)	Dr: -4.2x WL: -2.4x	-	-	
Osmotic Potential (MPa)	Dr: -82% WL: -42%	-	-	
Adjusted osmotic potential (Mpa)	ns	-	-	
Proline leaf (µmol/g FW)	Dr: 76% WL: -34%	T2: 28% T3: 44% T5: 46% T8: 56%	Dr T2: 76% Dr T3: 96% Dr T5: 2.4x Dr T8: 2.5x	WL T1: -24% WL T2: -41% WL T3: -39% WL T5: -41% WL T8: -46%
Proline root (µmol/g FW)	Dr: 32% WL: -41%	T2: 29% T3: 31% T5: 36% T8: 24%	Dr T1: -19% Dr T2: 60% Dr T3: 59% Dr T5: 75% Dr T8: 61%	WL T1: -31% WL T2: -44% WL T3: -42% WL T5: -51% WL T8: -56%

Drought (Dr), Waterlogging (WL), 'Scoresby Dwarf' (SBD), harvesting time (T), non-significant (ns), fold (x)

4.5.1.2 Interaction effects

Proline levels in leaves increased continuously under desiccation, from 76% higher levels two days after the imposition of drought stress to 2.5-fold at the end. Drought-induced increases in proline levels from day 2 were less pronounced and less continuous in roots (60%-75%) and (Table 4.5.1.a, b, figure 4.5.1.a, b, c and Appendix D). In contrast, waterlogging decreased proline levels from Day1 to Day8 in both leaf (-24% to -46%) and root tissues (-31% to -56%).

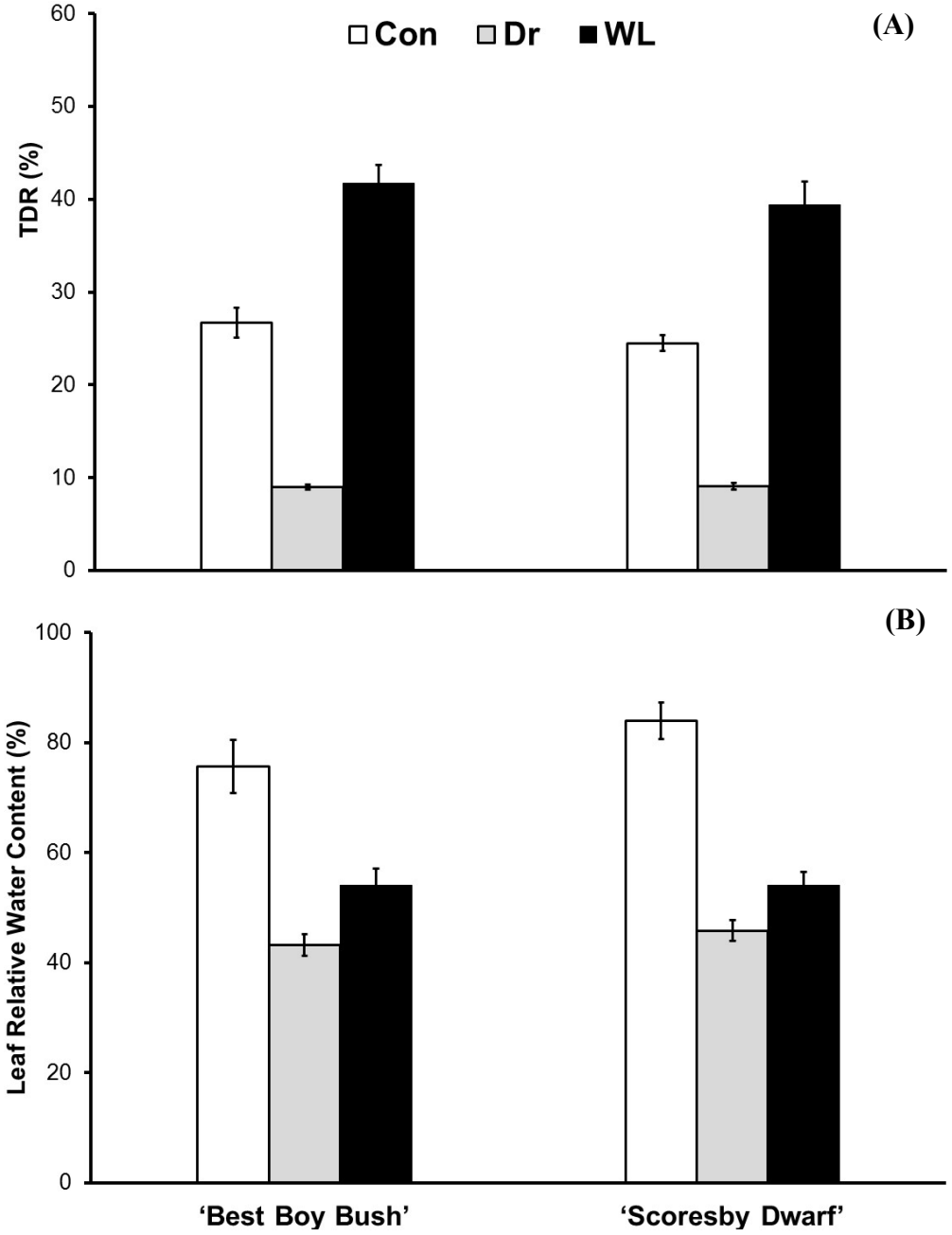


Figure 4.5.1.a TDR (A) and leaf relative water content (B) of tomato cultivars, 'Scoresby Dwarf' and 'Best Boy Bush' after eight days of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

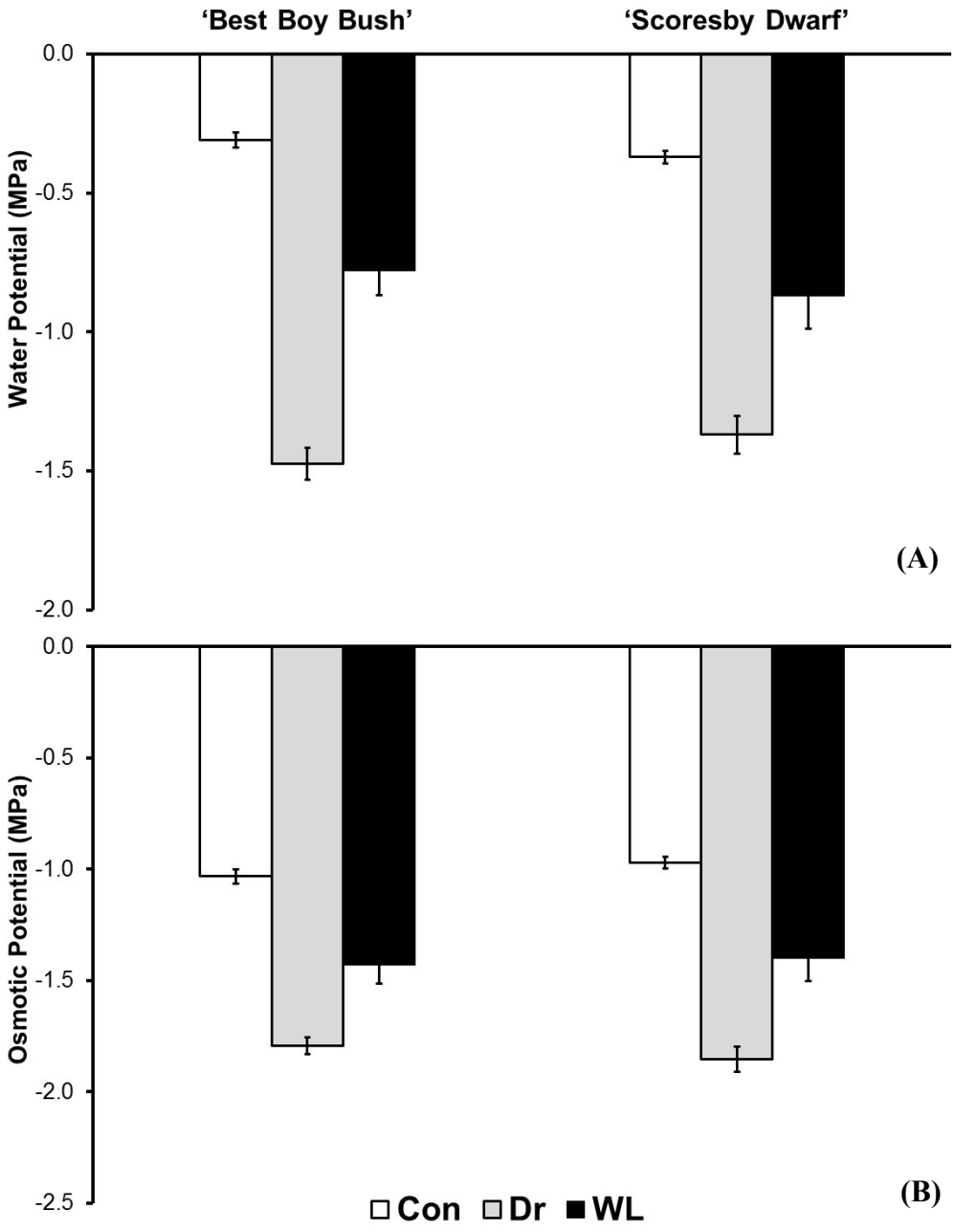


Figure 4.5.1.b Water potential (A) and osmotic potential (B) of tomato cultivars, 'Scoresby Dwarf' and 'Best Boy Bush' after eight days of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

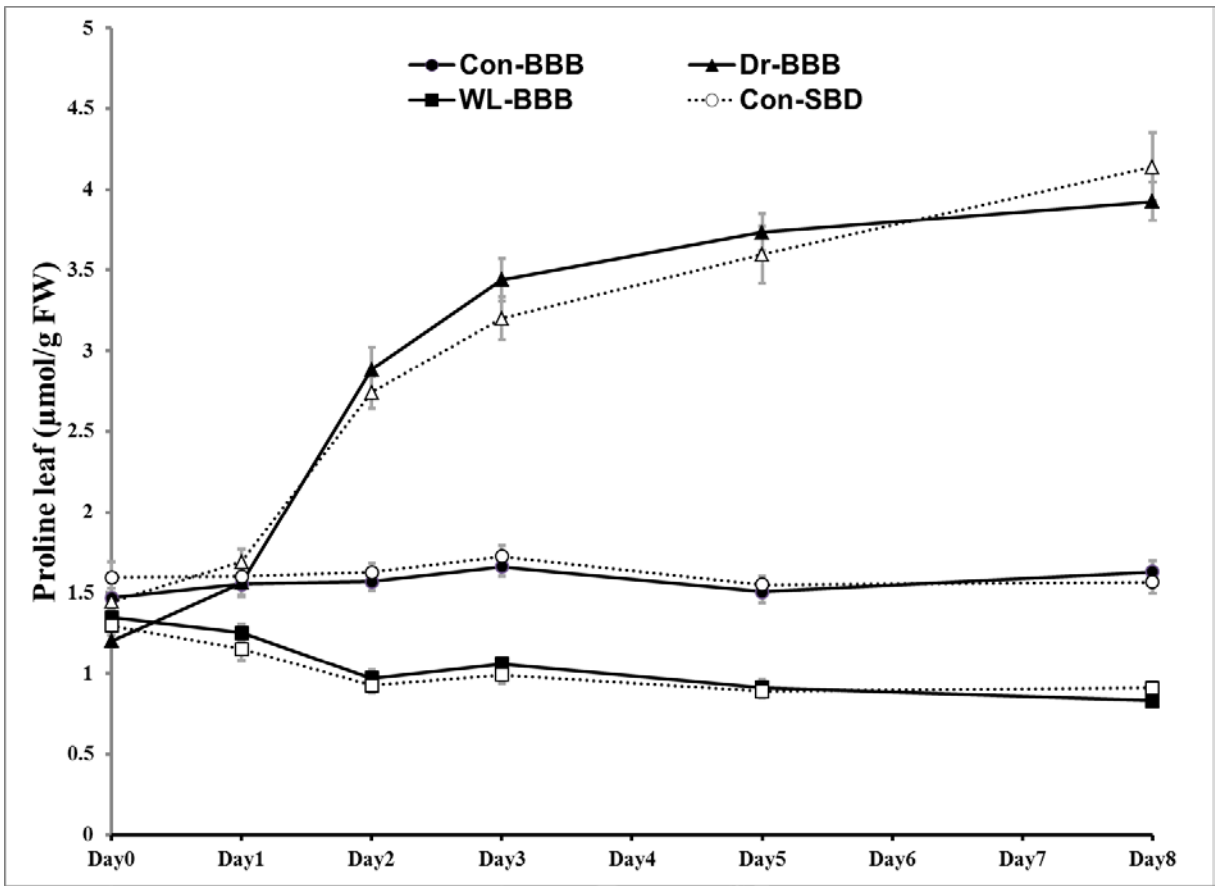


Figure 4.5.1.c Proline content in leaf tissue of two tomato cultivars, 'Scoresby Dwarf' and 'Best Boy Bush', during eight days of exposure to well-watered control conditions (Con), drought (Dr), and waterlogging (WL); values are means (n=10) ± standard error.

4.5.1.3 Summary of the key findings

- ❖ Eight days after exposure to water stress extremes, plant water status was reduced under both water deficit and to a lesser degree also under waterlogging.
- ❖ Proline levels increased strongly and progressively in leaf tissues and to a lesser degree in root tissues under water deficit, whilst waterlogging decreased the levels of this free amino acid.

4.5.2 Leaf gas exchange

Leaf gas exchange including photosynthesis rates, stomatal conductance and transpiration rates all decreased under water deficit (-87% to -92%) and under waterlogging (-55% to -62%) (Table 4.5.2.a, b, Figure 4.5.2.a, b and Appendix D). 'Scoresby Dwarf' had 23% lower transpiration rates relative to 'Best Boy Bush'.

Table 4.5.2.a Summary of P values of the main effects and interactions of water treatment, cultivar and harvesting time for leaf gas exchange attributes.

Traits	Water	Cultivar	Water x Cultivar
Photosynthesis (µmol CO_2 m^{-2} s^{-1})	<.001	0.356	0.356
Stomatal conductance (mol H_2O m^{-2} s^{-1})	<.001	0.099	0.221
Transpiration (mmol H_2O m^{-2} s^{-1})	<.001	0.041	0.451
SPAD	0.232	0.840	0.399

Raw data are shown in Appendix B

Table 4.5.2.b Summary of percentage changes of the main effects and interactions with water treatment for leaf gas exchange attributes.

Traits	Water	Cultivar	Water x Cultivar
Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Dr: -91% WL: -62%	ns	ns
Stomatal conductance (mol H_2O m^{-2} s^{-1})	Dr: -92% WL: -62%	ns	ns
Transpiration (mmol H_2O m^{-2} s^{-1})	Dr: -87% WL: -55%	SBD: -23%	ns
SPAD	ns	ns	ns

Drought (Dr), Waterlogging (WL), 'Scoresby Dwarf' (SBD), harvesting time (T), non-significant (ns), fold (x)

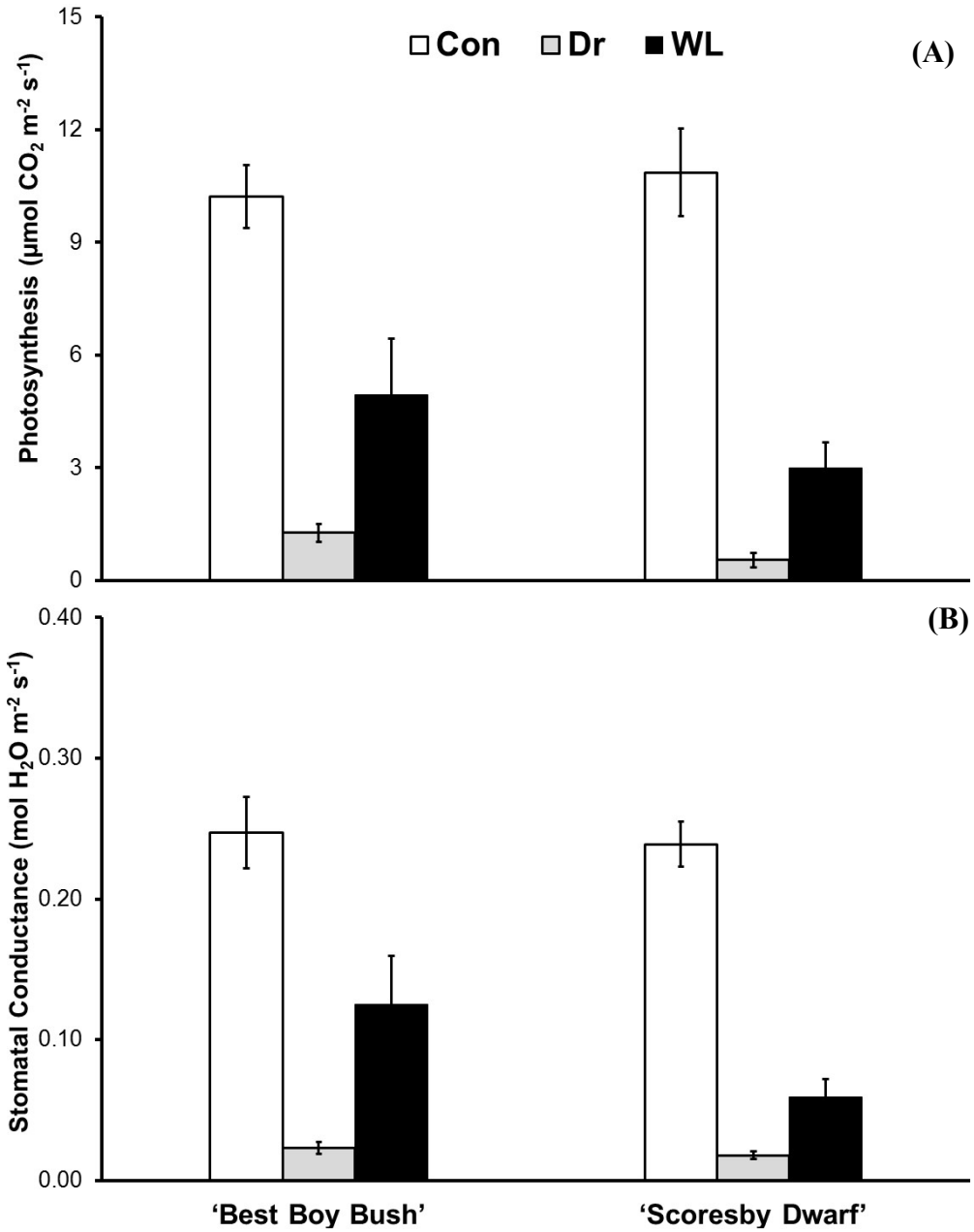


Figure 4.5.2.a Photosynthesis (A) and stomatal conductance (B) of tomato cultivars, 'Scoresby Dwarf' and 'Best Boy Bush' after eight days of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

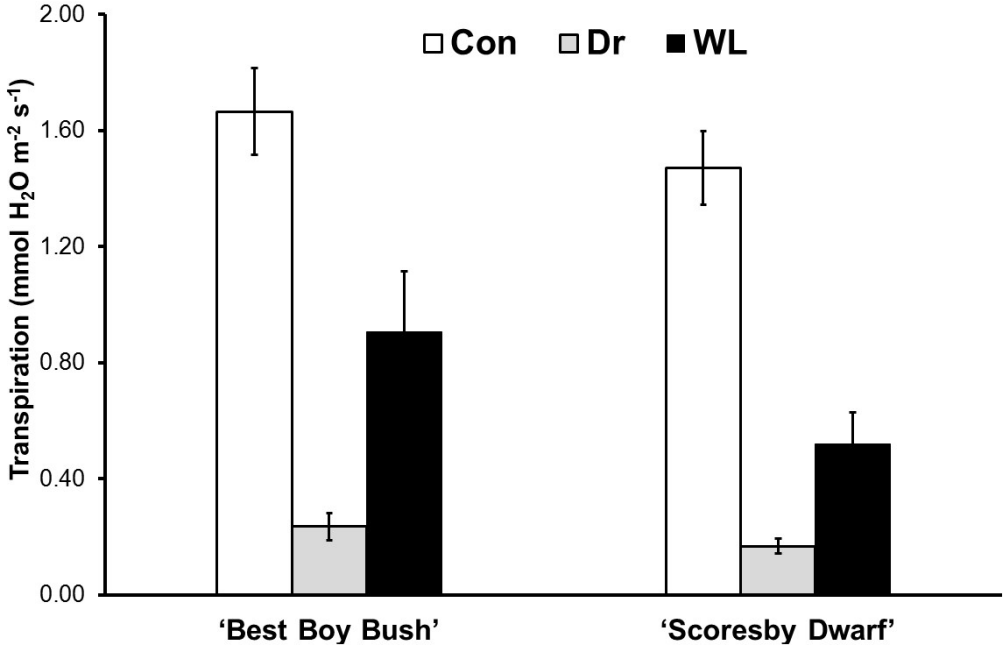


Figure 4.5.2.b Transpiration of tomato cultivars, 'Scoresby Dwarf' and 'Best Boy Bush', at the beginning of the treatment period (Day0) and after eight days of exposure to control conditions (Con), drought (Dr) and waterlogging (WL) conditions; values are means (n=10) ± standard error.

4.5.2.1 Summary of the key findings

- After eight days of exposure to water stress, water deficit and waterlogging caused reductions in all the components of leaf gas exchange. These reductions were more pronounced in plants subjected drought stress compared with plants subjected to waterlogging.

4.6 Multivariate traits response to the principal component analysis (PCA)

4.6.1 Plant trait responses to the effect of water stress in the glasshouse

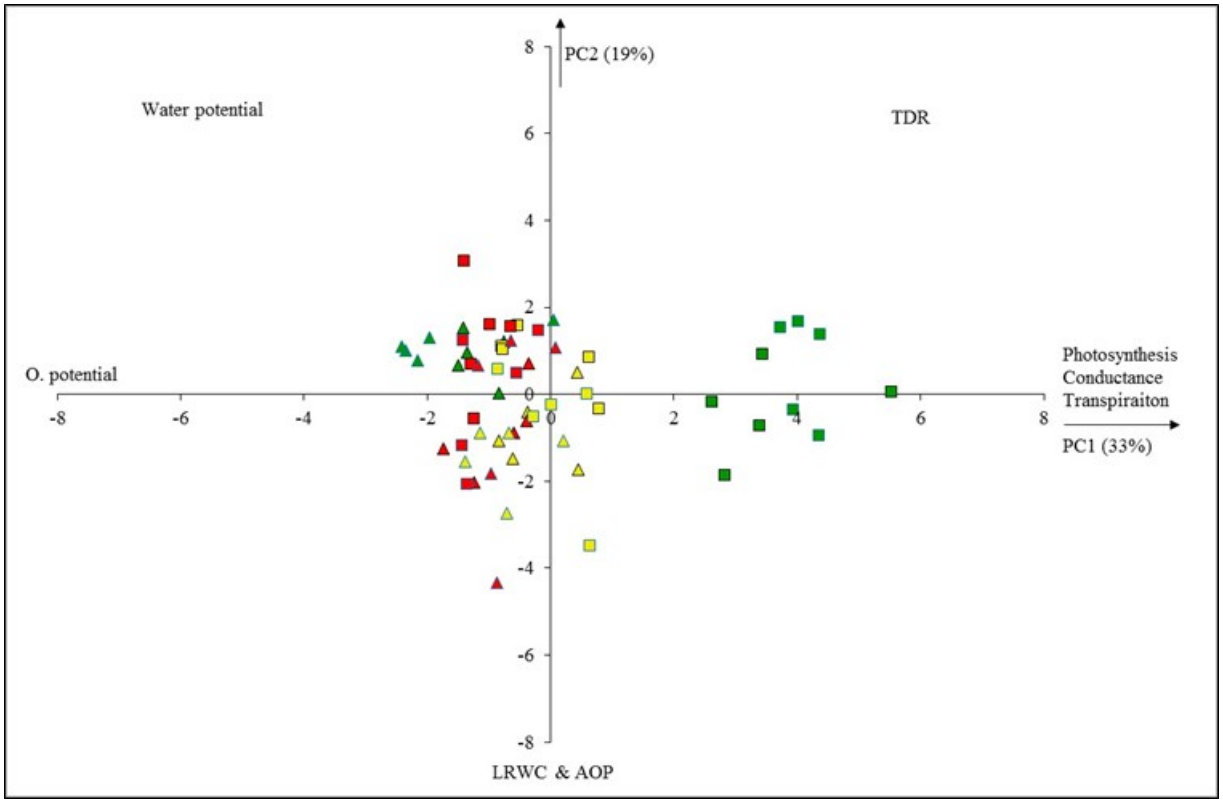


Figure 4.6.1.a Biplot of treatment responses (ratios of treatment/control) of two tomato cultivars at differential developmental stages. Triangles represent drought responses at the vegetative (▲), flowering (△), fruiting (▲) stages. Squares represent waterlogging responses at the vegetative (■), flowering (□), fruiting (■) stages.

4.6.1.1 Plant trait responses to water stress in the first principal component

The first principal component (PC1) accounted for 33% of all variance in the dataset (Figure 4.6.1.a). High positive PC1 scores associated with the leaf gas exchange responses. This was inversely correlated with osmotic potential changes at the negative part of this axis, which also showed an association with water potential changes, together with PC2. In addition, the positive end of PC1 and PC2 correlated with increasing TDR values.

4.6.1.2 Developmental stages and cultivar responses to water stress in the first principal component

PC1 separated waterlogging responses of both cultivars at the vegetative stage from the remainder of plant responses (Figure 4.6.1.a).

4.6.1.3 Plant trait responses to water stress in the second principal component

PC2 explained 19% of the variance in the dataset (Figure 4.6.1.a). Negative PC2 scores were associated with changes in leaf relative water content and adjusted osmotic potential.

4.6.1.4 Developmental stage and cultivar response to water stress in the second principal component

PC2 provided a developmental separation of drought responses for both cultivars, which showed positive scores at the vegetative stage and a majority of negative PC2 scores at the reproductive stages.

4.6.2 Plant trait responses to the effect of water stress in the field

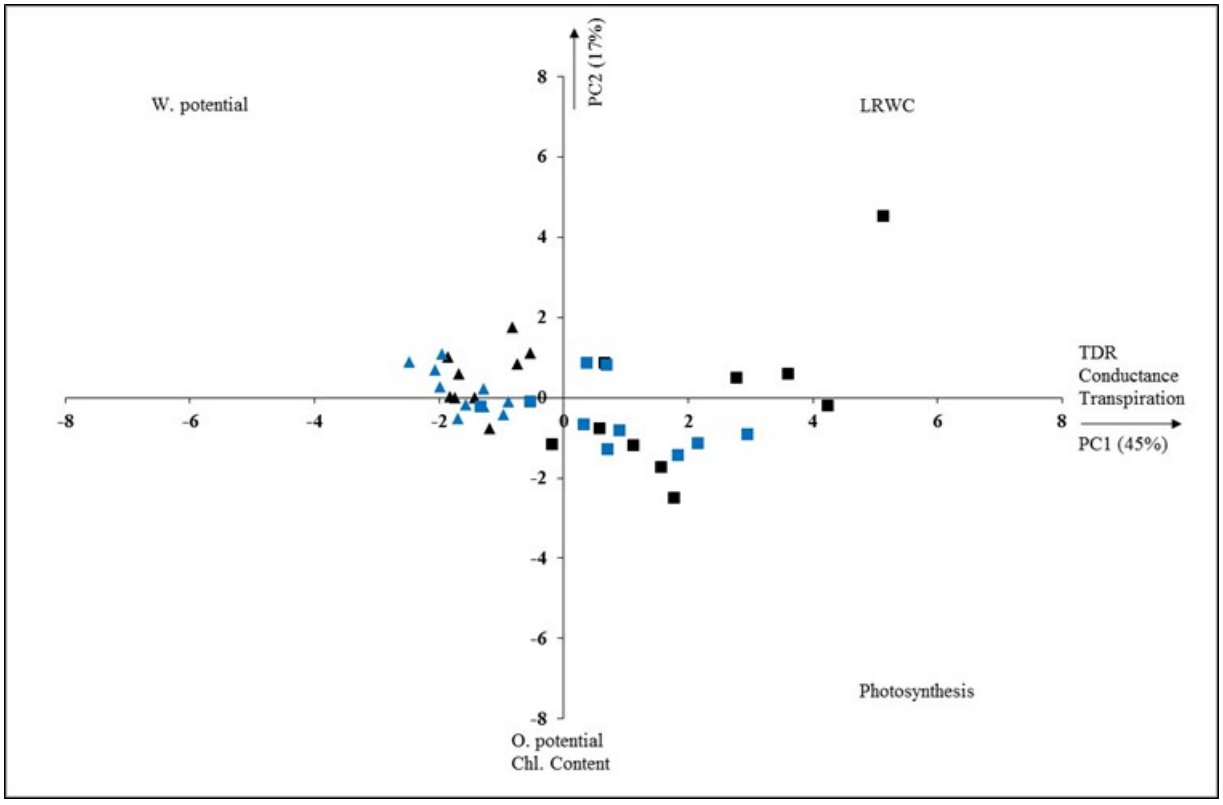


Figure 4.6.2.a Biplot of treatment responses (ratios of treatment/control) of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf' after eight days exposure to drought and waterlogging. Symbols: 'Best Boy Bush'- drought (▲) and waterlogging (■). 'Scoresby Dwarf'-drought (▲) and waterlogging (■).

4.6.2.1 Plant responses to water stress in the first principal component

PC1 explained 45% of the variance in the dataset, more than double than the next principal component (Figure 4.6.2.a). High scores of the axis were associated with increasing TDR values, stomatal conductance and transpiration rates. Photosynthesis rate was also correlated with this end of PC1 as well as with the negative end of PC2, and PC1 also correlated, together with PC2, with changes in water potential and LRWC. PC1 clearly separated waterlogging from

drought responses across both cultivars: plants subjected to waterlogging generally had positive PC1 scores, whilst plants under drought stress had negative PC1 scores (Figure 4.6.2.a).

4.6.2.2 Plant responses to water stress in the second principal component

The second principal component accounted for 17% of the variance in the dataset (Figure 4.6.2.a). Positive PC2 scores were related together with PC1 to changes in plant water status, including leaf relative water content and leaf water potential (Figure 4.6.2.a). The majority of drought response scores were located towards the positive part of PC2, and the opposite trend occurred for waterlogging responses.

4.7 Discussion

A number of studies have examined physiological responses of tomato plants to either water deficit or waterlogging (e.g: Calcagno et al., 2011; Horchani et al., 2008; Jackson et al., 2003; Zgallai et al., 2005). However, there appears to be no study that compares the physiological responses of these two stress factors (water deficit and waterlogging). Aloni and Rosenshtein (1982) suggested that complete root submergence induces a water deficit in the rhizosphere of tomato plants. Therefore, it is plausible that water deficit and waterlogging induce the same physiological responses. This chapter set out to test this hypothesis in plants harvested at the same time after exposure to the two water stress extremes.

4.7.1 Plant water status and osmotic adjustment

The effects of water deficit can vary depending on the different rates of imposition and severity of the stress, developmental stages, leaf age, as well as plant species (Chaves et al., 2003). It has been suggested that plants respond to water deficit via hydraulic signalling (Lipiec et al., 2013). This signalling follows a reduction in root water uptake, resulting in changes to leaf water and leaf turgor potential, followed by stomatal closure. Other typical responses include a reduction in leaf elongation and osmotic adjustment (Lipiec et al., 2013).

In this study, drought – and to a lesser degree waterlogging – decreased tomato plant water status in the form of decreases in plant water potential attributes and also in LRWC (Figure 4.4.1.b). This is in line with other findings of drought responses in tomato and other species (Calcagno et al., 2011; Sanchez-Rodriguez et al., 2010a; Yuan et al., 2010). Sanchez-Rodriguez et al. (2010a) suggested that plant water status measurements such as LRWC can be used to identify genotypes that are tolerant or susceptible to water stress in wheat crops. Leaf water potential depends on its component factors, especially osmotic potential and turgor potential, and also tissue rigidity (Calcagno et al., 2011).

The decreases in osmotic potential observed here under both water stress factors are in line with similar findings elsewhere, showing osmotic potential decreases in drought stressed tomato plants (Calcagno et al., 2011) and under waterlogging (Jackson et al., 1996). The results from the present study demonstrate that under drought, this increase in osmolality was not merely a passive result of water loss, but was due to the active accumulation of solutes in osmotic adjustment (Figure 4.4.1.c): the adjusted osmotic potential was lower (i.e. more negative) under drought than under control conditions in the tomato plants. Osmotic adjustment is considered a crucial mechanism for plant stress tolerance under limited water availability. This is in line with the multivariate component analysis in this study. In the PCA of the glasshouse studies, PC2

acted as a developmental ‘stress tolerance axis’ showing that decreasing adjusted osmotic potential (i.e. osmotic adjustment) under drought at the later developmental stages was related to maintenance of LRWC and to lesser decreases in water potential (Figure 4.6.1.a).

Osmotic adjustment is a slow process involving the accumulation of osmotically active solutes that are compatible with normal cell function such as proteins, amino acids (e.g. proline), quaternary ammonium compounds (QACs) and carbohydrates. This process facilitates water absorption into the cell, increasing cell turgor but does not improve plant growth or yield (Chaves et al., 2003; Selim & El-Nady, 2011). These osmo-regulators also can take the protective role of water molecules so that cellular proteins and membranes are stabilised (Chaves et al., 2003).

There is limited information available on osmotic adjustment responses in plants under waterlogging. Our findings showed that despite a strong dehydrating effect on plants under hypoxia, 'Scoresby Dwarf' was able to maintain its adjusted osmotic potential levels under waterlogging, whilst these levels increased in 'Best Boy Bush'. The latter cultivar was therefore unable to counter the overabundance of water under waterlogging. By increasing its adjusted osmotic potential it had less osmotically active cellular processes available, thus contributing to strong water status decreases.

As one of the compatible solutes in osmotic adjustment, proline acts as an osmo-regulator and also a ROS scavenger (Selim & El-Nady, 2011; Torrecillas et al., 1995). High levels of free proline in cells prevents proteins from denaturing and protects enzymes and cellular membranes (Chaves et al., 2003). Under drought, the increases in proline levels were less pronounced in ‘Best Boy Bush’ than in ‘Scoresby Dwarf’, further underlining the stress resistant nature of the latter cultivar.

In contrast to drought, proline levels decreased under waterlogging at all developmental stages in the glasshouse study and also in the kinetic analysis under field conditions (Figure 4.4.1.d). Aloni and Rosenshtein (1982) have observed increases and decreases of proline under waterlogging. The findings from the present study underline the high level of stress introduced by the waterlogging treatment in this study, demonstrated by the fact that the tomato plants were not able to produce this key osmoprotective amino acid against the desiccation effects of waterlogging (Ashraf, 2012).

4.7.2 Leaf gas exchange

Several studies have reported a reduction in stomatal conductance, photosynthetic and transpiration rates when tomato plants were subjected to drought stress (e.g: Haupt-Herting & Fock, 2000; Torrecillas et al., 1995; Yuan et al., 2010; Selim & El-Nady, 2011). In the PCA of the glasshouse studies, PC1 identified changes in leaf gas exchange as the main water stress response and also showed a clear developmental aspect as well as relationships with water potential parameters under waterlogging (Figure 4.6.1.a, b). Thus, increases of water potential and maintenance of osmotic potential under waterlogging at the vegetative harvest were related to the maintenance of leaf gas exchange parameters (highest PC1 scores). Conversely, reductions of leaf gas exchange parameters under hypoxia occurred progressively at the later developmental stages (low PC1 scores), in tandem with reductions in osmotic potential and water potential. The fact that waterlogged plants showed net respiration (i.e. CO_2 emission) demonstrated how stressed these plants were at fruiting (Figure 4.4.2.a). This could be attributed to the increases (less negative values) of adjusted osmotic potential mentioned above, thus reducing the protective effect of osmotic adjustment.

In the PCA of the field studies, PC1 provided a similar picture to PC1 of the glasshouse studies by identifying changes in leaf gas exchange parameters and in TDR values as the main stress response combination, and again juxtaposition of these traits with osmotic potential (Figure 4.6.2.a). In addition, it revealed a clear separation between drought and waterlogging responses. In this, increases in TDR values under waterlogging (high PC1 scores) correlated with less pronounced decreases in leaf gas exchange parameters. In contrast, pronounced decreases in gas exchange were associated with decreases in TDR values under drought, as well as decreases in osmotic potential (negative scores on PC1).
Evidence from the literature suggested that stomatal conductance played an integral role under either water stress extreme (drought or waterlogging) (Chaves et al., 2003) or waterlogging (Ashraf, 2012). The closure of stomata under water deficit minimises water loss, thereby protecting plant cells from dehydration. Ashraf (2012) proposed that stomatal closure under waterlogging was to limit water uptake by the root system.

Under drought stress, stomatal closure is mediated via root signalling of abscisic acid (ABA) (Chaves et al., 2003; Lipiec et al., 2013; Reddy et al., 2004). ABA is synthesised in the root and transported through the xylem to the leaves, where it triggers the closure of stomata and limits leaf expansion. The activity of ABA is thought to be a first order response to drought stress before any observable change in water status and nutrient status was seen (Lipiec et al., 2013).

There is, however, some conflict in the literature about whether ABA plays a role in stomatal closure when the roots are submerged in water for any length of time. About four decades ago, Jackson and Campbell (1976) suggested that stomatal closure in waterlogged plants was not triggered by ABA. The rationale was that the transporting pathway from root to shoot was inhibited under prolonged root hypoxic conditions. Recently, Ashraf (2012) reported that the stomatal closure of pea plants, which were suffering stress caused by flooding, was promoted by ABA.

Severe drought stress can inhibit photosynthetic activity because of a significant reduction in CO_2 diffusion into the inner leaf tissues (Chaves et al., 2003) or because there is an imbalance between light harvesting and utilisation (Reddy et al., 2004). This imbalance induces a change in quantum yield (F_v/F_m) which results in excessive light energy and an acceleration of ROS production. A limitation of the CO_2 supply to the leaf, and the alteration of photosystem activities may result in the accumulation of reactive oxygen species (ROS will be discussed in the next chapter) via the chloroplast Mehler-reaction (Reddy et al., 2004). The results from the present study suggest that it is CO_2 supply, rather than pronounced changes in F_v/F_m that characterise the stress response in the tomato plants.

4.7.3 Conclusions

The results from the field studies were in general agreement with those obtained under glasshouse conditions for the physiological parameters measured here. Both water deficit and waterlogging led to significant reductions in plant water status attributes, as well as decreased photosynthetic and transpiration rates as a consequence of stomatal closure. These effects were most pronounced under drought. Plant water status reductions became less pronounced under desiccation with increasing plant maturity, while the opposite was observed under waterlogging. This can be explained by the fact that classic drought tolerance mechanisms such as osmotic adjustment did not operate under waterlogging. Osmotic adjustment was even reduced in waterlogged 'Best Boy Bush' plants at fruiting, but not in 'Scoresby Dwarf'. That cultivar also showed higher increases in proline accumulation under drought, further underlining its stress tolerance. These cultivar differences occurred under glasshouse, but not under field conditions.

Chapter 5

The time-dependent influence of water deficit and waterlogging on oxidative stress attributes and antioxidant metabolism in two tomato cultivars

Abstract

This chapter investigated the biochemical responses of two tomato cultivars at three key developmental stages (vegetative, flowering and fruiting) to water stress (water deficit and waterlogging) in the glasshouse. These biochemical responses included the accumulation of reactive oxygen species, evidence of oxidative stress and activation of the antioxidant defence system (enzymatic antioxidants and non-enzymatic antioxidants) in leaves and roots, after 14 days of exposure to water stress. Levels of hydrogen peroxide were highly elevated in both leaves (92%) and roots (44%) of drought plants and in leaves (2.6-fold) of plants subjected to waterlogging. Oxidative damage was measured by the increasing production of lipid hydroperoxides (2.9-34.1-fold) and protein carbonyls (29% to 5-fold) in the tissues. Plants subjected to water deficit increased the activity of all antioxidant enzymes by 22-54%, while plants subjected to waterlogging generally suppressed the activity of these enzymes (-41% to -59%). Levels of non-enzymatic antioxidants all increased under drought stress and waterlogging, except for reduced ascorbate. The ascorbic acid content was increased by 48% in the fruit pericarp and by 18% in the skin of tomato fruits grown under water deficit relative to other fruits. Compared to the tomato cultivar 'Scoresby Dwarf', 'Best Boy Bush' was more sensitive as it contained higher levels of oxidative stress markers and had lower activity of enzymatic antioxidants and lower production of non-enzymatic antioxidants under stress. In conclusion, the results of this study provide strong proof of distinctive oxidative stress response patterns under the two water stress extremes with clear evidence for intraspecific differences in these responses. An overall multivariate analysis of all data from the glasshouse studies is also presented here, and will be discussed in the General Discussion section.

5.1 Introduction

Plants accumulate reactive oxygen species (ROS) under various biotic and abiotic stresses, including superoxide anions, singlet oxygen, hydrogen peroxide and hydroxyl radicals. When ROS are generated in excess they need to be removed to maintain normal plant growth and function (Eshdat et al., 1997). Among these ROS, H_2O_2 is a common signalling molecule as it is highly diffusible through membranes and relatively stable (Habibi, 2014; Hajiboland, 2014). The accumulation of hydrogen peroxide (H_2O_2) can cause lipid peroxidation when this compound attacks polyunsaturated fatty acids (Burritt & Mackenzie, 2003; Foyer et al., 1997; Habibi, 2014). If not detoxified, H_2O_2 can convert to $OH^{\bullet-}$, which is a highly toxic ROS that can oxidize proteins and damage DNA (Hajiboland, 2014). Therefore, plants have evolved a complex antioxidant defence system to cope with the hazardous effects of ROS (Burritt &

Mackenzie, 2003; Gill & Tuteja, 2010; Noctor et al., 2012). This defence system comprises enzymatic antioxidants (especially superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase and glutathione peroxidase) and non-enzymatic antioxidants (such as ascorbate and glutathione) (Gill & Tuteja, 2010). Oxidative stress in plants occurred when there was an imbalance between ROS production and the defence capacity of plants (Hajiboland, 2014).

Some research been undertaken on the production of H_2O_2 and the antioxidant system in tomato plants under water stress (both drought and waterlogging) (Lin et al., 2004; Sanchez-Rodriguez et al., 2012). Oxidative damage in plants has mostly been monitored by measuring the presence of malondialdehydes (secondary products of lipid peroxidation) (Ahsan et al., 2007; Sanchez-Rodriguez et al. 2012) but not lipid hydroperoxides (primary products of lipid peroxidation). In addition, these studies only investigated either leaf or root tissues or they failed to show the presence of stress markers. To the best of the author's knowledge, there has been no study that has compared biochemical responses from the two extremes of water stress (water deficit and waterlogging). Similarly, there have been no studies investigating the antioxidative systems under water stress or comparing the different developmental stages in the different tissues of tomato plants. The likely differences in terms of cultivar responsiveness to stress have already been mentioned in Chapter 3.

The general objective of this chapter was, therefore, to examine key oxidative plant responses at the various developmental stages of tomato plants under water stress (a water deficit and waterlogging). This included the investigation of the antioxidant defence system, including both enzymatic and non-enzymatic antioxidants. It was hypothesised that these responses will depend on the plants' developmental stage and will differ between the two tomato cultivars selected.

5.2 Material and methods

The tomato plants' growing conditions and the experimental design have already been outlined in Chapter 3 (3.2.1: Glasshouse experimental design for water stress). In addition, details of the analyses of specific plant biochemical compounds, including hydrogen peroxide, lipid hydroperoxides, protein carbonyls, the activities of antioxidant enzymes and levels of antioxidants present in various tissues of tomato plants are described below:

Leaves: Laminae from the second fully unfolded leaf (approx. 1000 mg of tissue) were sampled and immediately frozen in liquid nitrogen and stored at -80 °C prior to analysis.

Roots: About 1000 mg of washed roots were taken from the root system after a gentle wash with a water gun. The roots were placed in a 12.5 mL microtube and immediately frozen in liquid nitrogen. The frozen, fresh tissue was stored at -80 °C prior to analysis.

Fruit samples: Tomato fruits were halved and then half the fruit was peeled with a knife. The skin of the tomato fruit was frozen in liquid nitrogen. The other half of each fruit was chopped into small pieces and frozen in liquid nitrogen. The frozen fresh tissue of the skin and diced fruit was stored at -80 °C prior to analysis.

5.2.1 Extraction and assay of hydrogen peroxide and oxidative damage parameters

5.2.1.1 Hydrogen peroxide extraction and assay

Tomato tissue was ground to a fine powder in liquid nitrogen and 50 mg of powdered tissue was homogenised in 450 µL of ice-cold sodium phosphate buffer (100 mM phosphate buffer, pH 7.0) containing the catalase inhibitor, hydroxylamine (1 mM), and then centrifuged at 12 000 g for 15 min at 4°C (Burritt, 2012). H_2O_2 levels were determined colourimetrically as described by Cheeseman (2006). Briefly, the assay mixture contained 250 µM ferrous ammonium sulphate, 100 µM sorbitol, 100 µM xylenol orange in 25 µM H_2SO_4 and 1% ethanol. The absorbance was measured at 550 and 800 nM, and the difference in absorbance between the two values was calculated. A standard curve was generated using standards prepared from 30% H_2O_2 (Merck Ltd, Palmerston North, New Zealand). The concentration of H_2O_2 in all standards was checked by measuring the absorbance at 240 nm and by calculating the actual H_2O_2 concentration using an extinction coefficient of 43.6 M^{-1} cm^{-1}.

5.2.1.2 Lipid hydroperoxide extraction and assay

Tomato tissue was ground to a fine powder in liquid nitrogen. Fifty mg samples were homogenized in 0.3 mL of methanol:chloroform (2:1 v/v) and left for a minute. Chloroform

(0.2 mL) was added and mixed for 30 seconds. Deionised water (0.2 mL) was added and mixed for 30 seconds. The phases were allowed to separate and the (lower) chloroform phase was transferred to a new tube (Bligh & Dyer, 1959).

5.2.1.3 Protein carbonyl extraction and assay

Total protein was extracted using the method of Phang et al. (2011), with minor modifications, by grinding the tomato tissue to a fine powder in liquid nitrogen and homogenising 50 mg of the powdered tissue in 450 µL of 100 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM Na2 EDTA, 1mM PMSF and 0.05% Triton X-100 (Phang et al., 2011) and 2% Polyclar AT (Serva Chemicals Ltd.). The resulting homogenate was then centrifuged at 12 000 g for 15 min at 4°C (Phang et al., 2011). The protein extracts where subjected to ultrafiltration using Porvair (Porvair Filtration Group Inc., Ashland, Virginia, USA) filtration plates (96-well 10KD MWCO) according to the manufacturer's instructions and reconstituted with 100 mM potassium phosphate (pH 7.0). Protein carbonyls levels were determined using the 2.4-dinitrophenylhydrazine (DNPH) method (Reznick & Packer, 1994) adapted for microplates. The protein carbonyl content was determined using the extinction coefficient of DNPH at 370 nm (0.022 μM^{-1} cm^{-1}), corrected for the calculated path-length of the solution (0.6 cm). The protein content of the extracts was determined using the Lowry protein assay (Fryer et al., 1986) and the protein carbonyl content was expressed as nmol carbonyls/mg protein (Schweikert & Burritt, 2012).

5.2.2 Antioxidant enzyme extraction and assays

Ascorbate peroxidase (APOX) was extracted using the method of Phang et al. (2011) with minor modifications. Tomato tissue was ground to a fine powder in liquid nitrogen and 50 mg of the powdered tissue was homogenised in 450 µL100 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM Na_2EDTA, 1% PVP-40, 1 mM PMSF, 0.05% Triton X-100 and 2% Polyclar AT (Serva Chemicals Ltd.). The resulting homogenate was centrifuged at 12 000 g for 15 min at 4°C (Phang et al., 2011). For APOX analysis 50 mg of powdered tissue was homogenised in 450 µL of 100 mM sodium phosphate buffer (pH 7.0) containing 1 mM Na2 EDTA, 1% PVP-40, 1% PVP-40, 1mM PMSF, 0.05% Triton X-100 and 2% Polyclar AT (Serva Chemicals Ltd.), to which 5 mM ascorbate was added (Burritt, 2008). The resulting homogenate was centrifuged at 12 000 g for 15 min at 4°C. The protein content of the extracts was determined using the Lowry protein assay (Fryer et al., 1986).

5.2.2.1 Superoxide dismutase (SOD, EC 1.15.1.1)

SOD was assayed using the microplate assay described by Banowetz et al. (2004) with a minor modification (Lister et al., 2010). Briefly, 50 µL of extract, diluted extract or standard (prepared from bovine liver SOD (Sigma–Aldrich, St. Louis, MO, U.S.A.), where one unit of SOD corresponded to the amount of enzyme that inhibited the reduction of cytochrome C by 50% in a coupled system with xanthine oxidase at pH 7.8 and 25°C), was mixed with 125 µL of freshly prepared reaction solution containing piperazine-1,4-bis (2-ethanesulfonic acid) (Pipes) buffer, pH 7.8, 0.4 mM o-dianisidine, 0.5 mM diethylenetriaminepentaacetic acid (DTPA) and 26 µM riboflavin. The absorbance at 450 nm (A450) was measured immediately (t = 0 min) and samples were illuminated with an 18 W fluorescent lamp placed 12 cm above the plate for 30 min (t = 30 min) and the A450 was measured again. A regression analysis was used to prepare a standard line relating SOD activity to the change in A450 and SOD activities in the extracts, calculated with reference to the standard line and expressed as units of SOD per milligram of total protein.

5.2.2.2 Catalase (CAT, EC 1.11.16)

CAT was assayed using the chemiluminescent method of Maral et al. (1977), as modified by Janssens et al. (2000) for 96-well microplates. Briefly, extracts were subjected to ultrafiltration, as detailed above, and then 50 µL of extract, diluted extract or standard (purified bovine liver CAT (Sigma–Aldrich, St. Louis, MO, U.S.A.) in homogenization buffer) was mixed with 100 µL of 100 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaEDTA and 10^{-6}M H_2O_2. Samples were then incubated at 25°C for 30 minutes, after which 50 µL of a solution containing 20 mM luminol and 11.6 units mL−1 of horseradish peroxidase (Sigma–Aldrich, St. Louis, MO, U.S.A.) was injected into each well and the light emission (the intensity of which was proportional to the amount of H_2O_2 remaining in the mixture) was measured. A regression analysis was used to prepare a standard line relating standard CAT activities to the intensity of light emission. CAT activities in the extracts were calculated with reference to the standard line and expressed as µM of H_2O_2 consumed per minute per milligram of total protein (Schweikert & Burritt, 2012).

5.2.2.3 Ascorbate peroxidase (APOX, EC 1.11.1.11)

APOX was assayed by following the decrease in A290 when ascorbate vanished (Burritt & Mackenzie, 2003). APOX activity was assayed by following the decrease in absorbance at 290 nM as ascorbate disappeared (Rao et al., 1996). The reaction mixture (200 µL) contained 100

mM potassium phosphate (pH 7.0), 0.5 mM ascorbate, 0.2 mM H_2O_2 and up to 50 µL extract. APOX activity (µmol min-^{1}) was calculated using an extinction coefficient of 2.8 mM^{-1} cm^{-1}, corrected for the calculated path-length of the solution (0.6 cm) (Schweikert & Burritt, 2012).

5.2.2.4 Glutathione reductase (GR, EC 1.6.4.2)

GR was assayed following the method of Cribb et al. (1989) with slight modifications (Schweikert & Burritt, 2012). Briefly, 50 µL of extract, diluted extract or standard (GR from wheat germ, Sigma–Aldrich, St. Louis, MO, U.S.A., in homogenization buffer) was mixed with 150 µL of 100 mM sodium phosphate buffer (pH 7.6) containing 0.1 mM 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) and 10 µL of NADPH (10 mg/ml; 12 mM). The reaction was initiated by the injection of 10 µL of oxidized glutathione (GSSG) (1 mg/ml; 3.25 mM) and the absorbance at 415 nM (A415) was measured every 30 seconds for three minutes, with the plate shaken automatically before each reading. The rate of increase in A415 per minute was calculated and a regression analysis was used to prepare a standard line relating standard GR activities to the change in A415. GR activities in the extracts, calculated with reference to the standard line, were expressed as nM of oxidized glutathione reduced per minute per milligram of total protein.

5.2.2.5 Glutathione peroxidase (GPOX, EC 1.11.1.9)

The activity of GPOX was determined according to Paglia and Valentine (1967) and modified for a microplate reader (Phang et al. 2011). The reaction mix contained 170 µL of 50 mM Tris–HCl buffer (pH 7.6), 5 mM EDTA, 0.14 mM NADPH, 1 mM GSH, 3 units /mL GR (from wheat germ, Sigma–Aldrich; EC 1.6.4.2) and 20 µL enzyme extract (dH_2O as control). The reaction was initiated by the addition of 20 µL 2.1 mM t-butyl hydroperoxide. The absorbance was read at 340 nm for three minutes at 30 s intervals, by monitoring the consumption of NADPH. Plates were shaken automatically before each reading. A standard regression analysis was used to generate a standard curve for GPOX activity based on the change in A340 using bovine erythrocyte GPOX (Sigma–Aldrich, St. Louis, MO, USA). Specific GPOX activities in the extracts were expressed as µmol per minute per mg total protein.

5.2.3 Extraction and assay of non-enzymatic antioxidants

5.2.3.1 Ascorbate extraction and assay

Tomato tissue was ground to a fine powder in liquid nitrogen and 50 mg of powdered tissue was homogenised in 250 µL 5% (w/v) metaphosphoric acid. The resulting homogenate was centrifuged at 12 000 g for 15 min at 4°C (Schweikert & Burritt, 2012). The reduced form of ascorbate and total ascorbate levels were then measured using the microtitre plate based assay described by (Gillespie & Ainsworth, 2007). The amount of oxidised form of ascorbate (reduced AsA), dehydroascorbate (DHA) was estimated from the difference between total ascorbate and reduced ascorbate.

5.2.3.2 Glutathione extraction and assay

Tomato tissue was ground to a fine powder in liquid nitrogen and 50 mg of powdered tissue was homogenised in 250 µL of ice-cold 5% (w/v) sulfosalicylic acid, then centrifuged at 12 000 g for 15 min at 4°C (Burritt, 2012). Total glutathione and reduced glutathione (GSH) levels were measured by the enzymatic recycling method, using the microtitre plate based assay described by (Rahman et al., 2006). The content of the oxidised form of glutathione (GSSG) was calculated from the difference between total glutathione and reduced glutathione.

All the assays were carried out using a Perkin Elmer (Wallac) 1420 multilabel counter (Perkin Elmer, San Jose, CA, USA) controlled by a PC with a temperature control cell and an auto dispenser (Lister et al., 2010). Data were obtained and processed by the WorkOut 2.0 software package (Perkin Elmer) (Schweikert & Burritt, 2012).

5.3 Statistical analyses

Statistical analyses followed the procedures outlined for the glasshouse experiment in Chapter 3.

For conciseness, tables are used to show the statistical summaries of all main effects and their interactions with the water treatments, followed by the corresponding tables showing the significant treatment-induced changes. These responses are then summarised in the text to assist readability. Again, for conciseness, only significant results and representative graphs will be presented in this chapter. Supplementary information is in Appendix E.

The PCA biplot of the effects of water deficit and waterlogging on oxidative metabolism was based on the relative changes induced by each of the two water treatments (ratios of water treatment:control) in the two cultivars at three developmental stages (Ballizany et al., 2012). The overall PCA was based on all attributes measured in the glasshouse experiments (Chapters 3-5) under control, drought and waterlogging conditions (Ballizany et al., 2012).Only attributes that showed significant correlations with either PC1 and/or PC2 are presented in the PCAs.

The heatmap cluster analysis was built using conditional formatting similar to that described by Zhao et al. (2014). The values were calculated from the ratios between each of the two water treatments and the well-water control for each of the two cultivars. The dendrogram linked to the heat map was based on the Agglomerative Hierarchical Clustering (AHC) function in XLSTAT, version 2013.6.30 (Addinsoft).

5.4 Results

5.4.1 Stress markers: hydrogen peroxide production and oxidative damage

5.4.1.1 Main effects

Averaged across developmental stages and cultivars, stress marker levels in tomato leaf tissues increased with drought (66%-5.5-fold) and even more so under waterlogging (2.6-fold-13.1-fold), with most pronounced changes were for LOOHs (Table 5.4.1.a, b, Figure 5.4.1.a, b and Appendix E). The latter compounds also showed that the strongest increases in tomato roots were among the stress markers in response to drought, and even more so under waterlogging. H_2O_2 levels were the only stress markers that showed an average decrease in roots under excess water.

Averaged across water treatments and developmental stages, the levels of most stress markers were between 5%-24% and were lower in 'Scoresby Dwarf' compared to 'Best Boy Bush' (Table 5.4.1.a, b, Figure 5.4.1.a, b and Appendix E).

In comparison with the vegetative stage, the levels of all stress markers increased in tomato leaves at the later developmental stages, ranging from 19%-42% at flowering and from 40% to 2.3-fold levels (LOOHs) at fruiting. Increases in the roots were only observed for LOOHs at fruiting (Table 5.4.1.a, b, Figure 5.4.1.a, b and Appendix E).

Table 5.4.1.a Summary of P values of the main effects and interactions with water treatments for oxidative stress markers

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage	Water x Cultivar x Devel. stage
H_2O_2 leaf	<.001	0.043	<.001	0.061	<.001	<.001
H_2O_2 root	<.001	0.517	0.718	<.001	0.214	0.957
LOOHs leaf	<.001	<.001	<.001	0.011	<.001	0.002
LOOHs root	<.001	<.001	<.001	<.001	<.001	0.980
PCs leaf	<.001	0.005	<.001	0.224	<.001	<.001
PCs root	<.001	<.001	0.836	<.001	0.924	0.542

Raw data are shown in Appendix A

Table 5.4.1.b Summary of percentage changes of the main effects and interactions with water treatments for oxidative stress markers

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage	Water x Cultivar x Devel. stage
H_2O_2 leaf	Dr: 92% WL: 2.6x	SBD: -5%	H2: 22% H3: 40%	ns	Dr H1: 87%, H2: 89%, H3: 2x WL H1: 57%, H2: 2.7x, H3: 3.6x	**Dr BBB** H1: 2x, H2: 2.1x, H3: 2.1x **Dr SBD** H1: 73%, H2: 71%, H3: 85% **WL BBB** H1: 79%, H2: 2.5x, H3: 3.8x **WL SBD** H1: 36%, H2: 2.9x, H3: 3.4x
H_2O_2 root	Dr: 44% WL: -11%	ns	ns	Dr BBB: 62% Dr SBD: 28% WL SBD: -14%	ns	ns
LOOHs leaf	Dr: 5.5x WL: 13.1x	SBD: -14%	H2: 42% H3: 2.3x	Dr BBB: 6.7x Dr SBD: 4.4x WL BBB: 13.8x WL SBD: 12.4x	Dr H1: 5.2x, H2: 5.7x, H3: 5.5x WL H1: 6.2x, H2: 11x, H3: 22x	**Dr BBB** H1: 6x, H2: 7x, H3: 7x **Dr SBD** H1: 4.5x, H2: 4.4x, H3: 4.2x **WL BBB** H1: 6.5x, H2: 9.8x, H3: 25.8x **WL SBD** H1: 6x, H2: 12.1x, H3: 18.6x
LOOHs root	Dr: 2.9x WL: 34.1x	SBD: -24%	H3: 39%	Dr BBB: 3x Dr SBD: 2.8x WL BBB: 39x WL SBD: 29x	Dr H1: 3x, H2: 2.5x, H3: 3.3x WL H1: 30x, H2: 29x, H3: 45x	ns
PCs leaf	Dr: 66% WL: 2.9x	SBD: -8%	H2: 19% H3: 45%	ns	Dr H1: 65%, H2: 60%, H3: 72% WL H1: 70%, H2: 2.9x, H3: 4.1x	**Dr BBB** H1: 79%, H2: 61%, H3: 89% **Dr SBD** H1: 52%, H2: 58%, H3: 55% **WL BBB** H1: 74%, H2: 2.4x, H3: 4.7x **WL SBD** H1: 76%, H2: 3.3x, H3: 3.5x
PCs root	Dr: 29% WL: 5x	SBD: -21%	ns	Dr SBD: 39% WL BBB: 4.8x WL SBD: 5.2x	ns	ns

Drought (Dr), waterlogging (WL), developmental stage (H, harvest 1: vegetative, 2: flowering, 3: fruiting), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), fold (x).

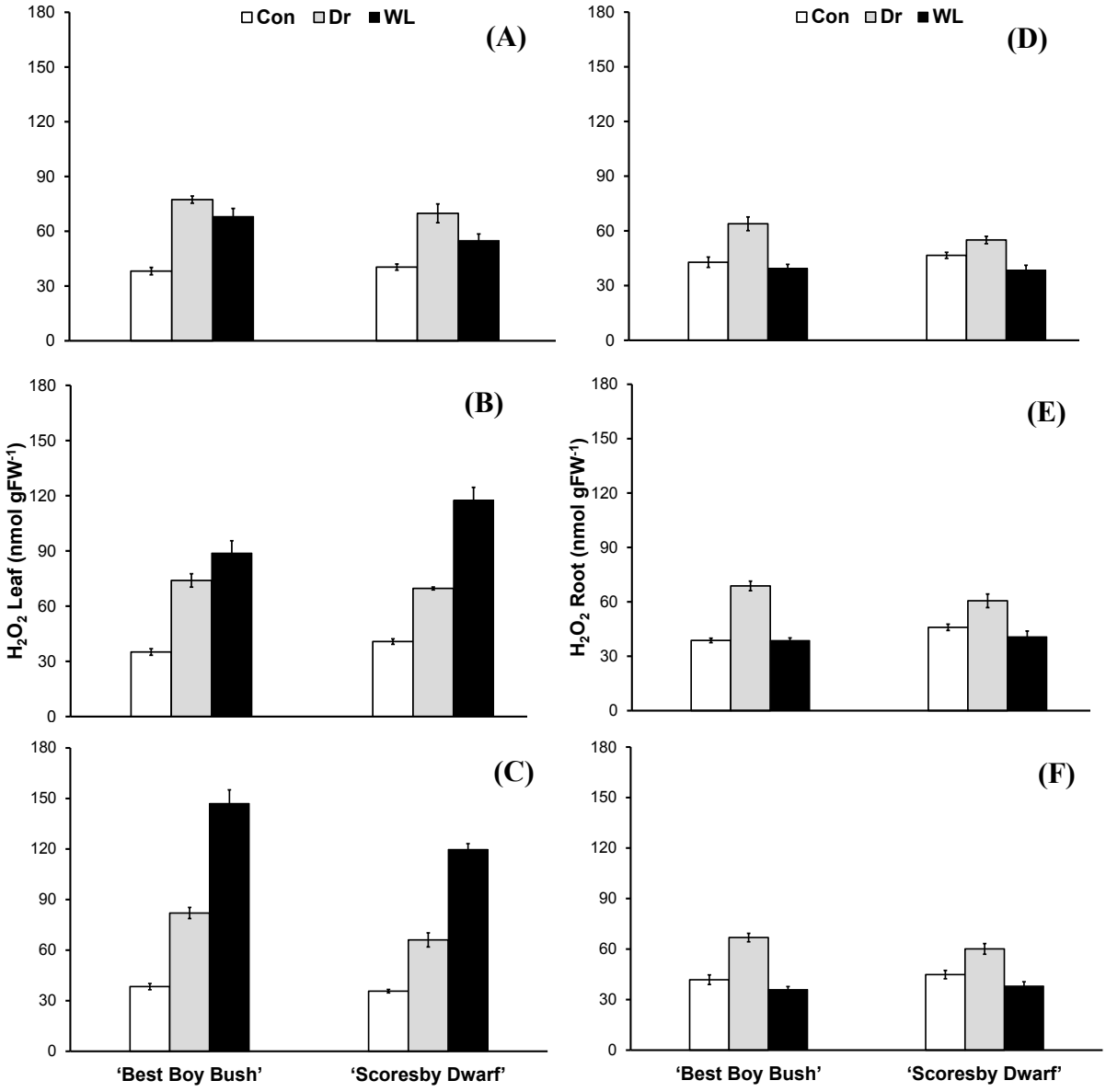


Figure 5.4.1.a Hydrogen peroxide (H_2O_2) in leaf tissues at the vegetative (A), flowering (B), fruiting (C) stages, and H_2O_2 in root tissues at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

5.4.1.2 Interaction effects

Water x Cultivar

Averaged across the developmental stages, stress-induced levels of leaf LOOHs differed between cultivars. While the leaf LOOH levels were strongly enhanced in both cultivars, these increases were less pronounced in 'Scoresby Dwarf' under both water treatments (Table 5.4.1.a, b, Figure 5.4.1.a, b and Appendix E). In roots, there were significant cultivar differences in the stress responses for all stress markers. Drought increased root levels of stress markers in 'Best Boy Bush' by 62% to 3-fold, and by 28% to 2.8-fold in 'Scoresby Dwarf', with highest increases

for LOOHs. Waterlogging, on the other hand, slightly decreased root H_2O_2 levels in 'Scoresby Dwarf', while it strongly increased average root PC levels and even more in root LOOH levels and, again, to a lesser degree in 'Scoresby Dwarf'.

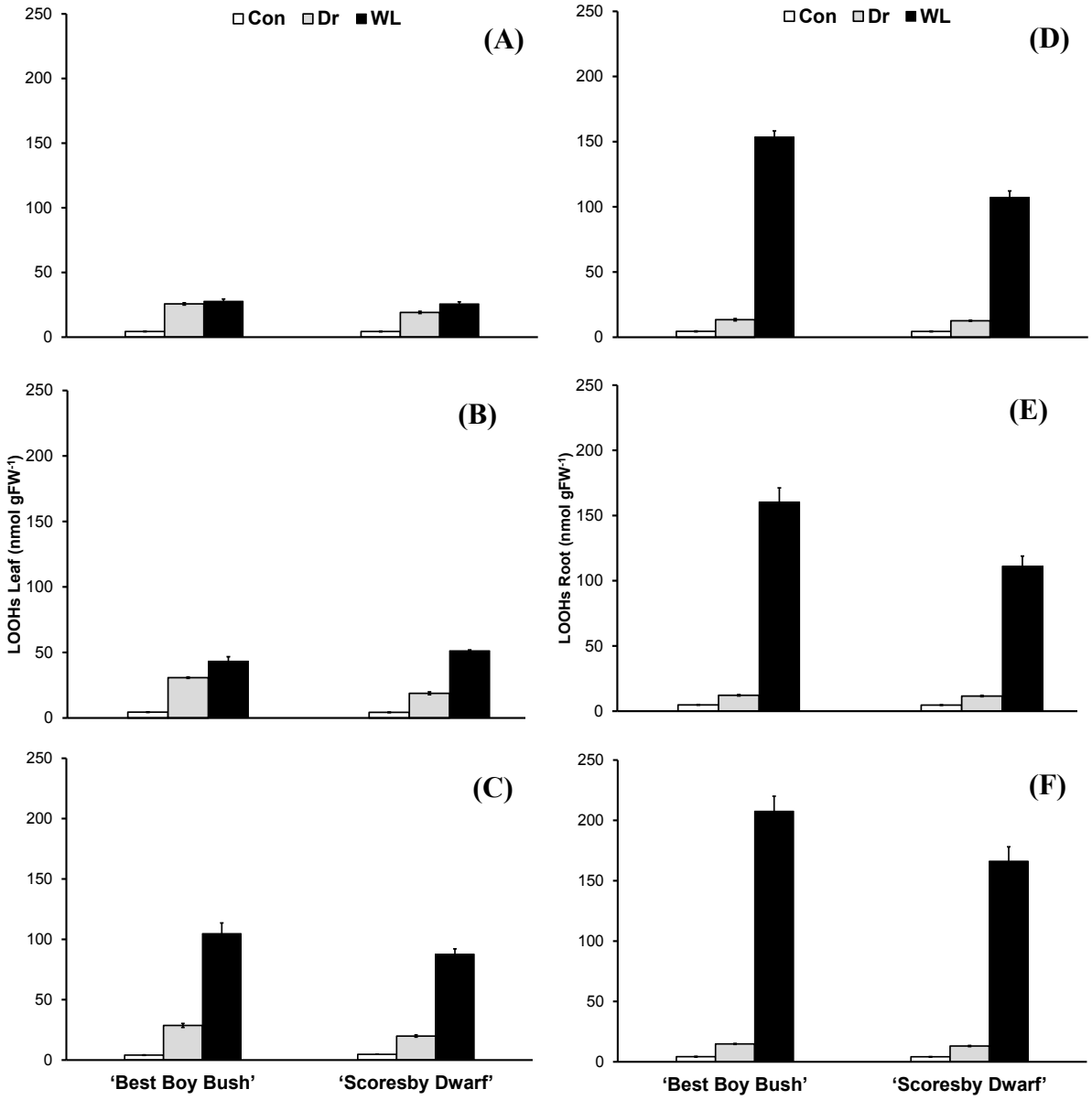


Figure 5.4.1.b Lipid hydroperoxides (LOOHs) in leaf tissues at the vegetative (A), flowering (B), fruiting (C) stages and LOOHs in root tissues at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error

Water x Developmental stage

There were significant waterlogging-induced increases in the levels of all oxidative stress markers in the leaves with increasing plant development showing 57% - 6.2-fold higher levels at the vegetative stage, but levels that were 3.6-fold – 22-fold higher at fruiting (Table 5.4.1.a, b, Figure 5.4.1.a, b and Appendix E). In contrast, drought-induced changes were comparable

across the developmental stages, with 60% - 5.2-fold increases at the vegetative stage and 72% - 5.5-fold changes at fruiting. In roots, this pattern was also was observed for levels of LOOHs, with drought-induced increases that were similar (2.5 - 3.3-fold) at all developmental stages, whereas under waterlogging they ranged from 29-fold increases at the vegetative stage to 45-fold at fruiting.

Water x Cultivar x Developmental stage

There were significant three-way interactions for stress marker levels in leaves, but not in roots. These interactions revealed higher drought-induced increases in stress marker accumulation for 'Best Boy Bush' at all developmental stages, with levels that were two times those of the control plants for H_2O_2 (73 - 85% for 'Scoresby Dwarf'), 6- – 7-fold for LOOHs (4.2 - 4.5-fold for 'Scoresby Dwarf') and 61 - 89% higher for PCs (52 - 58% for 'Scoresby Dwarf') (Table 5.4.1.a, b, Figure 5.4.1.a, b and Appendix E). Under water logging, no clear pattern emerged for cultivar differences in treatment responses in leaves until the fruiting stage, where 'Best Boy Bush' had higher increases of all three stress markers investigated, with 3.8-fold - 25.8-fold increases, compared to 3.4 - 18.6-fold for 'Scoresby Dwarf'.

5.4.1.3 Summary of the key findings

- Extremes in water stress increased the levels of all oxidative stress markers, especially LOOHs in the leaves and roots.
- Compared to plants exposed to drought, the levels of these stress markers became more elevated in waterlogged plants, particularly by the fruiting stage.
- Drought-induced levels of oxidative stress markers were less pronounced in the leaves of 'Scoresby Dwarf' compared with 'Best Boy Bush'.
- Waterlogging generally resulted in less pronounced increases in oxidative stress markers (and even decreases in root H_2O_2 levels) in 'Scoresby Dwarf' compared with 'Best Boy Bush'.

5.4.2 Antioxidant enzymes

5.4.2.1 Main effects

Averaged across cultivars and developmental stages, water deficit increased activities of all antioxidant enzymes measured here by 16% - 37% in leaves and by 14% - 25% in roots (Table 5.4.2.a, b, Figure 5.4.2.a and Appendix E). Waterlogging, on the other hand, suppressed the activities of these enzymes in both leaves and roots by 22% - 48%.

Averaged across water treatments and developmental stages, the activity of all the antioxidant enzymes was 19% - 51% higher in 'Scoresby Dwarf' than in 'Best Boy Bush' (Table 5.4.2.a, b, Figure 5.4.2.a and Appendix E).

Differences between the vegetative development stage and the reproductive development stages were observed in the activities of all enzymatic antioxidants, which were reduced at the flowering stage (relative to the vegetative stage) in both leaf and root tissues by 12%-23% (Table 5.4.2.a, b, Figure 5.4.2.a and Appendix E). At the fruiting stage, average reductions in enzyme activity by 17% - 28% were observed in leaf and root tissues.

Table 5.4.2.a Summary of P values of the main effects and interactions with water treatments for enzymatic antioxidant activities

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage	Water x Cultivar x Devel. stage
SOD Leaf	<.001	<.001	<.001	<.001	<.001	0.017
SOD Root	<.001	<.001	<.001	<.001	<.001	0.001
CAT Leaf	<.001	<.001	<.001	0.005	<.001	0.050
CAT Root	<.001	<.001	<.001	0.003	<.001	0.002
APOX Leaf	<.001	<.001	<.001	<.001	<.001	0.003
APOX Root	<.001	<.001	<.001	<.001	<.001	0.043
GR leaf	<.001	<.001	<.001	<.001	<.001	<.001
GR root	<.001	<.001	<.001	<.001	<.001	<.001
GPOX Leaf	<.001	<.001	<.001	<.001	<.001	0.001
GPOX Root	<.001	<.001	<.001	<.001	<.001	0.024

Raw data are shown in Appendix A

Table 5.4.2.b Summary of percentage changes of the main effects and interactions with water treatments for enzymatic antioxidant activities

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage		Water x Cultivar x Devel. stage
SOD Leaf	Dr: 25% WL: -41%	SBD: 39%	H2: -14% H3: -22%	Dr BBB: 9% Dr SBD: 37% WL BBB: -49% WL SBD: -34%	Dr H1: 30% Dr H2: 14% Dr H3: 31%	WL H2: -53% WL H3: -63%	**Dr BBB** H1: 17%, H3: 15% **Dr SBD** H1: 40%, H2: 28%, H3: 45% **WL BBB** H1: -21%, H2: -65%, H3: -58% **WL SBD** H2: -42%, H3: -67%
SOD Root	Dr: 25% WL: -25%	SBD: 51%	H2: -21% H3: -23%	Dr SBD: 41% WL BBB: -59% WL SBD: -44%	Dr H1: 25% Dr H2: 23% Dr H3: 27%	WL H2: -71% WL H3: -82%	**Dr SBD** H1: 43%, H2: 35%, H3: 45% **WL BBB** H2: -86%, H3: -79% **WL SBD** H1: 15%, H2: -60%, H3: -85%
CAT Leaf	Dr: 16% WL: -35%	SBD: 34%	H2: -14% H3: -17%	Dr SBD: 24% WL BBB: -42% WL SBD: -28%	Dr H1: 11% Dr H2: 22% Dr H3: 15%	WL H2: -37% WL H3: -62%	**Dr BBB** H2: 18% **Dr SBD** H1: 23%, H2: 24%, H3: 24% **WL BBB** H1: -16%, H2: -50%, H3: -61% **WL SBD** H2: -26%, H3: -63%
CAT Root	Dr: 11% WL: -43%	SBD: 32%	H2: -19% H3: -24%	Dr SBD: 22% WL BBB: -55% WL SBD: -31%	Dr H3: 16% WL H2: -58%	WL H3: -80%	**Dr SBD** H1: 19%, H3: 31% **WL BBB** H2: -69%, H3: -79% **WL SBD** H1: 34%, H2: -48%, H3: -80%
APOX Leaf	Dr: 37% WL: -22%	SBD: 22%	H2: -12% H3: -22%	Dr BBB: 20% Dr SBD: 54% WL BBB: -41%	Dr H1: 35% Dr H2: 42% Dr H3: 34%	WL H1: 19% WL H2: -29% WL H3: -55%	**Dr BBB** H2: 22%, H3: 24% **Dr SBD** H1: 56%, H2: 63%, H3: 45% **WL BBB** H2: -56%, H3: -54% **WL SBD** H1: 51%, H3: -55%
APOX Root	Dr: 22% WL: -46%	SBD: 19%	H2: -23% H3: -24%	Dr BBB: 10% Dr SBD: 33% WL BBB: -55% WL SBD: -37%	Dr H1: 19% Dr H2: 26% Dr H3: 20%	WL H2: -55% WL H3: -81%	**Dr BBB** H3: 13% **Dr SBD** H1: 28%, H2: 44%, H3: 28% **WL BBB** H1: -19%, H2: -64%, H3: -81% **WL SBD** H1: 13%, H2: -47%, H3: -80%
GR leaf	Dr: 36% WL: -22%	SBD: 21%	H2: -19% H3: -24%	Dr BBB: 23% Dr SBD: 48% WL BBB: -43%	Dr H1: 41% Dr H2: 33% Dr H3: 33%	WL H1: 20% WL H2: -33% WL H3: -54%	**Dr BBB** H1: 18%, H2: 19%, H3: 35% **Dr SBD** H1: 66%, H2: 49%, H3: 31% **WL BBB** H1: -17%, H2: -59%, H3: -56% **WL SBD** H1: 60%, H3: -53%
GR root	Dr: 20% WL: -45%	SBD: 24%	H2: -16% H3: -26%	Dr SBD: 37% WL BBB: -56% WL SBD: -34%	Dr H2: 27% Dr H3: 28%	WL H2: -62% WL H3: -80%	**Dr SBD** H1: 21%, H2: 41%, H3: 50% **WL BBB** H1: -22%, H2: -68%, H3: -81% **WL SBD** H1: 33%, H2: -57%, H3: -80%

GPOX Leaf	Dr: 29% WL: -46%	SBD: 24%	H2: -20% H3: -20%	Dr SBD: 51% WL BBB: -45%	Dr H1: 28% Dr H2: 30% Dr H3: 29%	WL H1: 15% WL H2: -36% WL H3: -57%	**Dr BBB** H3: 16% **Dr SBD** H1: 51%, H2: 60%, H3: 42% **WL BBB** H1: -19%, H2: -58%, H3: -59% **WL SBD** H1: 50%, H3: -56%
GPOX Root	Dr: 14% WL: -48%	SBD: 24%	H2: -17% H3: -28%	Dr SBD: 29% WL BBB: -59% WL SBD: -38%	Dr H1: 17% Dr H2: 12% Dr H3: 13%	WL H2: -62% WL H3: -82%	**Dr SBD** H1: 34%, H2: 31%, H3: 22% **WL BBB** H1: -23%, H2: -70%, H3: -83% **WL SBD** H1: 19%, H2: -53%, H3: -81%

Drought (Dr), waterlogging (WL), developmental stage (H, harvest 1: vegetative, 2: flowering, 3: fruiting), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), fold (x).

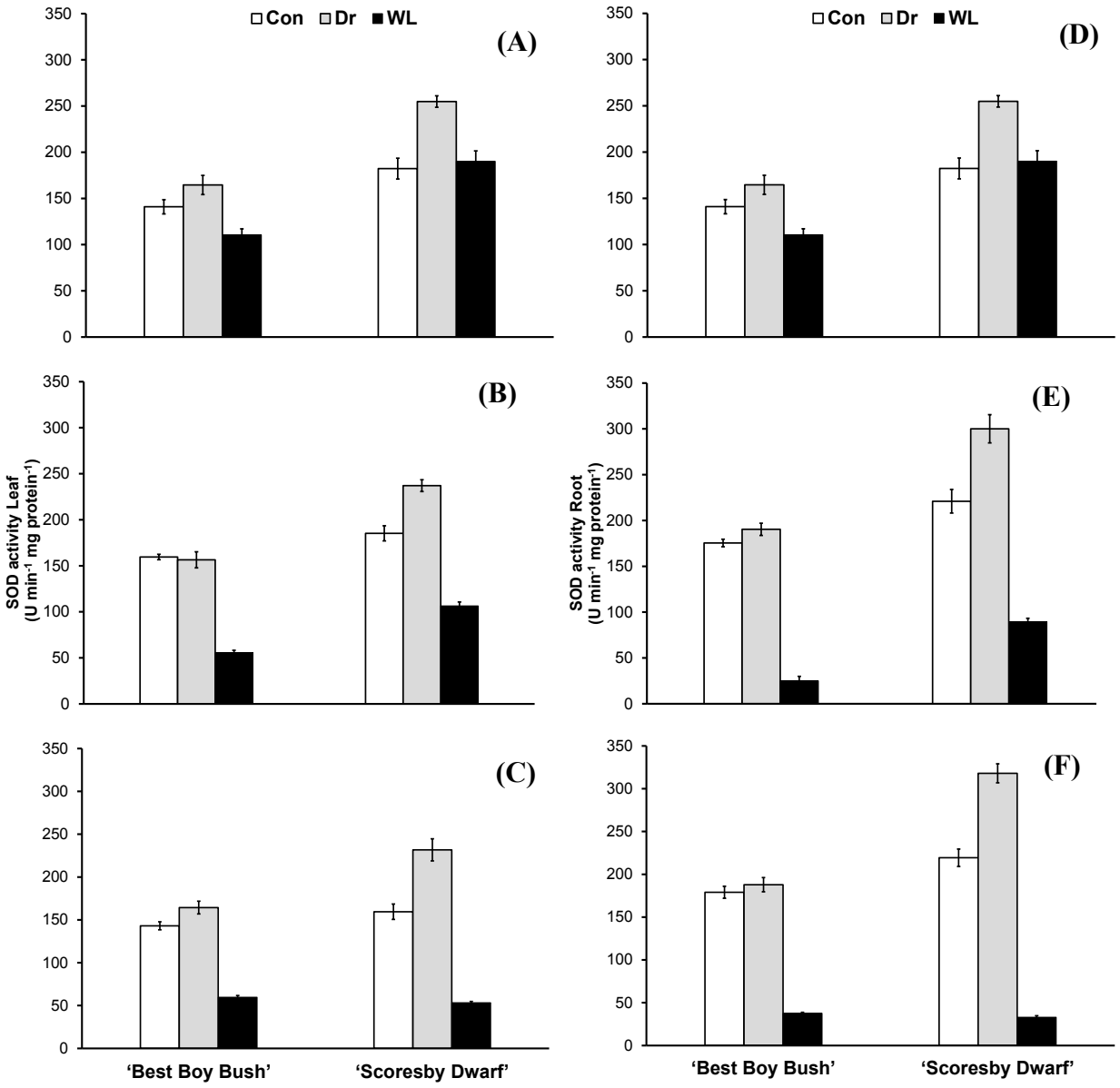


Figure 5.4.2.a Superoxide dismutase (SOD) activity in leaf tissues at the vegetative (A), flowering (B), fruiting (C) stages and SOD activity in root tissues at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

5.4.2.2 Interaction effects

Water x Cultivar

Averaged across developmental stages, water deficit induced increases in antioxidant enzyme activities in 'Scoresby Dwarf' leaves and roots by 22% - 54%, while, in most cases, there were no overall changes in 'Best Boy Bush' and only increases of 9% - 23% in SOD leaf, APOX leaf and root and GR leaf levels (Table 5.4.2.a, b, Figure 5.4.2.a and Appendix E). Waterlogging

caused reductions in the enzyme activities in both leaf and root tissues of 'Best Boy Bush' by 43% - 59%. In contrast, there were no changes in enzymatic antioxidant levels in 'Scoresby Dwarf' leaves for the activities of APOX, GR and GPOX, and reductions of 28%-44% in the remainder of samples.

Water x Developmental stage

The activity of all enzymatic antioxidants was stimulated by drought (11% - 42%) but with no consistent pattern at the developmental stages, while hypoxia suppressed the activities of all these enzymes progressively during reproductive development with reductions of 29% - 71% at flowering and of 54%-82% at fruiting (Table 5.4.2.a, b, Figure 5.4.2.a and Appendix E). In contrast, there were no hypoxia-induced changes for most enzymatic antioxidant activities at the vegetative stage, except for increases of 15%-20% for APOX, GR and GPOX in leaves.

Water x Cultivar x Developmental stage

Under drought, 'Scoresby Dwarf' increased the activity of all antioxidant enzymes measured here at all developmental stages and in all tissues by 19% - 66% (with the one exception of unchanged CAT activity in root tissues at flowering) (Table 5.4.2.a, b, Figure 5.4.2.a and Appendix E). In 'Best Boy Bush', on the other hand, drought did not affect antioxidant enzyme activities in the roots, except for a small increase in APOX activity. In 'Best Boy Bush' leaves, smaller drought-induced enzyme activity increases of 13% - 35% were noted, and often towards the later stages of plant development.

In 'Scoresby Dwarf', waterlogging also increased the enzymatic antioxidant activities, in most cases, at the vegetative stage (by 13% - 60%, except for no changes in SOD and CAT in leaves), while these activities decreased by 26% - 60% at flowering (but no change for leaf activities of APOX, GR and GPOX at flowering) and by 53% - 85% at fruiting. This was in contrast to the waterlogging responses of 'Best Boy Bush' where, in most cases, antioxidant enzyme activities decreased, except for no changes at the vegetative stage in the activities of SOD in roots, CAT in roots and APOX in leaves. This included decreases of 16% - 21% in leaves and roots at the first harvest, reductions of 50% - 65% in leaves and of 64% - 86% in roots at the flowering stage and reductions of 54% - 61% in leaves and of 79% - 83% in roots at the fruiting stage.

5.4.2.3 Summary of the key findings

- The activities of all antioxidant enzymes increased under drought stress throughout plant development, but decreased in plants subjected to waterlogging, particularly during reproductive development (flowering and fruiting).
- In plants subjected to water deficit, the activity of all antioxidant enzymes increased in both leaf and root tissues of 'Scoresby Dwarf' at all developmental stages, whereas there were no, or only minor, activity increases in 'Best Boy Bush' leaves and roots.
- Waterlogging suppressed all antioxidant enzyme activities in both leaf and root tissues of 'Best Boy Bush' at all developmental stages while, in 'Scoresby Dwarf', this suppression was mostly found at the fruiting stage, with less pronounced decreases at flowering and even enzyme activity increases or no change at the vegetative stage.

Plate 4. Tomato tissue grinding in liquid nitrogen

5.4.3 Non enzymatic antioxidants

5.4.3.1 Main effects

Averaged across cultivars and developmental stages, drought increased the levels of most non-enzymatic antioxidants in the leaves by 20% - 44% and by 9% - 30% in root tissues (Table 5.4.1.a, b, and Figure 5.4.3.a, b, c, d, e). Waterlogging increased total AsA, GSH and total GSH levels by 20% to 50% in the leaves and by 13% (total AsA) to 91% (total GSH) in roots. AsA levels, on the other hand, showed no significant change in leaves and a 30% decrease in roots under hypoxia. Plants subjected to a water deficit stress produced fruit with increased total AsA content in the fruit pericarp by 48% and in the skin fraction by 18%. Waterlogging, on the other hand, decreased this compound by 11% and by 37% in the pericarp and skin of tomato fruits, respectively.

Table 5.4.3.a Summary of P values of the main effects and interactions with water treatments for non-enzymatic antioxidant levels

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage	Water x Cultivar x Devel. stage
Reduced AsA leaf	<.001	<.001	0.354	<.001	0.546	0.832
Reduced AsA root	<.001	<.001	0.878	<.001	0.144	0.482
Total AsA leaf	<.001	<.001	0.605	<.001	0.468	0.361
Total AsA root	<.001	<.001	0.632	<.001	0.293	0.242
GSH leaf	<.001	<.001	0.169	<.001	0.047	0.789
GSH root	<.001	0.003	0.950	0.001	0.539	0.813
Total GSH leaf	<.001	<.001	0.600	<.001	0.302	0.837
Total GSH root	<.001	0.005	0.403	<.001	0.503	0.685
Total AsA pericarp	<.001	0.091	-	0.002	-	-
Total AsA skin	<.001	0.767	-	0.043	-	-

Raw data are shown in Appendix A

Averaged across water treatments and developmental stages, 'Scoresby Dwarf' had higher antioxidant accumulation than 'Best Boy Bush' in roots (8% - 22%) and leaves (25% - 59%). Furthermore, 'Scoresby Dwarf' had higher levels of reduced AsA in both leaf tissue (59%) and root tissue (19%). Total AsA levels of 'Scoresby Dwarf' were higher in both leaf tissue and root tissue by 51% and 22%, respectively. 'Scoresby Dwarf' also had greater levels of GSH leaves (40%), GSH roots (10%), total GSH in leaves (25%) and roots (8%), compared with 'Best Boy Bush'.

Table 5.4.3.b Summary of percentage change of the main effects and interactions with water treatments for non-enzymatic antioxidant levels

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage	
Reduced AsA leaf	Dr: 20%	SBD: 59%	ns	Dr SBD: 42% WL BBB: -35% WL SBD: 18%	ns	
Reduced AsA root	Dr: 9% WL: -30%	SBD: 19%	ns	Dr SBD: 21% WL BBB: -41% WL SBD: -20%	ns	
Total AsA leaf	Dr: 32% WL: 32%	SBD: 51%	ns	Dr SBD: 55% WL SBD: 55%	ns	
Total AsA root	Dr: 16% WL: 13%	SBD: 22%	ns	Dr SBD: 27% WL SBD: 30%	ns	
GSH leaf	Dr: 25% WL: 20%	SBD: 40%	ns	Dr SBD: 45% WL SBD: 45%	Dr H1: 19% Dr H2: 19% Dr H3: 38%	WL H1: 26% WL H2: 19%
GSH root	Dr: 28% WL: 28%	SBD: 10%	ns	Dr BBB: 26% Dr SBD: 30% WL SBD: 45%	ns	
Total GSH leaf	Dr: 44% WL: 50%	SBD: 25%	ns	Dr BBB: 33% Dr SBD: 54% WL BBB: 33% WL SBD: 65%	ns	
Total GSH root	Dr: 30% WL: 91%	SBD: 8%	ns	Dr BBB: 30% Dr SBD: 30% WL BBB: 70% WL SBD: 2.1x	ns	
Total AsA pericarp	Dr: 48% WL: -11%	ns	ns	Dr BBB: 34% Dr SBD: 65% WL BBB: -12% WL SBD: -10%	ns	
Total AsA skin	Dr: 18% WL: -37%	ns	ns	Dr SBD**:** 28% WL BBB: -37% WL SBD: -38%	ns	

Drought (Dr), waterlogging (WL), developmental stage (H, harvest 1: vegetative, 2: flowering, 3: fruiting), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), fold (x)

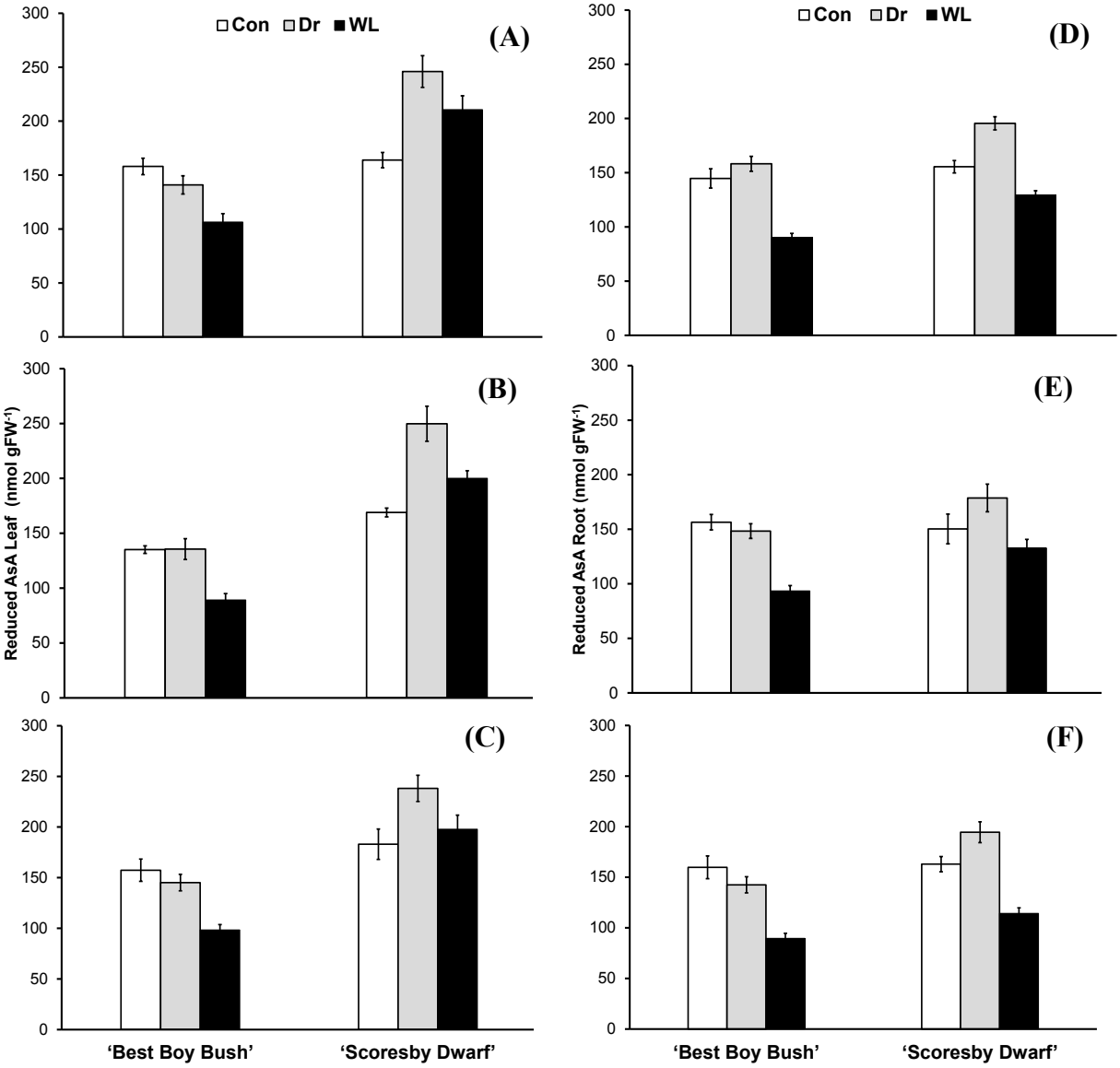


Figure 5.4.3.a Reduced AsA content in leaf tissues at the vegetative (A), flowering (B), fruiting (C) stages and reduced AsA content in root tissues at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

5.4.3.2 Interaction effects

Water x Cultivar

Drought increased the levels of reduced AsA, total AsA, GSH and total GSH by 42% - 55% in the leaves of 'Scoresby Dwarf' but not in 'Best Boy Bush', except for a 33% increase for total GSH (Table 5.4.1.a, b, and Figure 5.4.3.a, b, c, d). The level of these four antioxidants were also elevated in drought-exposed 'Scoresby Dwarf' roots by 21% - 30% whereas, in 'Best Boy Bush' roots, only GSH and total GSH levels increased under drought (by 26% - 30%). Waterlogging decreased reduced AsA levels in 'Best Boy Bush' leaves and roots by 35% to 41% whereas, in waterlogged 'Scoresby Dwarf' plants, these levels increased in leaves (18%)

and decreased in roots (-20%). Waterlogging also selectively increased antioxidant production in 'Scoresby Dwarf' for total AsA and GSH levels in leaves and roots by 30% - 55%. While total GSH levels increased in waterlogged 'Best Boy Bush' leaves and roots by 33% and 70%, these increases were double or more in 'Scoresby Dwarf'.

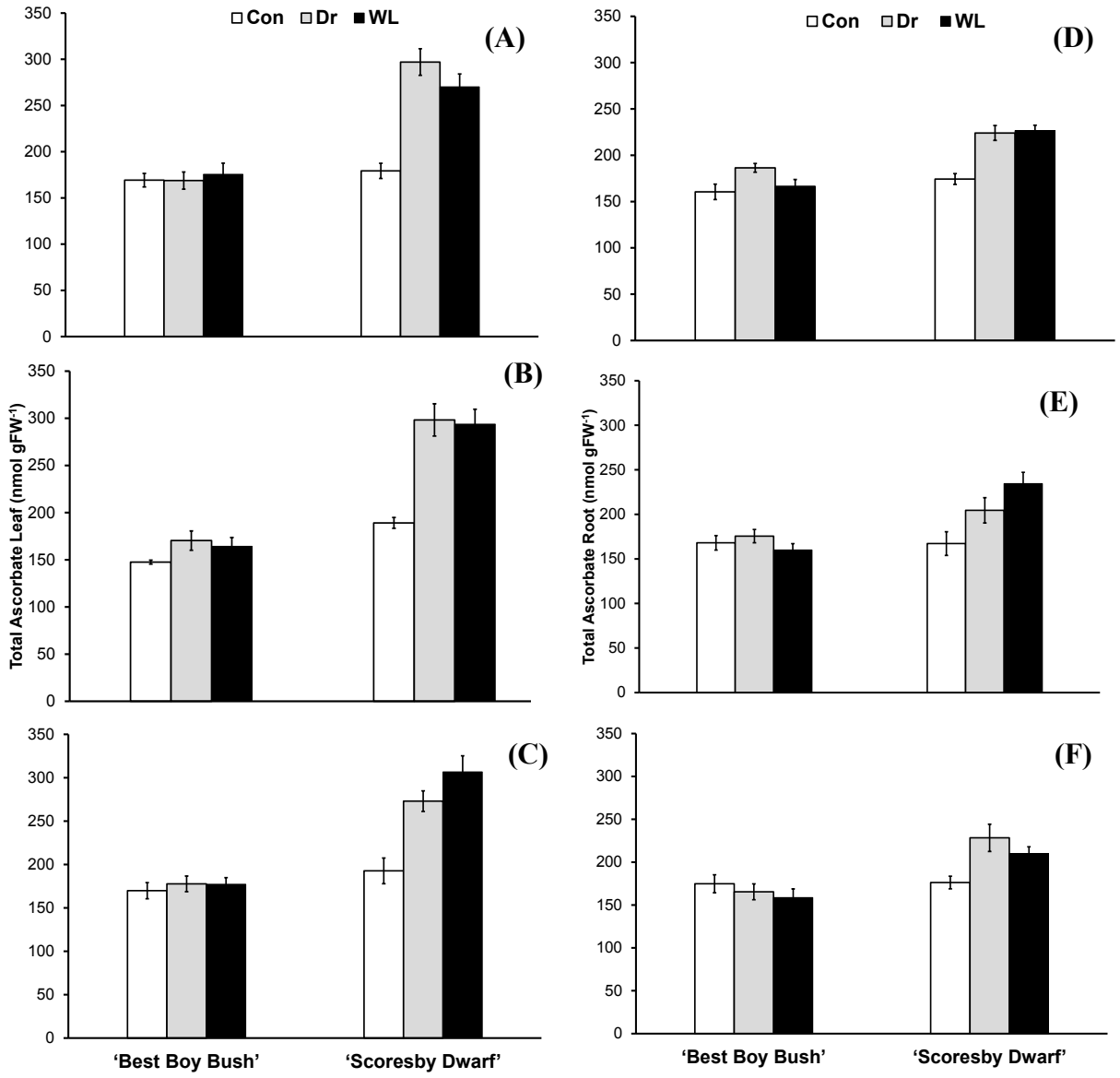


Figure 5.4.3.b Total AsA content in leaf tissues at the vegetative (A), flowering (B), fruiting (C) stages and total AsA content in root tissues at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

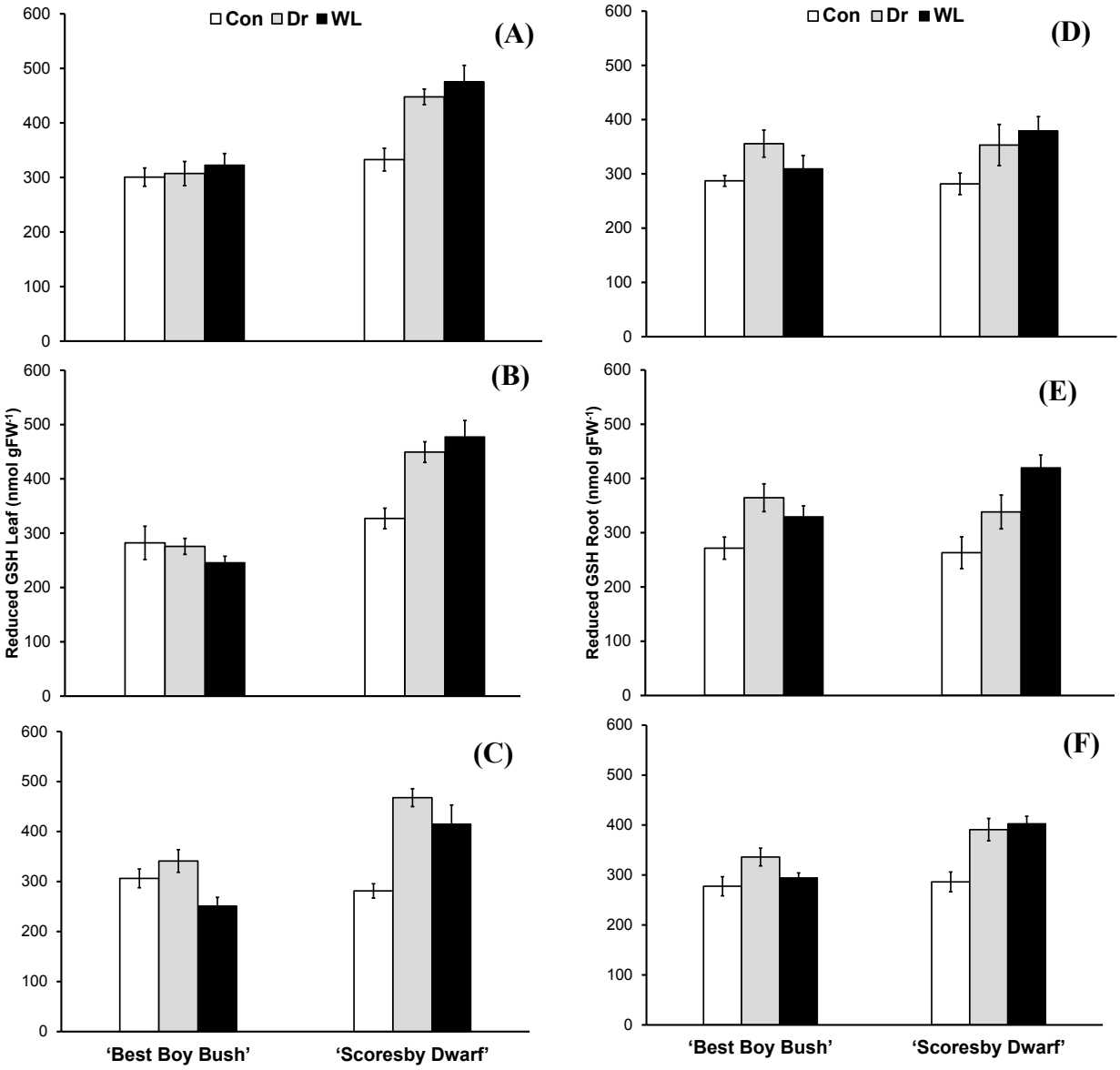


Figure 5.4.3.c Reduced glutathione (GSH) content in leaf tissues at the vegetative (A), flowering (B), fruiting (C) stages and reduced GSH content in root tissues a the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

'Scoresby Dwarf' showed drought-induced increases of total AsA levels in the fruit pericarp that were double those of 'Best Boy Bush'. 'Scoresby Dwarf' also had increased total AsA levels in the fruit skin under drought. Waterlogging decreased total AsA levels in both cultivars by 10% - 12% in the pericarp and by 37% - 38% in the fruit skin.

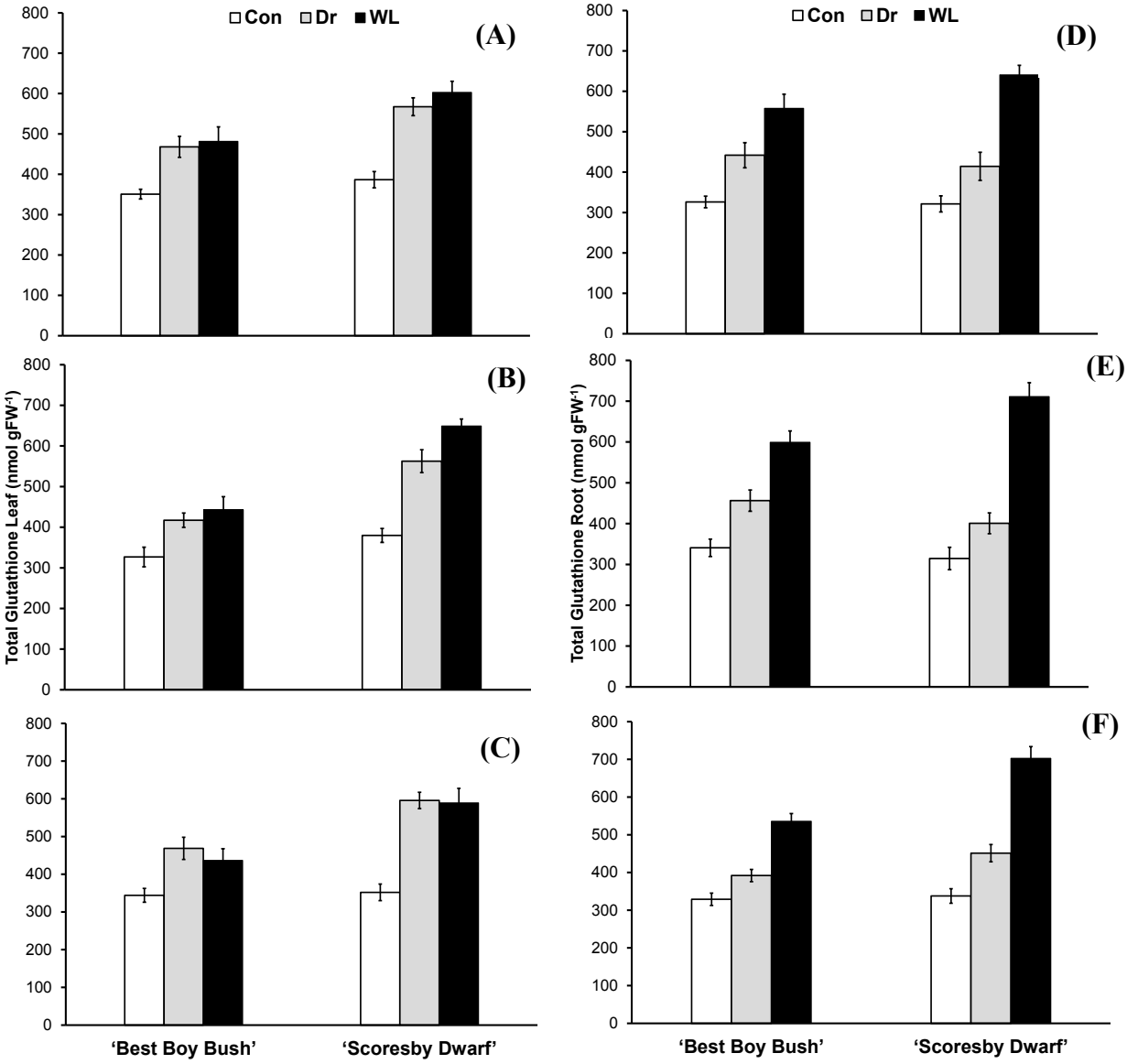


Figure 5.4.3.d Total glutathione content in leaf tissues at the vegetative (A), flowering (B), fruiting (C) stages and total glutathione content in root tissues at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

Water x Developmental stage

Averaged across cultivars, drought-induced increases in GSH levels in leaves of 19% at the vegetative stage had increased 2-fold by fruiting, whereas under waterlogging, a 26% increase at the vegetative stage had disappeared at fruiting (Table 5.4.1.a, b, and Figure 5.4.3.a, b, c, d, e).

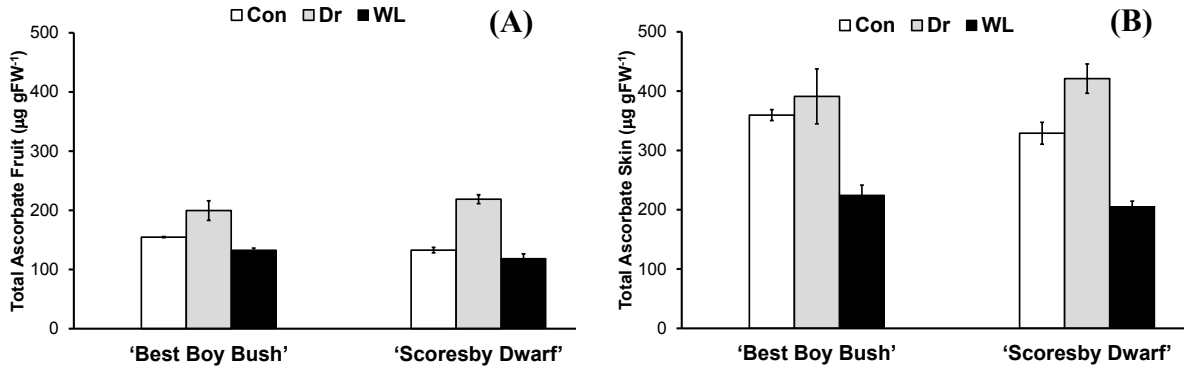


Figure 5.4.3.e Total AsA content in tomato fruit (A) and total AsA content in tomato skin (B) of two tomato cultivars, 'Scoresby Dwarf' and 'Best Boy Bush' grown under control conditions (Con), drought (Dr) and waterlogging (WL); values are means ± standard error.

5.4.3.3 DHA and GSSG

Main effects

The levels of the oxidised antioxidants DHA and GSSG increased markedly under drought: by 2.6-fold - 2.8-fold in leaves and by 38% - 83% in roots (Table 5.4.1.c, d, and Figure 5.4.3.f, g). Waterlogging had a even stronger effect on levels of the oxidised antioxidants, increasing these by 3.3-fold – 6-fold in the leaves and roots. Averaged across water treatment and developmental stage, 'Scoresby Dwarf' had 25% - 30% higher levels of DHA relative to 'Best Boy Bush' (Table 5.4.1.c, d, and Figure 5.4.3.f, g).

Table 5.4.3.c Summary of P values of the main effects and interactions with water treatments of oxidised forms of ascorbate and glutathione

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage	Water x Cultivar x Devel. stage
DHA leaf	<.001	<.001	0.026	0.219	<.001	0.006
DHA root	<.001	<.001	0.512	<.001	0.975	0.315
GSSG leaf	<.001	0.133	0.482	0.036	0.054	0.988
GSSG root	<.001	0.539	0.016	<.001	0.217	0.606

Raw data are shown in Appendix A

Table 5.4.3.d Summary of percentage change of the main effects and interactions with water treatments of oxidised forms of ascorbate and glutathione

Traits	Water	Cultivar	Devel. stage	Water x Cultivar	Water x Devel. stage		Water x Cultivar x Devel. stage
DHA leaf	Dr: 2.8x WL: 6x	SBD: 25%	H2: 22% H3: 18%	ns	Dr H1: 3x Dr H2: 2.6x Dr H3: 3x	WL H1: 4.8x WL H2: 5.2x WL H3: 8.5x	**Dr BBB** H1: 2.5x, H2: 2.8x, H3: 2.6x **Dr SBD** H1: 3.3x, H2: 2.4x, H3: 3.6x **WL BBB** H1: 6.2x, H2: 6.1x, H3: 6.3x **WL SBD** H1: 3.9x, H3: 4.6x, H3: 11.3x
DHA root	Dr: 83% WL: 5.6x	SBD: 30%	ns	Dr BBB: 85% Dr SBD: 80% WL BBB: 5x WL SBD: 6x	ns		ns
GSSG leaf	Dr: 2.6x WL: 3.3x	ns	ns	Dr BBB: 3.2x Dr SBD: 2x WL BBB: 4.1x WL SBD: 2.7x	ns		ns
GSSG root	Dr: 38% WL: 5.3x	ns	H2: 14%	Dr BBB: 47% WL BBB: 4.8x WL SBD: 6x	ns		ns

Drought (Dr), waterlogging (WL), developmental stage (H, harvest 1: vegetative, 2: flowering, 3: fruiting), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), fold (x).

Relative to the vegetative stage, plants at later developmental stages had about 20% higher DHA levels in leaves and 14% higher GSSG levels in roots at the flowering stage (Table 5.4.1.c, d, and Figure 5.4.3.f, g).

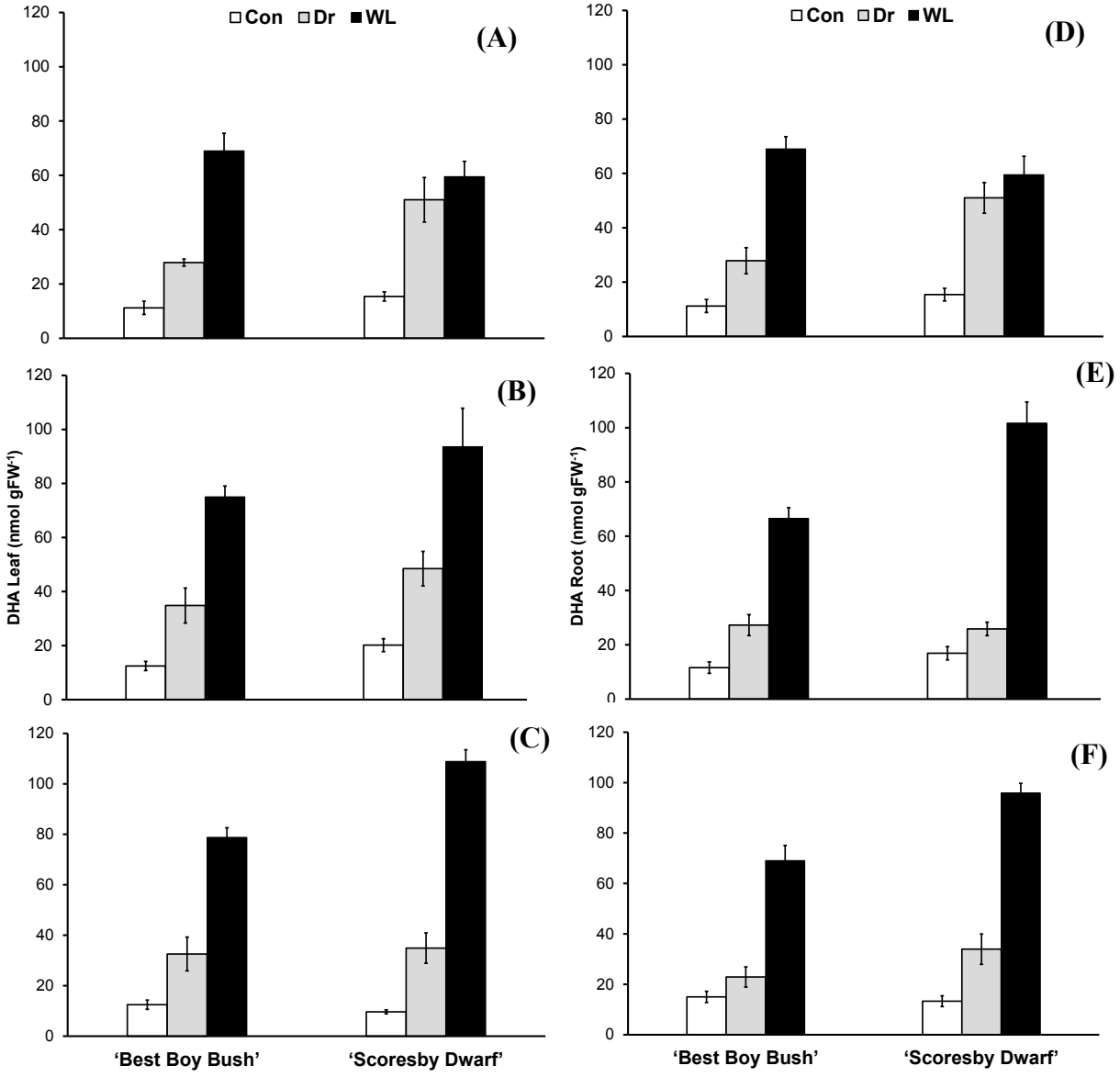


Figure 5.4.3.f Oxidised ascorbate (DHA) content in leaf tissues at the vegetative (A), flowering (B), fruiting (C) stages and DHA content in root tissues at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

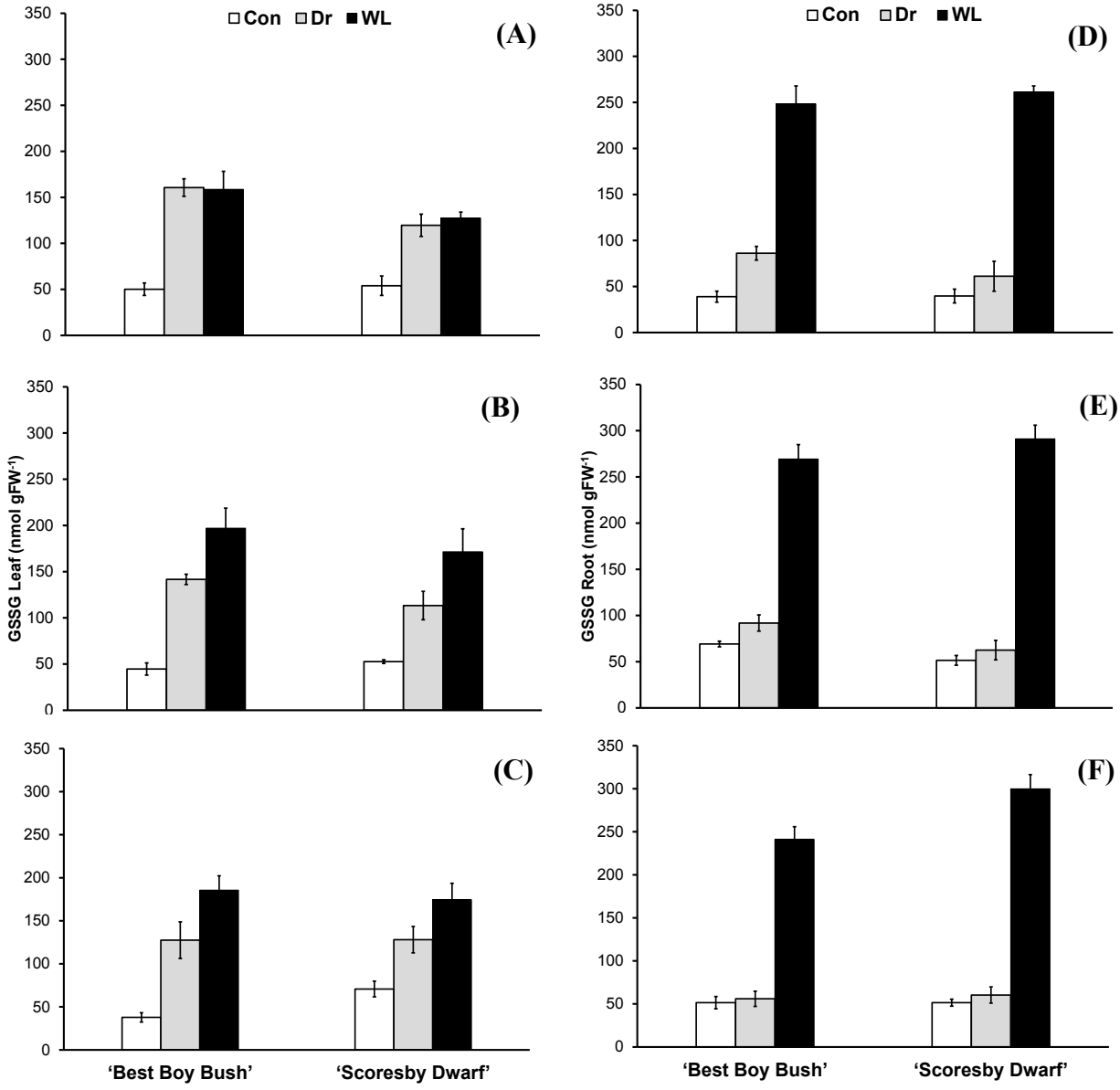


Figure 5.4.3.g Oxidised glutathione (GSSG) content in leaf tissues at the vegetative (A), flowering (B), fruiting (C) stages and GSSG content in root tissues at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

Interaction effects

Water x Cultivar

Intraspecific differences in the stress response of oxidised antioxidants were highlighted by stress-induced 3.2-fold – 4.1-fold increases in leaf GSSG levels in 'Best Boy Bush' compared to 2-fold - 2.7-fold increases in 'Scoresby Dwarf' (Table 5.4.1.c, d, and Figure 5.4.3.f, g). In the

roots, drought increased GSSG levels in 'Best Boy Bush' (by 47%) but not in 'Scoresby Dwarf', whereas the latter cultivar showed a 6-fold increase in this compound under waterlogging, compared to a 4.8-fold increase in 'Best Boy Bush'. This was similar to the observed increases in DHA root levels.

Water x Developmental stage

Averaged across cultivars, increases in leaf DHA levels of about 3-fold were the same at the first and last harvest under water deficit, whereas under waterlogging there were strong 4.8-fold increases at the vegetative stage that increased nearly 2-fold further by fruiting (Table 5.4.1.c, d, and Figure 5.4.3.f, g).

Water x Cultivar x Developmental stage

The only significant three-way interaction was detected for DHA in leaves. Compared to 6.1-fold - 6.3-fold increases under waterlogging in 'Best Boy Bush', DHA levels increased in 'Scoresby Dwarf' to a lesser degree (3.9-fold - 4.6-fold) at the first two developmental stages, while increases in DHA levels were nearly double those of 'Best Boy Bush' at fruiting (Table 5.4.1.c, d, and Figure 5.4.3.f, g).

5.4.3.4 Summary of the key findings

- Levels of all non-enzymatic antioxidants increased under drought and waterlogging except for reduced ascorbate levels which under waterlogging decreased in ‘Best Boy Bush’ leaves and in the roots of both cultivars, with more pronounced decreases for ‘Best Boy Bush’.
- Under water deficit, 'Scoresby Dwarf' increased the levels of all other antioxidants, in contrast to 'Best Boy Bush' which showed no changes for total ascorbate levels and for GSH accumulation in leaves and lower increases of total glutathione levels in leaves than 'Scoresby Dwarf'.
- Under waterlogging, 'Scoresby Dwarf' increased the levels of total ascorbate and of all glutathione attributes, in contrast to 'Best Boy Bush' which showed no changes for total ascorbate levels and for GSH accumulation, and lesser increases in total glutathione levels than 'Scoresby Dwarf'.

- In fruits, drought increased total ascorbate levels more in the pericarp than in the skin, and particularly so in ‘Scoresby Dwarf’. Waterlogging decreased total ascorbate levels more in the skin than in the pericarp in both cultivars.
- There were strong increases in the levels of the oxidised antioxidants DHA and GSSG under water deficit and these increases were even more pronounced under waterlogging.
- ‘Scoresby Dwarf’ showed less pronounced increases in leaf GSSG levels under both stress factors, compared to ‘Best Boy Bush’, whereas in roots only the latter cultivar increased GSSG levels under drought.

5.5 Principal component analysis and heatmap clustering analysis

5.5.1 Multivariate biochemical responses to the effect of water stress

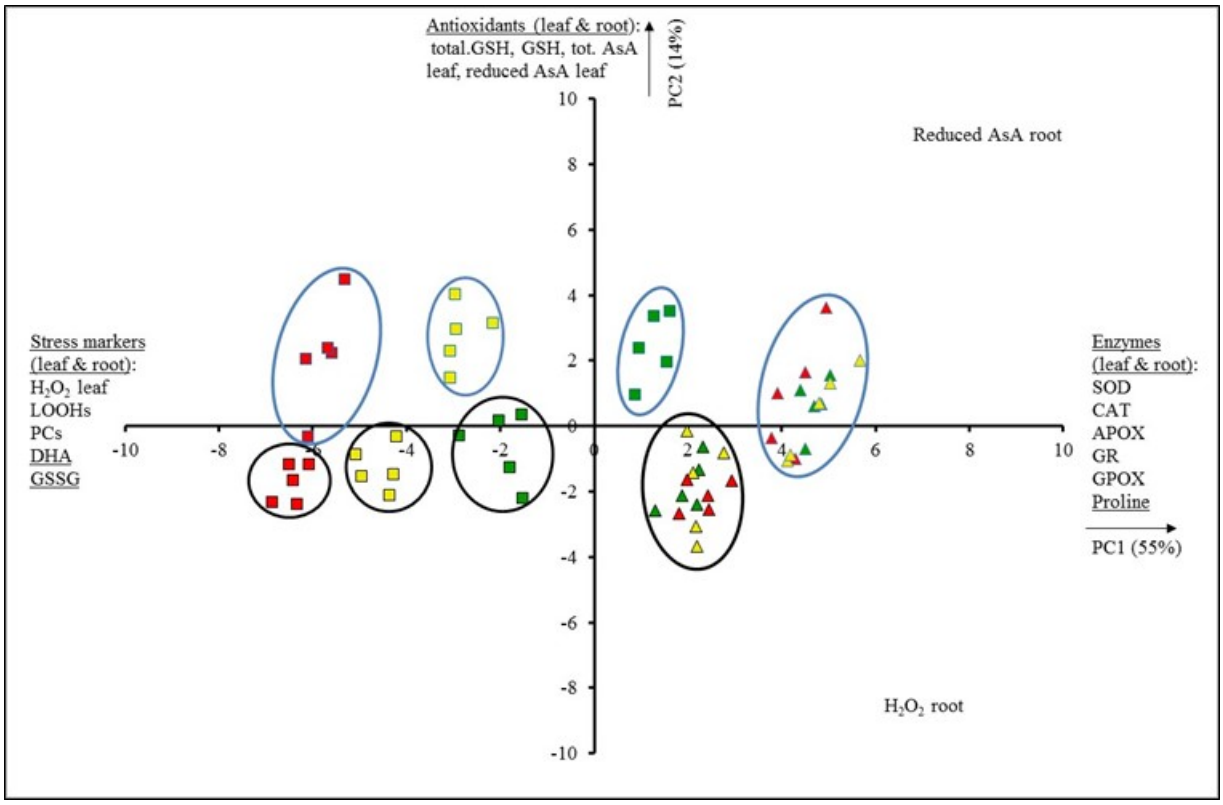


Figure 5.5.1.a Biplot of treatment responses (ratios of treatment/control) in two tomato cultivars at different developmental stages. 'Best Boy Bush' responses are circled in black and 'Scoresby Dwarf' responses are circled in blue. Triangles represent drought responses at the vegetative (▲), flowering (△) and fruiting (▲) stages. Squares represent –waterlogging responses at the vegetative (■), flowering (□) and fruiting (■) stages.

5.5.1.1 Plant trait responses to water stress in the first principal component

The PCA biplot revealed clear oxidative stress response patterns. The first principal component (PC1) explained the majority (55%) of the variance in the dataset. High scores on PC1 were characterised by increases in all enzymatic antioxidants and free proline in the leaf and root tissues (Figure 5.5.1.a). This was inversely correlated with increases in the stress markers in the leaf and root tissues (with the exception of H_2O_2 in root tissues), which were all located towards the negative end of the PC1 axis. Changes in reduced AsA levels in root tissues were positively associated with both PC1 and PC2, while DHA and GSSG held an intermediate position

between enzymatic antioxidants and stress markers. Waterlogging responses were generally located towards the negative part of the PC1 axis, while drought responses always had positive PC1 scores.

5.5.1.2 Developmental stages and cultivar responses to water stress in the first principal component

The waterlogging responses were further characterised in PC1 by distinctive developmental patterns, with the most negative scores at fruiting, less negative scores at flowering and higher scores at the vegetative stage. PC1 also separated the 'Best Boy Bush' hypoxia responses from those of 'Scoresby Dwarf', with the latter having the highest PC1 scores in this treatment at the vegetative stage. Thus, oxidative damage from waterlogging was less pronounced in 'Scoresby Dwarf' than in 'Best Boy Bush' at the vegetative stage, while the damage pattern was similar for both cultivars at fruiting. Under drought stress, however, 'Scoresby Dwarf' had the highest PC1 scores at all developmental stages.

5.5.1.3 Plant trait responses to water stress in the second principal component

The second principal component (PC2) accounted for 14% of the variance in the dataset. There were eight traits linked to PC2, all of which were found at the positive end of PC2 (Figure 5.5.1.a). These consisted primarily of antioxidant responses in the ascorbate and glutathione pool such as increased levels of reduced AsA and of total AsA in leaves, as well as increased levels of DHA, GSH, GSSG and total GSH in both leaf and root tissues.

5.5.1.4 Developmental stages and cultivar responses to water stress in the second principal component

PC2 clearly separated 'Best Boy Bush' (mainly negative PC2 scores) from 'Scoresby Dwarf' (mainly positive scores) across the developmental stages and water stress treatments (Figure 5.5.1.a).

The results from PC2 revealed a higher stress tolerance for 'Scoresby Dwarf', with overall higher increases in non-enzymatic antioxidant levels under both water stress extremes and at all three developmental stages compared to 'Best Boy Bush'.

5.5.2 Similarities of biochemical responses

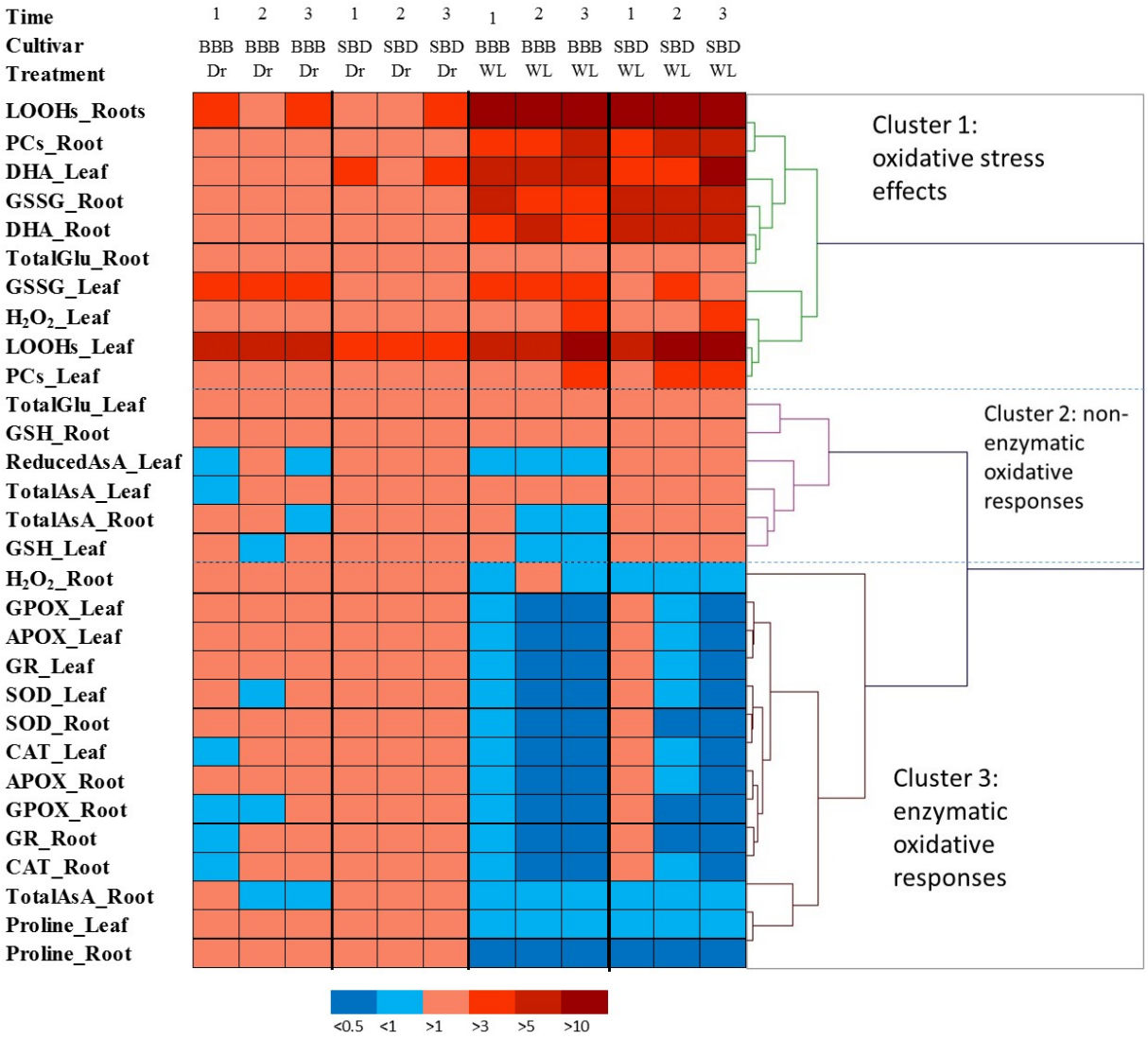


Figure 5.5.2.a Heat map and agglomerative hierarchical clustering of average biochemical changes in two tomato cultivars, 'Best Boy Bush' (BBB) and 'Scoresby Dwarf' (SBD) at the vegetative (Time 1), flowering (Time 2) and fruiting (Time 3) stages in response to drought stress (Dr) and waterlogging (WL). The list of biochemical traits is shown on the left and the similarity clustering is shown on the right.

The HCA identified three distinctive stress response clusters among the biochemical traits (Figure 5.5.2.a). The first can be described as the 'oxidative effects' cluster as it comprised stress-induced increases in the markers and in the oxidised forms of the non-enzymatic antioxidants. This cluster was subdivided by HCA into two groups. The first group consisted mainly of oxidative effects in roots, including increases in the stress markers LOOHs and PCs, as well as in GSSG and DHA (leaves and roots) and in total root glutathione levels. The second group consisted of leave stress markers, H_2O_2, LOOHs and PCs as well as of oxidised

glutathione (GSSG). The heatmap of the first cluster analysis revealed more pronounced increases under waterlogging than under drought, particularly in roots (Group 1).

The second cluster was charaterised by non-enzymatic antioxidant responses, such as reduced AsA (leaves), total ascorbate (leaves and roots), GSH (leaves and roots) and total GSH (leaves). All traits in this cluster increased in 'Scoresby Dwarf' under both water stress treatments, while several decreased in 'Best Boy Bush' under both stress factors.

The third cluster mainly comprised increases in antioxidant drought responses and decreases under waterlogging. This cluster was subdivided by HCA into a main group and two smaller groups. The latter included root responses of: (i) H_2O_2; and (ii) of total ascorbate and proline (in leaves as well). The main cluster group, however, identified by HCA was the enzymatic antioxidant group. The heat map of the drought responses showed increases in all enzymatic antioxidant activities in 'Scoresby Dwarf' and some decreases in 'Best Boy Bush'. The heat map of the waterlogging effects in 'Scoresby Dwarf' highlighted increases in these enzyme activities at the vegetative growth stage, followed by decreases at flowering and the most pronounced decreases at the fruiting stage. In contrast, these enzyme activities decreased in 'Best Boy Bush' at all developmental stages, with pronounced changes setting in from flowering.

5.5.3 Multivariate analysis of morphological, physiological and biochemical traits

5.5.3.1 Plant traits response to water stress in the first principal component

PC1 of the overall PCA accounted for 31% of the variance in the dataset, nearly double to the next principal component (Figure 5.5.3.a). PC1 was characterised by traits related to oxidative effects and antioxidative responses. The positive end of this axis was characterised by high levels of all stress markers in leaves and roots (with the exception of H_2O_2 in the roots) as well as by high levels of the oxidised antioxidants, DHA and GSSG, and of root GSH. High levels in these traits were inversely correlated with high activities of enzymatic antioxidants in the leaf and root tissues, which were all located towards the negative end of this axis. Traits measured under waterlogging were located towards the positive end of the PC1 axis, while traits measured under drought always had negative PC1 scores. In addition, the negative end of PC1 was characterised by the accumulation of non-enzymatic antioxidants, such as proline, and the reduced form of ascorbate in leaves and roots, as well as high levels of osmotic potential.

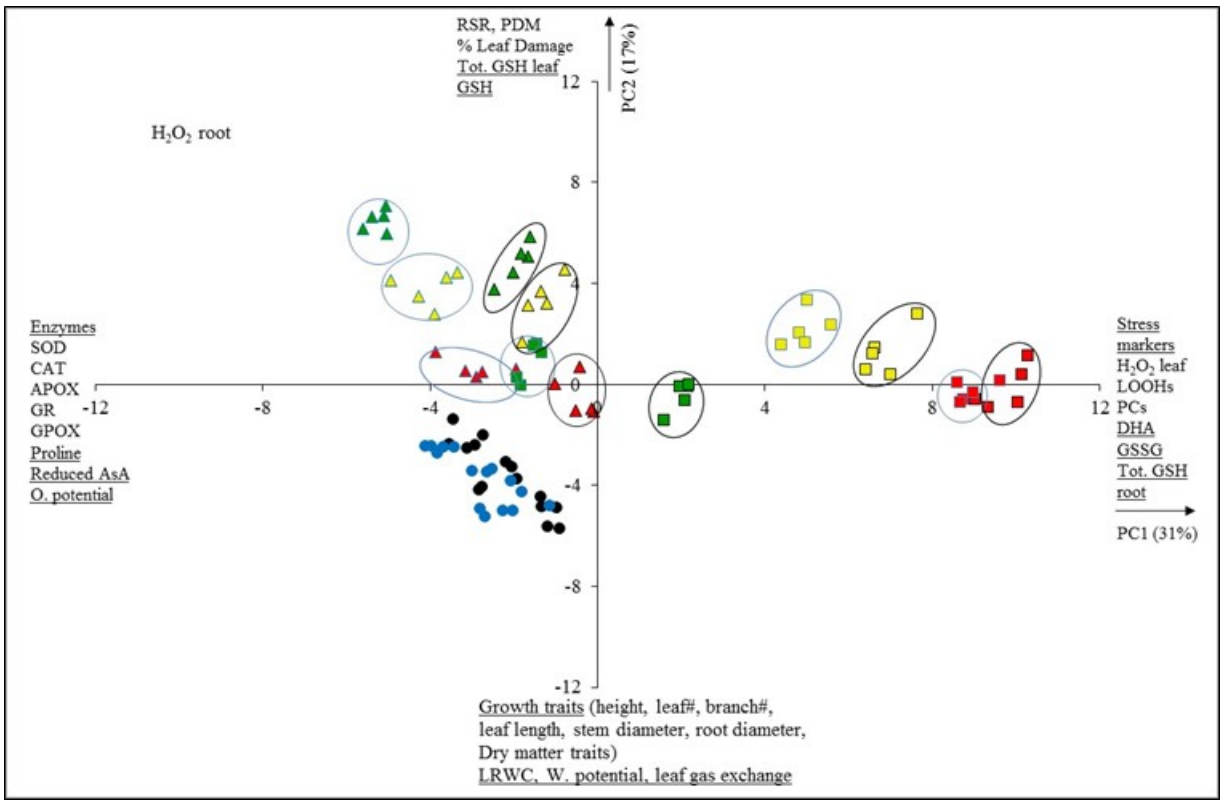


Figure 5.5.3.a Biplot representing plant traits in two tomato cultivars at different developmental stages. 'Best Boy Bush' traits are circled in black and 'Scoresby Dwarf' traits are circled in blue. Round shapes represent traits measured under control conditions for 'Best Boy Bush' (●) and 'Scoresby Dwarf' (●). Triangles represent traits measured under drought at the vegetative (▲), flowering (▲) and fruiting (▲) stages. Squares represent traits measured under waterlogging at the vegetative (■), flowering (■) and fruiting (■) stages.

5.5.3.2 Developmental stages and cultivar responses to water stress in the first principal component

The waterlogging responses were further characterised in PC1 by distinctive developmental stages, with the highest positive scores at fruiting, less postive scores for flowering and the lowest scores for the vegetative stage (Figure 5.5.3.a). PC1 also separated the 'Best Boy Bush' hypoxia traits from those of 'Scoresby Dwarf', with the latter having more negative PC1 scores in this treatment at the vegetative stage. Therefore, oxidative damage from waterlogging was more pronounced in 'Best Boy Bush' than in 'Scoresby Dwarf' at the vegetative stage, while the damage pattern was similar for both cultivars at fruiting. Plants subjected to a water deficit had negative PC1 scores, with 'Scoresby Dwarf' showing the lowest PC1 scores at all developmental stages. Furthermore, all the control plants were found on the negative side of PC1.

5.5.3.3 Plant trait responses in the second principal component

The second principal component (PC2) explained 17% of the variance in the dataset (Figure 5.5.3.a). Morphological and physiological traits largely separated the dataset in PC2. High PC2 scores were characterised by high root:shoot ratios, high percentage dry matter and high % leaf damage. Positive PC2 scores were also characterised by high levels of GSH in both leaf and root tissues and of total GSH in leaves. This was inversely related at the negative end of PC2 to a number of morphological and growth related traits (high values for plant height, numbers of leaves and branches, leaf length, stem and root diameters and dry matter traits), as well as to physiological parameters such as high relative water content in leaves, high water potential and high levels of all the components of leaf gas exchange. The production of root H_2O_2 level was located at an intermediate position between the negative PC1 and the positive PC2 axis.

5.5.3.4 Developmental stages and cultivar responses to water stress in the second principal component

In plants exposed to drought, the vegetative stage had the highest PC2 scores, closely followed by the flowering stage, while the fruiting stage had the lowest PC2 scores. There was no clear developmental separation of waterlogging-induced traits in PC2 and no cultivar differences for either water stress treatment in PC2. Control plants had the lowest PC2 scores compared to any of the water stress treatments. These results will be discussed in the General Discussion section of this thesis.

5.6 Discussion

This is the first study that specifically compares plant oxidative metabolism under water deficit and waterlogging at different growth and developmental stages in various tissues. There is also comparatively little known about oxidative damage and the antioxidant responses in water stressed tomatoes. The overall results from multivariate analysis (PC1 in the PCA) (Figure 5.5.1.a) clearly separated desiccation from waterlogging responses, showing that the latter were more affected by oxidative stress than the drought responses, which were also characterised by higher stress mitigation in the form of increased activity of enzymatic antioxidants (Figure 5.5.1.a).

5.6.1 Effects of water stress on oxidative damage markers

Oxidative damage of plant tissues is associated with the accumulation of reactive oxygen species. Consequently, some researchers have monitored oxidative damage in plants by measuring the presence of hydrogen peroxide (H_2O_2) and malondialdehydes (secondary products of lipid peroxidation) in plant tissues (Ahsan et al., 2007; Sanchez-Rodriguez et al., 2012). However, lipid hydroperoxides (primary products of lipid peroxidation) and protein carbonyls (an index of protein oxidation) have not, to date, been investigated in tomatoes under water stress.

In this present study, plants subjected to stress had, in general, higher levels of H_2O_2 and more oxidative damage, as measured by the production of LOOHs and PCs. Plants under waterlogging had higher concentrations of H_2O_2 and of the other oxidative damage markers compared to those exposed to drought (Figure 5.4.1.a, b and Appendix E).

In the plant cell, hydrogen peroxide is most often formed from the dismutation of the superoxide anion and catalysed by the enzyme superoxide dismustase (Sharma et al., 2012). Hydrogen peroxide is produced in cells as a result of normal plant metabolism, but levels increase if plants are exposed to biotic or abiotic stressors (Gill & Tuteja, 2010; Sharma et al., 2012). If cellular H_2O_2 increases, due to insufficient scavenging of ROS, and in the presence of Fe^{2+} and Fe^{3+} ions, the Fenton/Haber-Weiss reactions will be enhanced. The reaction results in the production of highly toxic hydroxyl radicals within the cells (Sharma et al., 2012; Tuteja et al., 2009). Hydroxyl radicals cause lipid peroxidation, protein oxidation and DNA damage and when this occurs the cells are in a state of oxidative stress (Gill & Tuteja, 2010).

Of further interest was the fact that in this study H_2O_2 did not accumulate in the hypoxic roots that have been directly exposed to waterlogging stress, while the leaves which had only indirectly been exposed to stress had high H_2O_2 levels (Figure 5.4.1.a). A reduction in H_2O_2 was also reported in hypoxic roots of pigeon peas by Kumutha et al. (2009). The authors suggested that reduction in ROS production in hypoxic roots might be caused by a shift from aerobic respiration to fermentation because the main ROS production area was in the mitochondrial electron transfer chain in non-green tissues (Kumutha et al., 2009). Ahsan et al. (2007) reported that foliar H_2O_2 levels gradually increased with the imposition of waterlogging and then slightly reduced on the third day of stress exposure, perhaps due to the activation of H_2O_2 scavengers. It may be possible that a less efficient antioxidant scavenging system, particularly the enzymes CAT and APOX, was responsible for a significant H_2O_2 continuing presence in waterlogged leaf tissues. In addition, it is likely that more superoxide anion was generated and this was converted into H_2O_2. Other studies have shown that this was the case and was, at least in part, due to increased levels of DPI-sensitive NADPH-oxidases (Kumutha et al., 2009). It was noteworthy that compared to elevated H_2O_2 levels, the concentrations of LOOHs and PCs were particularly elevated in hypoxic roots (Figure 5.4.1.a, b), suggesting value for using these measurements in future stress marker studies.

5.6.2 Enzymatic antioxidant responses

The ability of plants to maintain a balance between the formation and detoxification of reactive oxygen species is associated with tolerance to oxidative stress (Lin et al., 2004; Sanchez-Rodriguez et al., 2012). The juxtaposition with these stress marker responses on PC1 underlined the role of enzymatic antioxidants, rather than non-enzymatic antioxidants, for oxidative stress mitigation (Figure 5.5.1.a). High SOD activity is usually associated with high tissue H_2O_2 contents because SOD catalyses H_2O_2 production from $O_2^{\bullet -}$ (Sanchez-Rodriguez et al. 2012; Zgallai et al., 2006). The activities of CAT, APOX, GR and GPOX are all essential for the elimination of H_2O_2 under stressful conditions. Catalase, APOX and GPOX showed decreased activities in the tissues sampled from waterlogged plants (Appendix E). Therefore, H_2O_2 that was not detoxified, could have been converted into hydroxyl radicals in the presence of Fe^{2+} and Fe^{3+} ions and, concomitantly, LOOHs and PCs were generated (Burritt & Mackenzie, 2003). Catalase can directly convert H_2O_2 to water and oxygen (Gill & Tuteja, 2010). Similar to catalase, APOX is involved in the conversion of H_2O_2 to water by utilising reduced ascorbate as the electron donor (Gill & Tuteja, 2010). GR is also an important enzyme in maintaining a

recycling glutathione pool. This enzyme uses NAD(P)H as a co-factor to convert GSSG to GSH and generate $NAD(P)^+$ (Gill & Tuteja, 2010). It has been suggested that GPOX can effectively reduce H_2O_2 and lipid hydroperoxides (Gapinska et al., 2008; Griffiths et al., 2000; Yoshimura et al., 2004). The fact that tissue taken from water deficit-treated plants in the present study had a lower content of LOOHs could indicate the efficiency of this enzyme in handling oxidative damage. Gapinska et al. (2008) reported that the activity of GPOX was apparent in the first hour of the experiment and was associated with rising H_2O_2 levels. Later, GPOX activity increased in response to elevated lipid peroxidation (Gapinska et al., 2008). Low GPOX activity may also result in further membrane damage by GPOX, which uses GSH as a co-factor (Hall & Bosken, 2009) and can, like CAT and APOX, neutralise H_2O_2 and aid in terminating the autocatalytic propagation process associated with lipid peroxidation (Gill &Tuteja, 2010; Shamim et al., 2013).

5.6.3 Non-enzymatic antioxidant responses

Non-enzymatic antioxidants also play important roles in ROS detoxification. This was highlighted in the present study by the fact that non-enzymatic antioxidant levels increased under both stress factors, except for levels of reduced AsA, which increased under drought but decreased under waterlogging in 'Best Boy Bush' leaves and in the roots of both cultivars, with more pronounced decreases for 'Best Boy Bush' (Figure 5.4.3.a). Hajiboland (2014) suggested that reduced AsA was a major ROS scavenger. This antioxidant can directly remove many types of ROS molecules including $O_2^{\bullet -}$, $OH^{\bullet -}$ and $^1O_2$ from plant cells. Additionally, reduced AsA is needed for the enzyme APOX to detoxify H_2O_2 (Gill & Tuteja, 2010; Hajiboland, 2014). A previous study suggested that lower levels of reduced AsA in tissues may result from insufficient regeneration of this compound from its oxidised form, DHA (Shalata et al., 2001). Dehydroascorbate can be lost by conversion to 2,3-diketegulonic acid (Kaur & Nayyar, 2014) if it was not reduced back to ascorbate, because DHA has a shorter half-life than ascorbate (Gill & Tuteja, 2010). In addition, the activity of APOX may also be responsible for a reduction in the levels of reduced AsA and an accumulation of DHA as observed in the present study (Appendix E). APOX uses ascorbate as a co-factor to detoxify H_2O_2 with the generation of two molecules of water and one molecule of DHA (Gill & Tuteja, 2010). However, APOX activity in the present study decreased under waterlogging (Appendix E).

Lower levels of ascorbate may be caused by the loss of GSH, which is known to participate in regeneration of reduced AsA from DHA (Hajiboland, 2014). GSH is also an important antioxidant participating in ROS detoxification (Foyer & Noctor, 2011; Hajiboland, 2014). GSH is an electron donor used to catalyse H_2O_2 breakdown by GPOX. The enzyme GPOX, uses two molecules of GSH in the reaction with lipid hydroperoxides and produces one molecule of GSSG. Foyer and Noctor (2011) reported that the glutathione pool was mostly reduced (GSH) under normal conditions and a shift to the oxidised form GSSG was a sign of increasing intercellular ROS levels. In the present study, a high GSSG content was found in hypoxic roots (Figure 5.4.3.f). 'Scoresby Dwarf' showed less pronounced increases in leaf GSSG levels under both stress factors, compared to 'Best Boy Bush', whereas in roots only the latter cultivar increased GSSG levels under drought. These findings again point at higher stress sensitivity for 'Best Boy Bush'.

ROS detoxification, as an on-going process, can also be followed by determining the redox state of the ascorbate and glutathione pools. This can be expressed using ratios of ascorbate:DHA or GSH:GSSG (Foyer & Noctor, 2011). These ratios are generally used to indicate the capacity a plant has for ROS detoxification (Sanchez-Rodriguez et al., 2012). A high ratio can be interpreted as plants possessing an effective scavenging system. In the present study, drought stressed plants mostly had higher ratios of both ascorbate:DHA and GSH:GSSG, whereas the waterlogged plants had lower values for these ratios. These ratios could have decreased for two reasons (Foyer & Noctor, 2011). First, the lower ratios could be caused by an increase in H_2O_2 in the tissues. Secondly a decrease in the recycling capacity of the ascorbate-glutathione pool could cause a reduction in these ratios (Foyer & Noctor, 2011). The intermediate position between enzymatic antioxidants and stress markers in the PCA responses of DHA and GSSG, and the pronounced stress responses of these oxidised antioxidants, underlines their role as indicators of oxidative stress (Figure 5.5.1.a).

In addition, tomato fruits are excellent sources of vitamin C. Vitamin C (ascorbic acid) has well-established beneficial effects on human health due to its powerful ROS scavenger capacity. The ascorbic acid levels in tomato fruit can be altered by light, heat (Chandra et al., 2012) and drought (Murshed et al., 2013). However, information on the effects of abiotic stress on different tomato fruit components is still limited. Ascorbate is a powerful water soluble antioxidant (Gill & Tuteja, 2010). The health benefit of this antioxidant includes preventing scurvy, assisting in wound healing, improving the immune system and preventing

cardiovascular disease (Kader et al., 2012). Humans cannot synthesize ascorbic acid endogenously, and thus a dietary intake of this compound is recommended (Kader et al., 2012). Ascorbic acid is present in some fruits and vegetables and also available as a dietary supplement. Ascorbic acid is present abundantly in tomato fruits grown under optimal growing conditions (Chandra et al., 2012) and can be modified by drought stress (Murshed et al., 2013) and reduced under root hypoxia (Horchani et al., 2010). In this study, the tomato fruit pericarp and tomato skins of drought-treated plants contained the highest levels of total AsA (Figure 5.4.3.e). Ascorbate synthesis genes in tomato could be influenced by light irradiance (Massot et al., 2012). The author suggested that the higher level of ascorbate synthesis in the tomato skin or epidermis was expected because these tissues were more exposed to light (Massot et al., 2012). This helps explain the finding here that the skin fraction of tomato fruits contained twice as much total AsA as in the pericarp (Figure 5.4.3.e).

5.6.4 Cultivar differences in stress responses

'Scoresby Dwarf' showed, in general, lower production of H_2O_2 and less damage to lipids (lower LOOHs) and proteins (lower PCs) relative to 'Best Boy Bush' under both stress factors. Under drought, 'Scoresby Dwarf' had increased activities of enzymatic antioxidants (SOD, CAT, APOX, GR and GPOX) relative to 'Best Boy Bush' (Table 5.4.2.b). Furthermore under waterlogging, the latter cultivar showed reduced antioxidant enzyme activities at all harvests, whereas in 'Scoresby Dwarf' this occurred at the fruiting and – to a lesser degree – at the flowering stages (Table 5.4.2.b). The findings are in line with suggestions that plant tolerance to oxidative stress is associated with the levels of antioxidant enzymes (Hameed et al., 2014). Gill and Tuteja (2010) reported that overexpression of SOD in many transgenic plants enhanced abiotic stress tolerance. For example, overexpression of Mn-SOD in transformed tomato plants resulted in the plants' improved response to salt stress (Gill & Tuteja, 2010). The author also reported that transgenic rice plants exhibited tolerance to drought stress due to high CAT activity following overexpression of *OsMT1a*. APOX and GR are enzymes in the ascorbate-glutathione pathway and increased levels of these enzymes have been shown to confer tolerance against oxidative stress induced by water deficit and waterlogging stresses (Lin et al., 2004; Sanchez-Rodriguez et al., 2012). In addition, Hameed et al. (2014) reported that increased activities of GR and SOD improved oxidative stress tolerance in transgenic tobacco plants. Sanchez-Rodriguez et al. (2012) have recently investigated the response of antioxidant enzymes

and their roles in drought tolerant cherry tomatoes. Their findings showed – similar to the findings in the present study – that antioxidant enzyme activity was strongly increased and more consistent in stress tolerant genotypes, whereas in drought- sensitive cultivars, enzyme activity was limited and there was substantial damage caused by oxidative stress. In addition, Lin et al. (2004) suggested that the activity of ascorbate peroxidase in tomato roots play an essential role on promoting stress tolerance in plants experiencing waterlogging.

As outlined above, non-enzymatic antioxidants also play an important role in the enhancement of plant tolerance to oxidative stress. In the present study, 'Scoresby Dwarf' increased the levels of most non-enzymatic antioxidants under both water stress factors, in contrast to 'Best Boy Bush' there were either no changes or lower increases (Table 5.4.3 b, d). This is similar to findings of high levels of reduced AsA in tolerant cherry tomatoes subjected to drought stress (Sanchez-Rodriguez et al., 2012). Similarly, Hossain et al. (2014), showed that plant tolerance to oxidative stress caused by salinity was often associated with efficient ROS scavenging systems and high GSH and AsA levels. Under oxidative stress it is crucial to maintain the redox state of GSH by enhancing activity of GR (Foyer & Noctor, 2011; Hameed et al., 2014). Reduced AsA and GSH are fundamental cofactors of many enzymes involved in ROS detoxification. This is supported by the results from PC2 (Figure 5.5.1.a), which revealed higher stress tolerance for ‘Scoresby Dwarf’, with overall higher increases of non-enzymatic antioxidant levels under both water stress extremes and at all three developmental stages compared to ‘Best Boy Bush’. This finding clearly demonstrates that oxidative stress tolerance in ‘Scoresby Dwarf’ is – at least partly – due to the higher stress-induced efficiency of glutathione and ascorbic acid metabolism.

This was also highlighted in the second cluster on HCA, where the heatmap revealed increases for all non-enzymatic antioxidant levels in 'Scoresby Dwarf' under water stress, whereas several non-enzymatic antioxidant levels showed decreases in 'Best Boy Bush' under both stress factors (Figure 5.5.2.a). Non-enzymatic antioxidants can thus be seen as a constitutive stress buffer in ‘Scoresby Dwarf’, while enzymatic antioxidants are specifically involved in drought protection, highlighted by drought-induced increases in all enzymatic antioxidant activities in 'Scoresby Dwarf'. The results also suggest that ‘Scoresby Dwarf’ is able to withstand waterlogging at the early stages of plant development via increases in enzymatic antioxidant activities. Taken together, this cultivar can be considered more tolerant in terms of its oxidative responses to drought stress and waterlogging compared to ’Best Boy Bush’.

5.6.5 Influence of developmental stages

While the developmental stage did not affect the oxidative plant responses to water deficit, such responses to the waterlogging treatment varied between the vegetative growth and reproductive developmental stages. Plants at the vegetative growth stage had higher tolerance to stress induced by waterlogging. Analysis of hypoxic plant tissues at the vegetative stage showed low foliar H_2O_2 levels and less oxidative damage (LOOHs and PCs) compared with tissues of plants at the reproductive stage of development (Figure 5.4.1.a, b and Appendix E). There are two possible explanations for this. First, and most importantly, this might be attributed to the activities of enzymatic antioxidants which were still relatively high, especially in 'Scoresby Dwarf', in both leaf and root tissues of waterlogged plants at the vegetative stage. Secondly, the formation of adventitious aerial roots was observed mostly in plants at the earlier growth stage. Availability of oxygen from these roots could help alleviate the oxidative stress from waterlogging (Horchani et al., 2008; Sairam et al., 2008). At the reproductive developmental stages, waterlogged plants were characterised by a significant increase in H_2O_2 content in leaf tissues, accompanied by high levels of lipid hydroperoxides and protein carbonyls in both leaf and root tissues. Concomitantly, the activities of all the major antioxidant enzymes were all reduced at the later developmental stages. The susceptibility of tomato plants to waterlogging in the reproductive phase was also observed by Horchani et al. (2009). The authors suggested that flowers and fruits have a higher priority for carbon allocation compared to carbon investment into the rest of the plant. Therefore, waterlogged plants at the reproductive stage might have less energy to invest in vegetative growth to tackle damaging stress effects (Horchani et al., 2009). The waterlogging results showed relative tolerance from oxidative damage for 'Scoresby Dwarf' compared to 'Best Boy Bush' at the vegetative stage, but not at fruiting (Figure 5.5.1.a). This finding suggests that the draw on plant resources at the latter developmental stage overrides any cultivar-specific stress tolerance. In contrast under drought stress, the PC1 distribution of cultivar responses consistently showed increased enzymatic antioxidant activity for 'Scoresby Dwarf' relative to 'Best Boy Bush', demonstrating maintenance of relative drought tolerance in 'Scoresby Dwarf' throughout all developmental stages.

5.6.6 Conclusions

In summary, the oxidative plant response to drought was characterised by increasing activities of enzymatic antioxidants, as well as of higher non-enzymatic antioxidant levels as a response

to the stress-induced formation of oxidative stress markers. Waterlogging, on the other hand, was generally characterised by a rise in oxidative stress markers and suppression of these enzymes. Plants at the reproductive stage were more susceptible to waterlogging when compared with the earlier vegetative stage. The results support the view that 'Scoresby Dwarf' was more tolerant to water stress compared to 'Best Boy Bush'. Under stress, 'Scoresby Dwarf' exhibited a lower oxidative load (H_2O_2) and was also less affected by oxidative stress, indicated by lower stress-induced increases of lipid hydroperoxides, protein carbonyls, and oxidised antioxidant levels. Compared to 'Best Boy Bush', 'Scoresby Dwarf' had higher stress-induced activities of the enzymatic antioxidants and strongly increased non-enzymatic antioxidant levels.

Chapter 6

The time-dependent influence of water deficit and waterlogging on oxidative stress, nutritional quality and bioactivity of tomatoes

Abstract

The oxidative responses of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf', to extremes of water stress (water deficit and waterlogging) at the fruiting stage, were kinetically investigated under field conditions. The effects of water stress treatments were noticeable soon after stress imposition in both leaf and root tissues by increases for all stress markers measured. Levels of these stress markers increased by 33% to 5.2-fold in plants exposed to drought, while their levels became more elevated in waterlogged plants over time (on Day 5 by 67% to 7.1-fold and on Day 8 by 2.1- to 8.1-fold). Tomato plants responded to oxidative stress by activating enzymatic antioxidants. Increases in these activities were higher (by 29-63%) in drought stressed plants than in waterlogged plants, which plateaued at Day 5 with increases of 30% in most cases. In addition, levels of non-enzymatic antioxidants largely increased by 6% to 4.1-fold under water deficit but decreased under waterlogging by 10-31%, except for total glutathione and the oxidised antioxidants. The activity of glyoxalase enzymes (GLOX1 and GLOX2) increased under water stress by 2.1-2.9-fold, in parallel with rising methylglyoxal levels but were higher in the hypoxic plants, increasing by 2.4-3-fold, compared to 2.1-fold under drought. Total carotenoid content decreased by nearly 40% in the pericarp of 'Best Boy Bush' fruits under the two water stress treatments, but not in 'Scoresby Dwarf' fruits. Total antioxidant activity (TAC) increased in the pericarp of drought stress tomato fruits (25%), but decreased in the pericarp of hypoxic fruits (16%). In addition, the viability of Caco-2 cells under oxidative stress improved when pre-incubated with digests of drought-stressed tomato fruits. This suggests value for the targeted application of drought before harvesting of tomato plants for added health benefits for consumers. There were no cultivar differences in treatment responses for most of the traits measured in this chapter. An overall multivariate analysis of all data from the field studies of this thesis is also presented here and will be discussed in the General Discussion section.

6.1 Introduction

A serious imbalance between ROS accumulation and antioxidant defence results in oxidative damage in plants. Reactive oxygen species can damage DNA, oxidise proteins and cause lipid peroxidation (Burritt & Mackenzie, 2003; Foyer & Noctor, 2011; Gill & Tuteja, 2010). Methylglyoxal (MG) is a highly toxic compound that accumulates as a result of metabolic activities, including glycolysis, and as a consequence of lipid peroxidation (Hoque et al., 2012b). The activity of glyoxalase enzymes in MG detoxification is crucial because high levels of MG can inactivate antioxidant defence systems (Yadav et al., 2005b). As mentioned in Chapter 5, the primary effect of waterlogging is oxygen deprivation or hypoxia. The hypoxic

conditions can be determined by measuring the activity of alcohol dehydrogenase in the roots (Wei et al., 2013).

Tomato fruits are excellent sources of vitamins, minerals and phytochemicals (Frusciante et al., 2007; Panthee et al., 2012). The term phytochemicals can be defined as bioactive non-nutrient components of fruits and vegetables, and can be divided into groups that include the carotenoids and polyphenols (Coates et al., 2007). These compounds have been found to lower the incidence of certain types of cancer, cardiovascular disease, eye diseases and enhance the immune system (Horchani et al., 2010; Panthee et al., 2012; Sanchez-Rodriguez et al., 2011). Carotenoids are pigments that are often responsible for the yellow, orange and red colours of fruit and vegetables (Frusciante et al., 2007; Kopsell & Kopsell, 2006). The assessment of the protective or antioxidant capacity of these phytochemicals to prevent damage to biological systems caused by excessive levels of oxidants is termed the total antioxidant capacity (Goerge et al., 2004).

The assessment of these phytochemicals using *in vitro* methods has gained current research interest because these approaches are simple, fast and less expensive than *in vivo* trials (Rodriguez-Roque et al., 2013). *In vitro* gastrointestinal digestion is designed to mimic the human digestive system and is a useful tool to assess the bio-accessibility or bioactivity of compounds (Rodriguez-Amay, 2010). Cilla et al. (2013) suggested that *in vitro* studies are unrealistic if they involved only a single compound at high concentrations far above the concentrations detected in *in vivo* and, therefore, bioactivity might be overestimated. However, using whole food samples or plant extracts instead of bioactive constituents to measure bioactivities can provide information close to real-life physiological situations (Cilla et al., 2013). These *in vitro* gastrointestinal digestion studies have been used in conjunction with Caucasian Colon Adenocarcinoma Cell (Caco-2 cell) culture systems. Caco-2 cell cultures can be used to provide an estimation not only to monitor nutrient uptake and transport of supplements and whole food samples but also to determine in digested plant-based foods have bioprotective capacity against oxidative damage (Aherne et al., 2007; Etcheverry et al., 2012; Kopsell & Kopsell, 2006). There has been no previous study that investigated the biological activity of water stressed tomato fruits using these methods.

Drought or excessive water commonly occurs in the natural environment. Therefore, a field study was conducted, following the glasshouse experience, to gain a better understanding of

plant responses to water stress under more natural environmental conditions. The general objective of this chapter was to examine the oxidative responses of tomato plants under drought and waterlogging. DNA oxidation, the activity of alcohol dehydrogenase and the activity of glyoxalase enzymes in response to accumulation of methylglyoxal will be examined, as well as the production of H_2O_2, oxidative damage (lipid hydroperoxides and protein carbonyls) and antioxidant defence system markers that were previously investigated in the glasshouse study. An additional aim of this chapter was to examine the nutritional quality of tomato fruits and the bioactivity of digested tomato fruits grown under water stresses (water deficit and waterlogging).

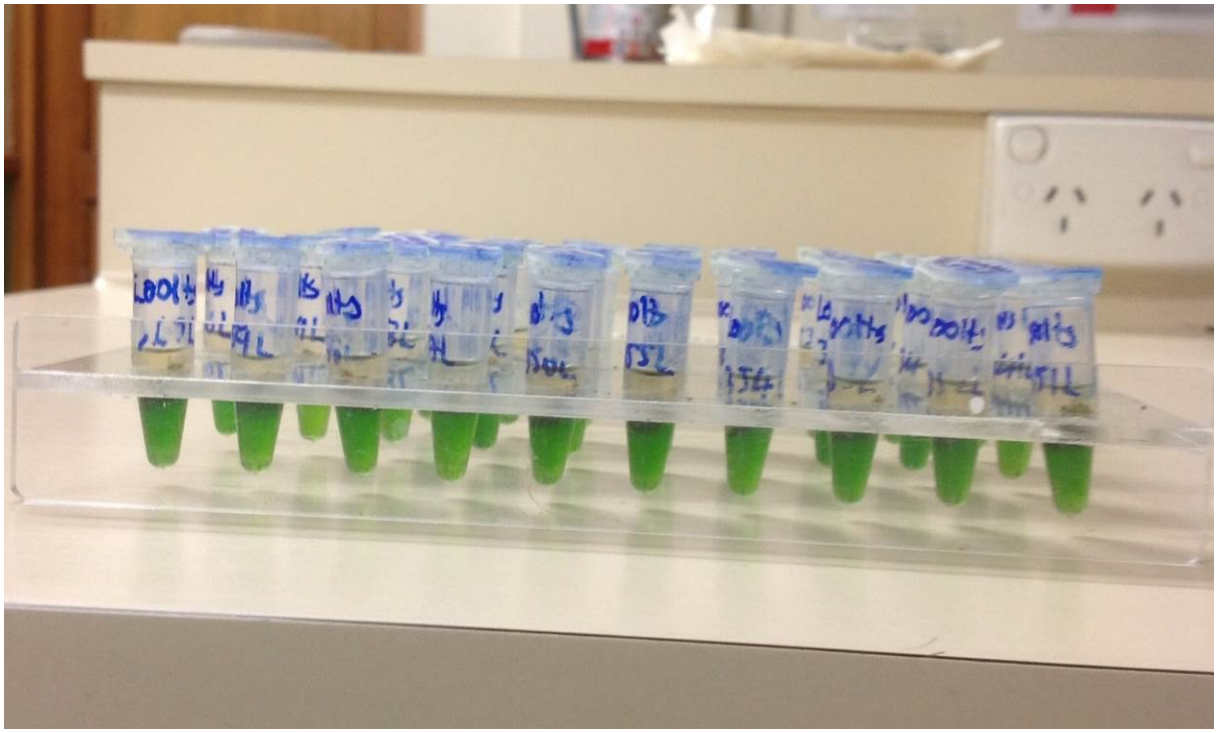


Plate 5. Extraction of lipid hydroperoxides: The process of chloroform phase separation of tomato leaf tissue.

6.2 Material and methods

The plant growing conditions and the experimental design have already been outlined in Chapter 3. In addition, details of the analyses of specific plant biochemical compounds, including hydrogen peroxide, lipid hydroperoxides, protein carbonyls and antioxidant enzymes and antioxidants, have already been shown in detail in Chapter 5. DNA damage, ADH, MG, glyoxalase enzymes, total carotenoids and total antioxidant capacity as well *in vitro* gastrointestinal digest coupled with Caco-2 cell culture method are described below.

6.2.1 DNA purification and assay

DNA extraction: DNA was extracted using a Plant DNA Mini Kit II (Bioline) with the following modifications. The lysis buffers were supplemented with 5 mM deferoxamine and 20 mM EDTA (Dany et al., 1999). The rest of the protocol followed the manufacturer's instructions. The quantity of DNA in each sample and its relative purity were determined by measuring the absorbance at 260 and 280 nm using a PerkinElmer VICTOR^3 multilabel plate reader. DNA purity was determined by the A_{260}/A_{280} ratio, with all values being within the range of 1.7–1.9 (Sambrook et al., 1989).

DNA measurement: The levels of 8-OHdG were determined by ELISA using mouse monoclonal antibodies N45.1, (Japan Institute for the Control of Aging, Shizuoka, Japan). A sample of extracted DNA was precipitated by the addition of 0.1 volume of 4M NaCl and 2.5 volumes of cold ethanol and digested as per Shigenaga et al. (1994) with modifications. Briefly, the precipitated DNA was re-dissolved in 200 ml of sterile DNA hydrolysis buffer (1 mM deferoxamine, 20 mM sodium acetate, pH 5). Nuclease P1 was added (4 μL; 3.3 mg ml^{-1}) and the samples were incubated at 65°C for 15 minutes. Alkaline phosphatase (4 U in 1 M Tris-HCl [pH 8]) was added and the samples were incubated at 37°C for 60 minutes. Finally, 20 μL of 3 M sodium acetate was added to each sample, followed by 20 μL of chelating solution (50 mM EDTA, 10 mM deferoxamine). The solutions were filtered through a 30 kDa cut-off filter-membrane and the filtered solutions containing the nucleotides, were collected for 8-OHdG analysis.

Digested DNA samples were analysed using high-performance liquid chromatography (HPLC) followed by UV detection of G and electrochemical detection (coulometric) of 8-OHdG. The procedure was performed, essentially, as described by Shigenaga et al. (1994), using a C18 reverse-phase (5 mm, 4.6 mm x 250 mm) column (JASCO, Ishikawa-cho, Hachioji-shi, Tokyo, Japan), a Perkin-Elmer HPLC system (Boston, U.S.A.) and an electrochemical detector (Model

5100, ESA, Chelmsford, MA). The oxidation potentials of the analytical cell of the electrochemical detector were set to 150 mV and 350 mV for electrodes 1 and 2, respectively, with the guard cell potential set at 400 mV. Unmodified nucleosides were detected by their absorbance at 260 nm. Separation of 50 ml of digested DNA was achieved using an isocratic mobile phase consisting of 50 mM potassium phosphate (pH 5.5) and 10% methanol, at a flow rate of 1 ml min^{-1}, with the column maintained at 30^{o}C. Peak data were collected and analysed using a DataCenter 4000 general-purpose laboratory data interface and Delta chromatography data acquisition and analysis software (DataworkX, Brisbane, Australia). The retention times for G and 8-OHdG were 12 and 17 minutes, respectively. Solutions of 8-OHdG and G (Sigma, Chemical Co, St Louis, MO, USA), prepared in HPLC-grade water (Merck, Darmstadt, Germany) and sterilised by passage through 0. 22 mm filters (Millipore, Bedford, MA, USA), were used as standards. For each sample, the amount of DNA injected onto the column was estimated using the signal for G and 8-OHdG was quantified by comparison to external standards.

6.2.2 Alcohol dehydrogenase extraction and assay

The extraction buffer consisted of 50 mM Tris HCl and 15 mM DTT, pH 8.5. Root tissue was first powdered with liquid nitrogen and then homogenised with extraction buffer (1:9, w/v). The extract was centrifuged at 12 000 g for 15 min at 4°C in a refrigerated centrifuge. The ADH was assayed following the method described by Chung and Ferl (1999) with minor modifications. Briefly, the 200 µL reaction mixture contained 50 mM Tris buffer pH 8.5, 0.867 mM NAD, 20% ethanol, 25 µL of extract, diluted extract or standard (prepared from (A3263) ADH from *Saccharomyces cerevisiae* (Sigma–Aldrich, St. Louis, MO, U.S.A.). NAD was added to initiate the reaction and the resultant increase in absorbance was recorded for three minutes at 340 nm. A regression analysis was used to prepare a standard line relating ADH activity to the change in A_{340} and ADH activities in the extracts, calculated with reference to the standard line.

6.2.3 Methylglyoxal and glyoxalase extraction and assay

6.2.3.1 Methylglyoxal

Methylglyoxal levels were determined by extracting 0.1 g leaf in 0.9 ml 0.5 M perchloric acid following the protocol described by Yadav et al. (2005b) with minor modifications. Before the assay was conducted the supernatant was decolorized by incubation with activated charcoal and

centrifuged at 11 000 g for 10 min. The supernatant was then neutralised by the addition of a known volume of saturated potassium carbonate and centrifuged again at 11 000 g for 10 min. The assay mixture (0.2 mL) contained: 50 µL 7.2 mM 1, 2-diaminobenzene, 20 µL 5 M perchloric acid and 130 µL diluted sample extract. The absorbance was read at 340 nm. The final concentration of MG was calculated from a standard curve and expressed in terms of umol/g FW.

6.2.3.2 Glyoxalase

Crude enzyme extracts were subjected to ultra-filtration using Porvair (Porvair Filtration Group Inc., Ashland, Virginia, USA) filtration plates (96-well 10KD MWCO) according to the manufacturer's instructions and the concentrated protein extracts were reconstituted in 100 mM potassium phosphate buffer (pH 7.0) for glyoxalase I or 100 mM Tris–HCl buffer (pH 7.2) for glyoxalase II.

Glyoxalase I (EC: 4.4.1.5) activity was determined according to Hossain et al. (2010), with minor modifications. The assay mixture contained 100 mM potassium phosphate buffer (pH 7.0), 15 mM magnesium sulfate, 1.7 mM GSH and 3.5 mM methylglyoxal (MG) and 25 µL of extract, diluted extract or standard (prepared from (G4252) GLOX I from *Saccharomyces cerevisiae* (Sigma–Aldrich, St. Louis, MO, U.S.A.), to a final volume of 200 µL. The reaction was started by the addition of MG and the increase in absorbance was recorded at 240 nm for two minutes.

Glyoxalase II (EC: 3.1.2.6) activity was determined according to the method of Principatoa et al. (1987), with minor modifications, by monitoring the formation of GSH at 410 nm for two minutes. The reaction mixture contained 100 mM Tris–HCl buffer (pH 7.2), 0.2 mM DTNB, 1 mM S-D-lactoylglutathione (SLG) and 25 µL enzyme extract, to a final volume of 200 µL. The reaction was started by the addition of SLG and the activity was calculated using the extinction coefficient of 13.6 mM^{-1} cm^{-1}, which was corrected for path-length (0.552).

6.2.4 Nutritional quality and bioactivity analysis

6.2.4.1 Total carotenoid extraction and assay

Tomato tissue was ground to a fine powder in liquid nitrogen. The total carotenoids were analysis using the method described by Chappelle et al. (1992), with minor changes. Briefly, 5 mL of dimethyl sulfoxide (DMSO) was added to grounded powder of exocarp (skin) (15 mg) and pericarp (25 mg) of tomato fruit and extracting for 20 hours in darkness at 25°C (ProBlot

hybridization oven, Labnet). The absorption spectra of the DMSO extract of tomato skin and fruit pericarp was determined using UV/VIS Spectrometer (JASCO-V-550, Science & Technology (NZ), Ltd). The concentrations of the extracted pigments were calculated from the absorbance values at 664 nm, 648 nm and 470 nm using equations described by Lichtenthaler (1987).These equations are described as follows:

Chlorophyll a_c = 12.25 A664nm – 2.79A648nm,

Chlorophyll b_c = 21.50A648nm – 5.10A664nm,

Carotenoid s_c = (1000A470nm - 1.82Chl a_c – 85.02 chl b_c)/198

A = absorbance

C = pigment concentration (µg/mL of extract)

These equations are only for DMSO extracts where, chlorophyll a, chlorophyll b and carotenoids have absorption maxima at 664 nm, 648 nm and 470 nm, respectively (Chappelle et al. 1992).

6.2.4.2 Total antioxidant capacity extraction and assay

Tomato tissue was ground to a fine powder in liquid nitrogen. Total antioxidant activity was separated individually into the hydrophilic and lipophilic extracts of the exocarp (skin) and pericarp of tomato fruit. Briefly, 100 mg of finely ground fresh tissue was extracted three times using deionised water. Deionised water (400 µL) was added into the homogenised sample and thoroughly mixed using a shaking device for 10 minutes (IKA-Vibrax with an Eppendorf upper part) at 1400/minute. The resulting homogenate was centrifuge for five minutes at 1, 3000 rpm at 4°C. The supernatant was transferred to a clean Eppendorf tube. After the hydrophilic antioxidant was extracted, the pellet was continued to obtain for the lipophilic antioxidants. Acetone (700 µL) was added into the pellet and mixed thoroughly with a shaking device for about 15 minutes (IKA-Vibrax with an Eppendorf upper part) at 1400/minute. The acetone mixture was centrifuge for five minutes at 1, 3000 rpm at 4°C. The supernatant was transferred to a new clean tube and the pellet was extracted one more time following the same procedure. The total antioxidant capacity assay was carried out using a Perkin Elmer (Wallac) 1420 multilabel counter (Perkin Elmer, San Jose, CA, USA), controlled by a computer with temperature control cell and an auto dispenser (Lister et al., 2010). Data were obtained and processed by the WorkOut 2.0 software package (Perkin Elmer) (Schweikert & Burritt, 2012). The total antioxidant capacity was estimated from the sum of the amount of water soluble antioxidant and the lipid soluble antioxidant.

6.2.4.3 In vitro gastrointestinal digestion coupled with Caco-2 cell culture

In vitro gastrointestinal digestion of tomato samples

The simulated human gastrointestinal digestion of tomato fruit samples were prepared, as previously described by Asano et al. (2003) and by Glahn et al. (1998), with minor modification. Briefly, in the mastication phase, 1 g of ground tomato fruit sample was added to a 50 mL glass Schott bottle containing 14 mL of simulated buccal fluid (50 mM sodium maleate buffer (α-amylase [1.4 units/mL]), which had already been preheated at 37°C for 30 minutes (Asano et al., 2003). The slurry was then incubated for two minutes at 37°C in an oven with a shaking rate of 55 strokes per minute. The sample was acidified by adding 5 mL of HCl (0.1 mol/L) and 1 mL of simulated gastric digestion fluid, pepsin (40 mg/mL) in HCl (0.1 mol/L). This acidic sample was then incubated for 1 hour at 37°C in the oven with the same shaking rate, 55 strokes per minute, to mimic the contraction of the human stomach. In the last phase, the human intestinal phase, the pH of the acidic sample was raised to 5-5.5 by adding $NaHCO_3$ (1 M, drop wise) before adding 5 mL of simulated intestinal digestion fluid comprising $NaHCO_3$ (0.1M), pancreatin (2 mg/mL) and bile salts (12 mg/mL). The sample was then incubated for two hours at 37°C in the hybridization oven with a shaking rate at 55 strokes per minute to simulate the human small intestine during digestion. Finally, the pH of the sample was raised to 7.4 by adding NaOH (1 mL, drop wise) and the volume was brought to 40 mL with 120 mmol/L of NaCl. The sample was filtered through Whatman Grade 1 filter paper and stored as 5 mL aliquots at -80°C (Glahn et al. 1998).

Cell culture

Caco-2 cells (HTB-37, ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco), supplemented with 1% non-essential amino acids, 1% L-glutamine, 20% heat-inactivated foetal bovine serum (FBS) (Gibco), 100 units/mL of penicillin and 100 µg/mL of streptomycin (Sigma-Aldrich) in 250 mL plastic flasks (JET BIOFIL). Cells were cultured in a 5% CO_2 atmosphere at 37°C and the culture medium replaced every 72 hours. Cells were sub-cultured after reaching approximately 80% confluence, and used between passages 5 and 6.

Digest preparation

To remove digestive enzymes the crude digests were subjected to ultra-filtration using Porvair (Porvair Filtration Group Inc., Ashland, Virginia, USA) filtration plates (96-well 10KD

MWCO) according to the manufacturer's instructions and the filtered digests were diluted with culture medium (1:3, v:v).

Hydrogen peroxide challenge

Caco-2 cells were trypsinized (0.5% Trypsin-EDTA, Gibco) and transferred to flat bottom 96-well tissue culture plates (Greiner bio-one) at a seeding density of 104 cells per well and allowed to grow for six days under the conditions detailed above. The culture medium was removed and replaced with medium diluted digests (duplicated for each digest) and the cells were incubated for a further 24 hours. The culture medium was removed and the cells were then washed with fresh culture medium without FBS. Cells were then exposed, for one hour, to 2000 μM hydrogen peroxide, in fresh culture medium without FBS. The culture medium was removed and the cells were washed with fresh culture medium without FBS.

Cell viability assay

The 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to measure cell viability. Caco-2 cells were incubated with 0.5 mg/mL MTT, in culture medium without FBS, for 4 h at 37°C in a 5% CO_2 atmosphere. The medium was removed and the formazan crystals were dissolved in 10% SDS in 0.01 M HCl. The absorbance was then measured at 570 nm using a Perkin Elmer (Wallac Victor) 1420 multilabel counter. Cell viability was expressed as a % of the mean (n=4) reference value (cells cultured in medium without digest or hydrogen peroxide treatment). The means for each digest were calculated from the duplicate wells.

All the assays were carried out using a Perkin Elmer (Wallac) 1420 multilabel counter (Perkin Elmer, San Jose, CA, USA), controlled by a computer with temperature control cell and an auto dispenser (Lister et al., 2010). Data were obtained and processed by the WorkOut 2.0 software package (Perkin Elmer) (Schweikert & Burritt, 2012).

6.3 Statistical analyses

Statistical analyses followed the procedures outlined for the field experimentation in Chapter 3.

For conciseness, tables are used to show the statistical summaries of the main effects and their interactions with the water treatments, followed by the corresponding tables showing significant treatment-induced changes. These responses are then summarised in the text to assist readability. Again, for conciseness, only significant results and representative graphs will be presented in this chapter. Supplementary information is in Appendix F.

The PCA biplot of the effects of a water deficit and waterlogging on oxidative metabolism was based on the relative changes induced by each of the two water treatments (ratios of water treatment:control) in the two cultivars at the fruiting stage under field conditions (Ballizany et al., 2012). The overall PCA was based on all attributes measured in the field experiments (Chapters 3, 4 and the present chapter) under control, drought and waterlogging conditions (Ballizany et al., 2012). Some traits, including methylglyoxal, glyoxalase I, glyoxalase II and DNA damage, were excluded from the PCAs due to differences in the number of replicates in the majority of the other data. Furthermore, excluding these traits not investigated in the glasshouse studies made the PCAs of the field studies comparable with the glasshouse studies. Only attributes that showed significant correlations with either PC1 and/or PC2 were presented in the PCAs.

The Heatmap and the Agglomerative Hierarchical Clustering (AHC) of the attributes measured in the field experimentation followed the method described in Chapter 5.

Statistical analyses of experimental data of nutritional quality and bioactivity of tomato fruits were conducted with the General Analysis of Variance (ANOVA) procedure in Genstat 14, to examine the main effects and their interactions following the procedure outlined in Chapter 3.

6.4 Results

There was no main effect on cultivars for any of the parameters measured in this chapter, and the main effect of time was characterised by increases over the eight day measuring period for the oxidative damage markers and the enzymatic antioxidants (Table 6.4.1.a, Table 6.4.2.a and Table 6.4.3.a). The main effects of time for these parameters were, therefore, not specifically mentioned in the text of this results section. Similarly, only a few parameters showed a water x cultivar or water x cultivar x time interaction and, therefore, these interactions were only mentioned in the text when they were significant.

6.4.1 Stress markers: hydrogen peroxide production and oxidative damage

6.4.1.1 Main effects (water treatment)

Table 6.4.1.a Summary of P values of the main effects and interactions with water treatments for oxidative stress markers

Traits	Water	Cultivar	Time	Water x Cultivar	Water x Time	Water x Cultivar x Time
H_2O_2 leaf	<0.001	0.611	<0.001	0.785	<0.001	0.254
H_2O_2 root	<0.001	0.281	<0.001	0.099	<0.001	0.01
LOOHs leaf	<0.001	0.297	<0.001	0.171	<0.001	0.575
LOOHs root	<0.001	0.865	<0.001	0.980	<0.001	0.992
PCs leaf	<0.001	0.837	<0.001	0.797	<0.001	0.940
PCs root	<0.001	0.134	<0.001	0.107	<0.001	0.635
DNA damage leaf	<0.001	0.602	<0.001	0.572	<0.001	0.863
DNA damage root	<0.001	0.519	<0.001	0.764	<0.001	0.475

Raw data are shown in Appendix B

Averaged across cultivars and harvesting times, stress marker levels in tomato leaf tissues increased with drought (56% - 5.2 fold) and under waterlogging (31% - 6.4 fold), with the least pronounced changes in the levels of H_2O_2 and most pronounced changes for LOOHs (Table 6.4.1.a, b, c, Figure 6.4.1.a, b and Appendix F). A similar pattern was also observed in roots, where levels of stress markers were 33% to 5.1-fold higher under drought, and even greater increases were observed under waterlogging (2-fold to 7-fold).

Table 6.4.1.b Summary of percentage changes of the main effects and interactions with water treatment for hydrogen peroxides

Traits	Water	Time	Water x Time		Water x Cultivar x Time
H_2O_2 leaf	Dr: 56% WL: 74%	T2: 29% T3: 27% T5: 85% T8: 2.1x	Dr T2: 52% Dr T3: 34% Dr T5: 2.3x Dr T8: 2.3x	WL T2: 55% WL T3: 51% WL T5: 2.5x WL T8: 3.1x	ns
H_2O_2 root	Dr: 33% WL: 2x	T2: 30% T3: 35% T5: 2x T8: 2x	Dr T5: 2x Dr T8: 2x	WL T2: 2.1x WL T3: 77% WL T5: 3.2x WL T8: 3.1x	**Dr BBB** T5: 94%, T8: 88% **Dr SBD** T5: 2x, T8: 2.1x **WL BBB** T2: 2x, T3: 81%, T5: 2.8x, T8: 2.9x **WL SBD** T2: 2.2x, T3: 74%, T5: 3.7x, T8: 3.3x

Drought (Dr), waterlogging (WL), harvesting time (T), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), fold (x)

Table 6.4.1.c Summary of percentage changes of the main effects and interactions with water treatment for oxidative stress marker

Traits	Water	Time	Water x Time	
LOOHs leaf	Dr: 5.2x WL: 6.4x	T2: 3.6x T3: 4.8x T5: 6.6x T8: 8.4x	Dr T2: 4.1x Dr T3: 5.1x Dr T5: 6.5x Dr T8: 8.7x	WL T2: 3.3x WL T3: 4.5x WL T5: 6.8x WL T8: 8.1x
LOOHs root	Dr: 5.1x WL: 7x	T2: 4.1x T3: 4.3x T5: 7.4x T8: 7.5x	Dr T2: 4.6x Dr T3: 4.3x Dr T5: 7.8x Dr T8: 7.7x	WL T2: 3.7x WL T3: 4.3x WL T5: 7.1x WL T8: 7.3x
PCs leaf	Dr: 2.1x WL: 2.5x	T1: 19% T2: 81% T3: 2.1x T5: 2.5x T8: 2.8x	Dr T1: 26% Dr T2: 2.2x Dr T3: 2.3x Dr T5: 2.9x Dr T8: 3.1x	WL T1: 27% WL T2: 2.2x WL T3: 2.5x WL T5: 3.8x WL T8: 4x
PCs root	Dr: 2.1x WL: 2.9x	T1: 16%, T2: 85% T3: 95%, T5: 2.8x T8: 3x	Dr T1: 31% Dr T2: 2.6x Dr T3: 2.5x Dr T5: 2.8x Dr T8: 2.6x	WL T1: 31% WL T2: 2.2x WL T3: 2.3x WL T5: 4.6x WL T8: 6x
DNA damage leaf	Dr: 61% WL: 31%	T3: 34% T5: 73% T8: 82%	Dr T3: 49% Dr T5: 2.5x Dr T8: 2.5x	WL T5: 67% WL T8: 2.1x
DNA damage root	Dr: 2.1x WL: 2.1x	T2: 2.2x T3: 2x T5: 85% T8: 2.8x	Dr T2: 2.3x Dr T3: 3.2x Dr T5: 3.1x Dr T8: 2.6x	WL T2: 2.1x WL T3: 2.5x WL T5: 2.6x WL T8: 3.8x

Drought (Dr), waterlogging (WL), harvesting time (T), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), fold (x)

6.4.1.2 Interaction effects (Water treatment x Time)

Averaged across cultivars, water deficit induced increases in leaves by 52% to 2.3-fold for H_2O_2 and by 4.1-fold to 8.7-fold for LOOHs (and 4.6-fold to 7.7-fold in roots) from Day 2 to Day 8 (Table 6.4.1.a, b, c, Figure 6.4.1.a, b and Appendix F). H_2O_2 root levels showed 2-fold increases towards the final harvest. PC levels increased under water deficit (26% to 3.1-fold) and even more so under waterlogging (27% - 6-fold) from Day 1 to Day 8. Waterlogging-induced levels of H_2O_2 (55% - 3.2-fold) and LOOHs (3.3 – 8.1-fold) from Day 2 to Day 8 in both leaf and root tissues (and Appendix F). However, the H_2O_2 levels in roots plateaued after five days of waterlogging. Levels of DNA oxidation were stimulated by water stress by 49% - 2.5- fold in leaves. In roots, DNA oxidation increased 2.3-fold – 3.2-fold under drought with no consistent time-dependent pattern while, under waterlogging, DNA damage in the roots increased from Day 2 to Day 8 (2.1 – 3.8-fold). A significant three-way interaction indicated higher H_2O_2 levels in 'Scoresby Dwarf' roots under waterlogging compared to 'Best Boy Bush' on Day 5, however, no significant cultivar differences were observed in this trait under hypoxia at final harvest (Figure 6.4.1.a).

6.4.1.3 Summary of the key findings

- Extremes in water stress increased the levels of all oxidative stress markers mostly after two days of stress exposure.
- Compared to plants exposed to drought, the levels of these stress markers became more elevated in waterlogged plants over time (Day 5 and Day 8).

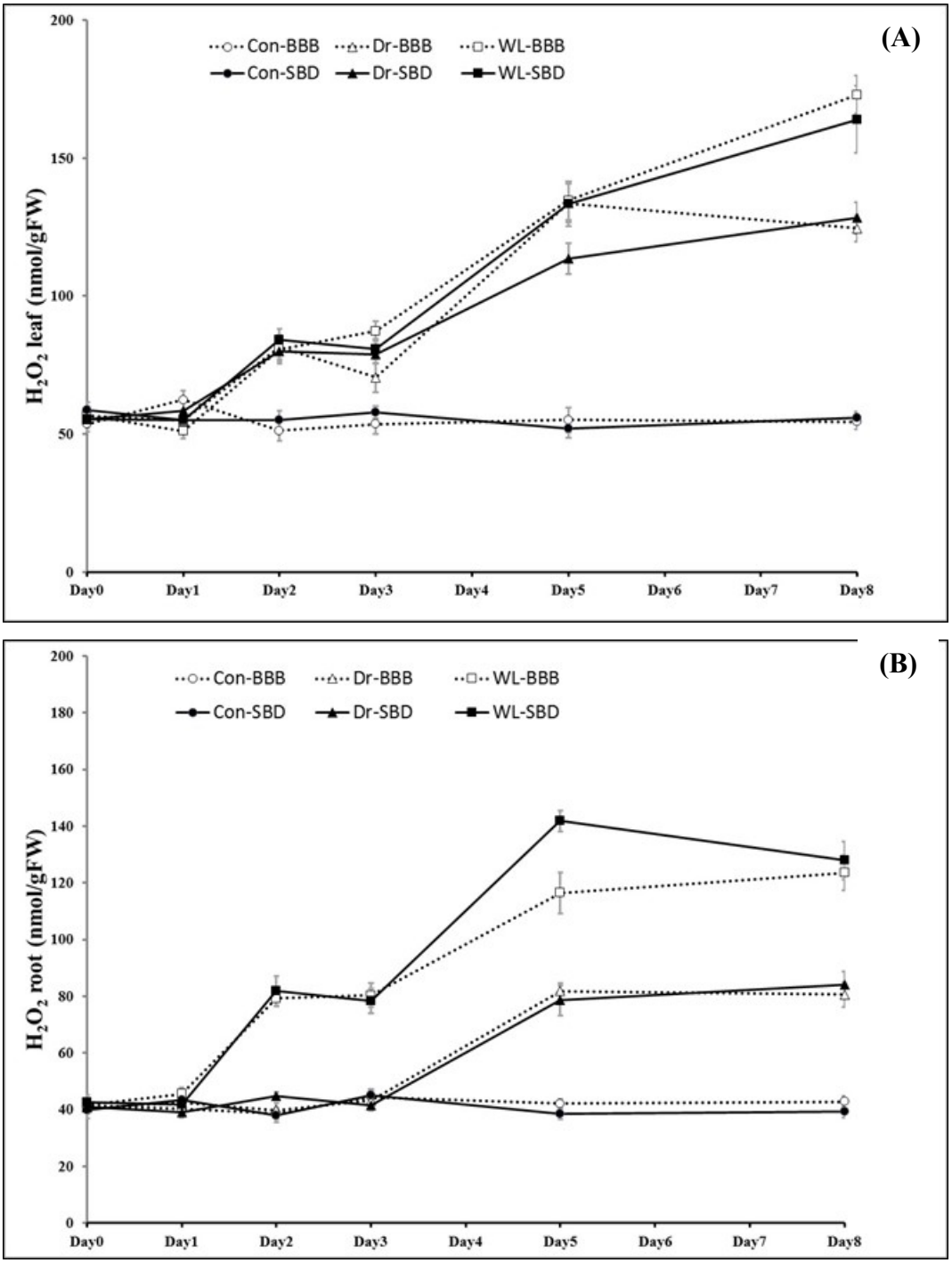


Figure 6.4.1.a Hydrogen peroxide (H_2O_2) content in leaf tissues (A) and root tissues (B) of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf', during eight days of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

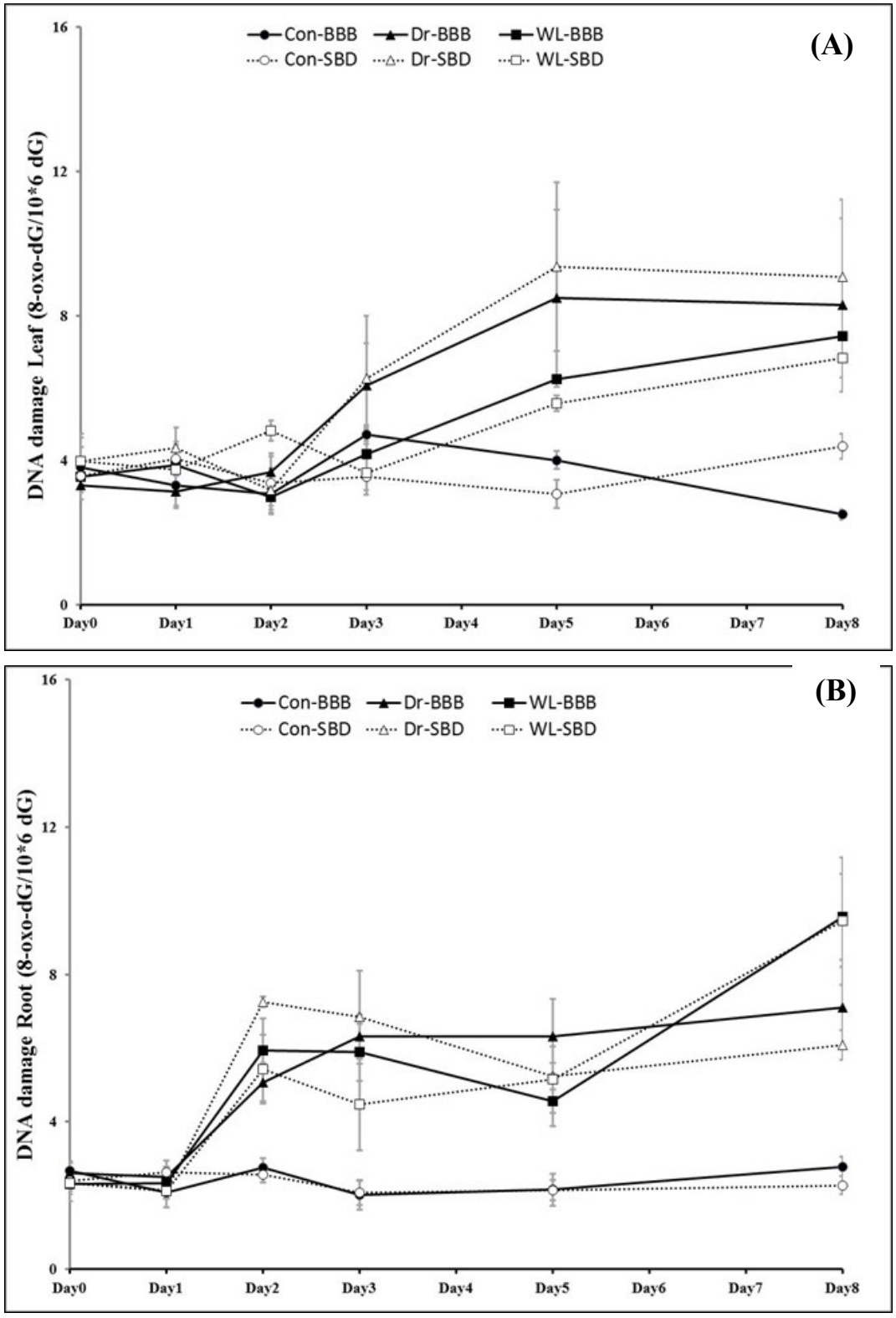


Figure 6.4.1.b DNA damage in leaf tissues (A) and root tissues (B) of two tomato cultivars 'Best Boy Bush' and 'Scoresby Dwarf', during eight days of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL) ; values are means (n=4) ± standard error.

6.4.2 Antioxidant enzymes

6.4.2.1 Main effects (water treatment)

Averaged across cultivars and harvesting times, waterlogging increased activities in leaf and root tissues of all the enzymatic antioxidants measured here by 25-30%, compared with the greater increases under drought stress (29% - 63%) (Table 6.4.2.a, b, c, Figure 6.4.2.a and Appendix F). The activity of ADH in the roots of plants subjected to waterlogging was 2.9- fold the activity of ADH in the roots of well-watered plants (Figure 6.4.2.b).

Table 6.4.2.a Summary of P values of the main effects and interactions with water treatments for enzymatic antioxidant activities.

Traits	Water	Cultivar	Time	Water x Cultivar	Water x Time	Water x Cultivar x Time
SOD leaf	<0.001	0.544	<0.001	0.754	<0.001	0.902
SOD root	<0.001	0.097	<0.001	0.655	<0.001	0.109
CAT leaf	<0.001	0.983	<0.001	0.394	<0.001	0.111
CAT root	<0.001	0.101	<0.001	0.177	<0.001	0.490
APOX leaf	<0.001	0.105	<0.001	0.616	<0.001	0.510
APOX root	<0.001	0.658	<0.001	0.084	<0.001	0.158
GR leaf	<0.001	0.884	<0.001	0.534	<0.001	0.847
GR root	<0.001	0.226	<0.001	0.846	<0.001	0.256
GPOX leaf	<0.001	0.452	<0.001	0.251	<0.001	0.349
GPOX root	<0.001	0.208	<0.001	0.091	<0.001	0.013
ADH root	<0.001	0.056	<0.001	0.183	<0.001	0.592

Raw data are shown in Appendix B

6.4.2.2 Interaction effects (Water treatment x Time)

Averaged across cultivars, enzymatic antioxidant activities were all elevated after two days of exposure to water stress. Activities of these enzymes increased under both stress factors from 10% (APOX activity in roots at Day 1 under waterlogging) to 83% (GPOX activity in roots under drought at Day 8), except for GPOX and APOX activity levels which were 2.5-fold to 3-fold higher in drought-exposed tomato leaves (Table 6.4.2.a, b, c, Figure 6.4.2.a and Appendix F).

Table 6.4.2.b Summary of percentage changes of the main effects and interactions with water treatments for enzymatic antioxidant activities

Traits	Water	Time	Water x Time	
SOD leaf	Dr: 34% WL: 28%	T3: 29% T5: 39% T8: 39x	Dr T2: 29% Dr T3: 44% Dr T5: 52% Dr T8: 81%	WL T2: 27% WL T3: 44% WL T5: 57% WL T8: 46%
SOD root	Dr: 29% WL: 30%	T2: 11% T3: 25% T5: 33% T8: 40%	Dr T2: 16% Dr T3: 28% Dr T5: 55% Dr T8: 71%	WL T2: 18% WL T3: 37% WL T5: 58% WL T8: 53%
CAT leaf	Dr: 33% WL: 27%	T2: 12% T3: 28% T5: 42% T8: 40%	Dr T2: 13% Dr T3: 49% Dr T5: 53% Dr T8: 78%	WL T2: 21% WL T3: 50% WL T5: 44% WL T8: 52%
CAT root	Dr: 31% WL: 28%	T2: 16% T3: 21% T5: 37% T8: 41%	Dr T2: 18% Dr T3: 43% Dr T5: 68% Dr T8: 63%	WL T2: 20% WL T3: 30% WL T5: 65% WL T8: 50%
APOX leaf	Dr: 63% WL: 30%	T2: 15% T3: 32% T5: 59% T8: 82%	Dr T2: 22% Dr T3: 41% Dr T5: 2x Dr T8: 3x	WL T2: 26% WL T3: 43% WL T5: 57% WL T8: 42%
APOX root	Dr: 32% WL: 29%	T2: 16% T3: 22% T5: 35% T8: 39%	Dr T2: 30% Dr T3: 31% Dr T5: 56% Dr T8: 70%	WL T1: 10% WL T2: 20% WL T3: 33% WL T5: 58% WL T8: 55%
GR leaf	Dr: 36% WL: 28%	T2: 13% T3: 27% T5: 40% T8: 42%	Dr T2: 29% Dr T3: 39% Dr T5: 40% Dr T8: 42%	WL T2: 19% WL T5: 41% WL T5: 67% WL T8: 41%
GR root	Dr: 34% WL: 27%	T2: 21% T3: 24% T5: 36% T8: 40%	Dr T2: 34% Dr T3: 40% Dr T5: 55% Dr T8: 72%	WL T2: 25% WL T3: 37% WL T5: 49% WL T8: 50%
ADH root	WL: 2.9x	T2: 55% T3: 65% T5: 2.8x T8: 3.3x	-	WL T2: 2.3x WL T3: 2.3x WL T5: 4.5x WL T8: 6x

Drought (Dr), waterlogging (WL), harvesting time (T), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), fold (x)

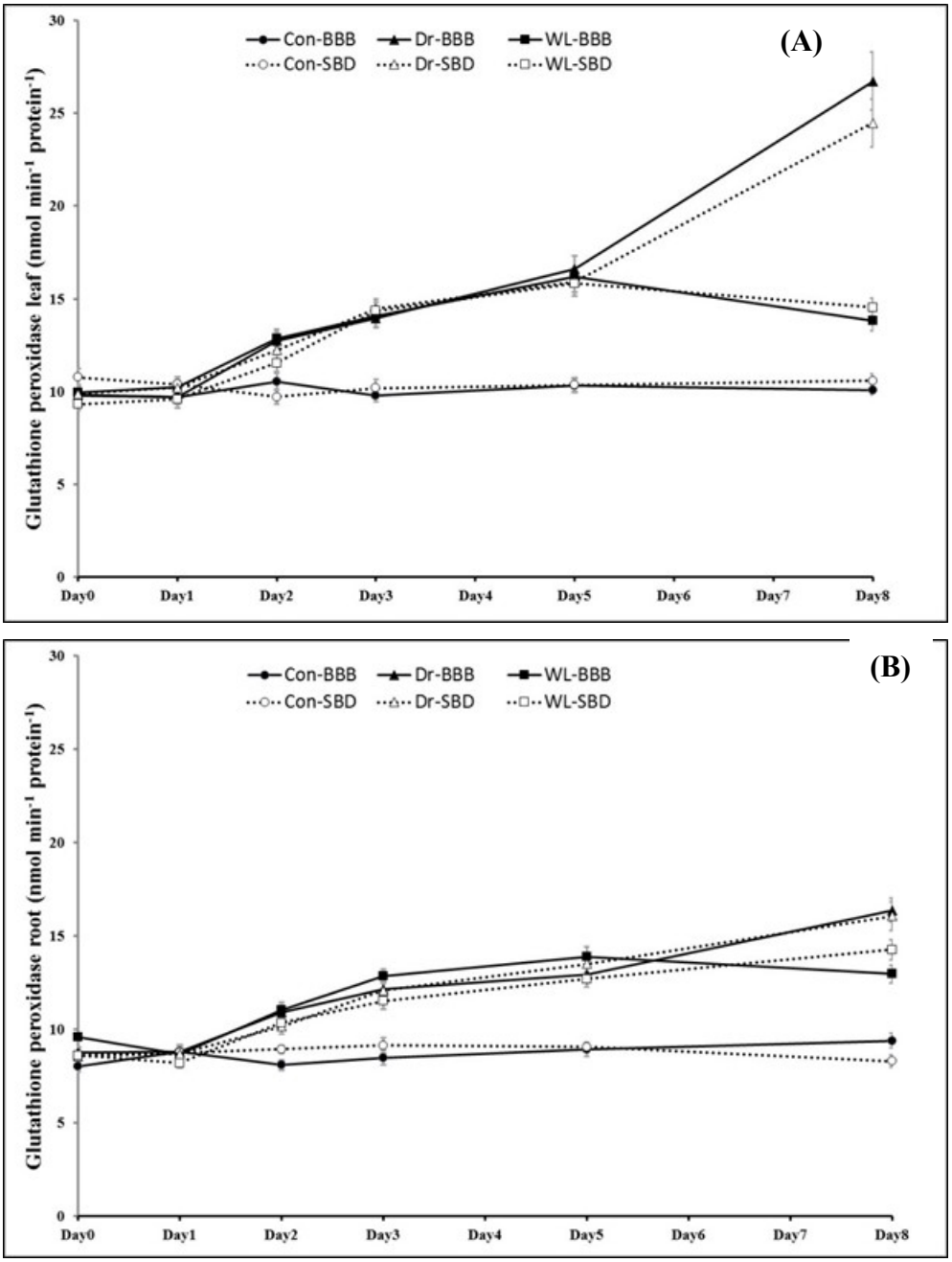


Figure 6.4.2.a Glutathione peroxidase (GPOX) in leaf tissues (A) and root tissues (B) of two tomato cultivars: 'Scoresby Dwarf' and 'Best Boy Bush', during eight days exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

However, there was a pattern in that the activities of these enzymes plateaued at Day 5 (except GR and GPOX in roots) under waterlogging. ADH activity progressively increased from 2.3-fold at Day 2 to 6-fold at Day 8. A significant three-way interaction indicated a lower GPOX activity compared to 'Best Boy Bush' in 'Scoresby Dwarf' roots after two days of water stress (drought and waterlogging), but higher enzyme activity in 'Scoresby Dwarf' after eight days of stress exposure (Figure 6.4.2.b).

Table 6.4.2.c Summary of percentage changes of the main effects and interactions with water treatments for GPOX activities

Traits	Water	Time	Water x Time		Water x Cultivar x Time
GPOX leaf	Dr: 45% WL: 25%	T2: 17% T3: 29% T5: 43% T8: 68%	Dr T2: 23% Dr T3: 43% Dr T5: 57% Dr T8: 2.5x	WL T2: 21% WL T3: 42% WL T5: 55% WL T8: 37%	ns
GPOX root	Dr: 33% WL: 29%	T2: 14% T3: 27% T5: 37% T8: 48%	Dr T2: 24% Dr T3: 37% Dr T5: 47% Dr T8: 83%	WL T2: 26% WL T3: 38% WL T5: 48% WL T8: 54%	**Dr BBB** T2: 35%, T3: 43%, T5: 45%, T8: 74% **Dr SBD** T2:14%, T3:32%, T5: 49%, T8: 93% **WL BBB** T2: 37%, T3: 51%, T5: 56%, T8: 38% **WL SBD** T2: 16%, T3: 26%, T5: 40%, T8: 72%

Drought (Dr), waterlogging (WL), harvesting time (T), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), fold (x)

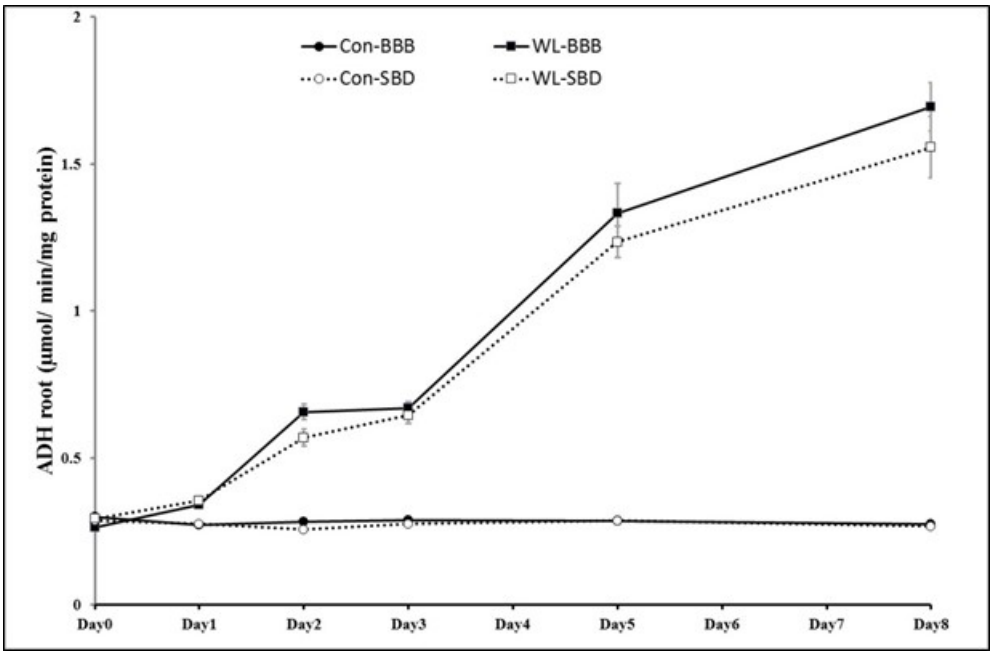


Figure 6.4.2.b Alcohol dehydrogenase (ADH) activity in root tissues of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf', during eight days of exposure to well-watered control conditions (Con) and waterlogging (WL); values are means (n=10) ± standard error.

6.4.2.3 Summary of the key findings

- ❖ The activity of all enzymes, including SOD, CAT, APOX, GR and GPOX, mostly increased after two days of the imposition of water stress and were all higher in plants subjected to drought stress than in plants grown under waterlogging.
- ❖ Under waterlogging, the activity of these enzymes plateaued at Day 5 and the increase became less pronounced at Day 8.
- ❖ The activity of ADH in hypoxic roots doubled at Days 2 and 3 and increased 6-fold on Day 8.

6.4.3 Non-enzymatic antioxidants

6.4.3.1 Main effects (water treatment)

Averaged across cultivars and harvesting times, the water deficit increased the levels of antioxidants in leaf and root tissues by 6% - 38% except for total GSH increases in the roots, of 76%, and slightly decreased GSH levels in the leaves (Table 6.4.3.a, b and Figure 6.4.3.a, b, c, d). Waterlogging, on the other hand, decreased levels of all antioxidants in both leaf and root tissues by 10% - 31%. Total GSH levels in leaves did not change while they increased by 15% in hypoxic roots. In contrast to the enzymatic antioxidants, there was no consistent overall time-dependent pattern of non-enzymatic antioxidant accumulation when averaged across treatments and cultivars.

Table 6.4.3.a Summary of P values of the main effects and interactions with water treatments for non-enzymatic antioxidants levels.

Traits	Water	Cultivar	Time	Water x Cultivar	Water x Time	Water x Cultivar x Time
Reduced AsA leaf	<0.001	0.610	<0.001	0.806	<0.001	0.839
Reduced AsA root	<0.001	0.341	<0.001	0.508	<0.001	0.972
Total AsA leaf	<0.001	0.575	<0.001	0.193	<0.001	0.855
Total AsA root	<0.001	0.104	<0.001	0.144	<0.001	0.911
GSH leaf	<0.001	<0.001	0.907	<0.001	0.101	0.111
GSH root	<0.001	<0.001	0.798	<0.001	0.522	0.673
Total GSH leaf	<0.001	0.879	<0.001	0.139	<0.001	0.161
Total GSH root	<0.001	0.811	<0.001	0.703	<0.001	0.704

Raw data are shown in Appendix B

6.4.3.2 Interaction effects (Water treatment x Time)

Water deficit increased leaf and root levels of reduced AsA at Day 1 by around 15%, but reduced these levels at Day 2 (by 16 to 20%) before increasing later (23% to 41%) in both tissues (Table 6.4.3.a, b and Figure 6.4.3.a). Waterlogging on the other hand caused consistent time-dependent reductions in the levels of this antioxidant (-20% down to -62%) from Day 1 in leaves and from Day 2 in roots.

Table 6.4.3.b Summary of percentage changes of the main effects and interactions with water treatments for non-enzymatic antioxidant levels

Traits	Water	Time	Water x Time	
Reduced AsA leaf	Dr: 6% WL: -31%	T2: -13% T3: -15% T5: -7% T8: -18%	Dr T1: 15% Dr T2: -16% Dr T5: 41%	WL T1: -20% WL T2: -25% WL T3: -39% WL T5: -45% WL T8: -53%
Reduced AsA root	Dr: 6% WL: -30%	T1: 9% T2: 12% T3: -7% T8: -10%	Dr T1: 16% Dr T2: -21% Dr T5: 23% Dr T8: 26%	WL T2: -38% WL T3: -37% WL T5: -44% WL T8: -62%
Total AsA leaf	Dr: 37% WL: -12%	T5: 16% T8: 14%	Dr T1: 22% Dr T2: 16% Dr T3: 36% Dr T5: 88% Dr T8: 73%	WL T1: -12% WL T3: -16% WL T5: -18% WL T8: -26%
Total AsA root	Dr: 34% WL: -10%	T1: 17% T3: 15% T5: 24% T8: 17%	Dr T1: 26% Dr T3: 30% Dr T5: 68% Dr T8: 75%	WL T1: 19% WL T2: -14% WL T3: -12% WL T5: -13% WL T8: -35%
GSH leaf	Dr: -9% WL: -25%	T1: -17% T2: -11% T5: -8% T8: -19%	Dr T1: -9% Dr T2: -29% Dr T3: -19%	WL T2: -30% WL T3: -18% WL T5: -29% WL T8: -68%
GSH root	Dr: 38% WL: -14%	T1: -7% T2: -7% T3: 31% T5: 22% T8: 12%	Dr T2: 20% Dr T3: 66% Dr T5: 65% Dr T8: 71%	WL T2: -18% WL T8: -60%
Total GSH leaf	Dr: 21%	T3: 11% T5: 19% T8: 14%	Dr T3: 12% Dr T5: 37% Dr T8: 67%	WL T3: 14% WL T5: 15% WL T8: -39%
Total GSH root	Dr: 76% WL: 15%	T2: 16% T3: 64% T5: 59% T8: 51%	Dr T2: 62% Dr T3: 2.2x Dr T5: 2.2x Dr T8: 2.4x	WL T3: 46% WL T5: 43%

Drought (Dr), waterlogging (WL), harvesting time (T), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), fold (x)

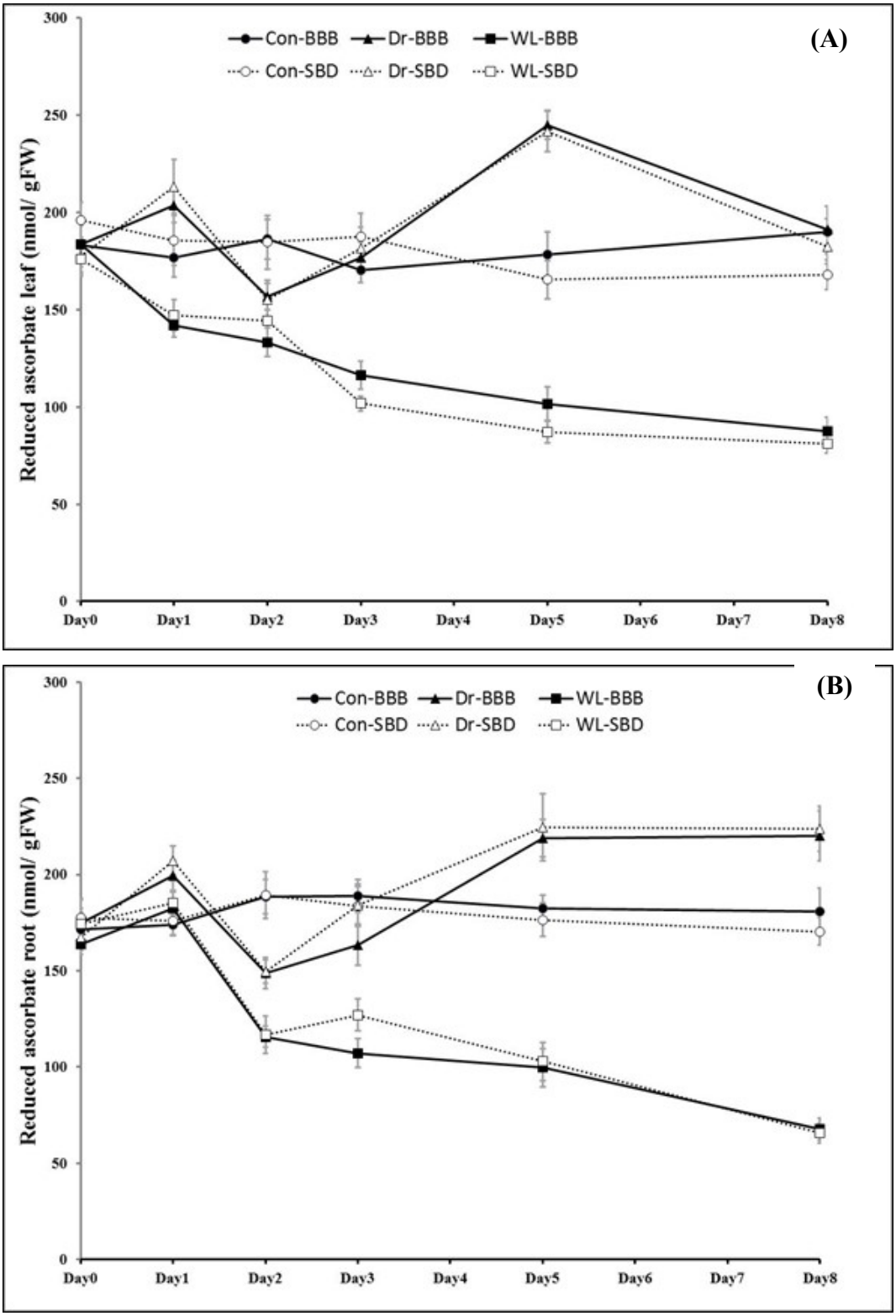


Figure 6.4.3.a Reduced ascorbate content in leaf tissues (A) and root tissues (B) of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf', during eight days of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

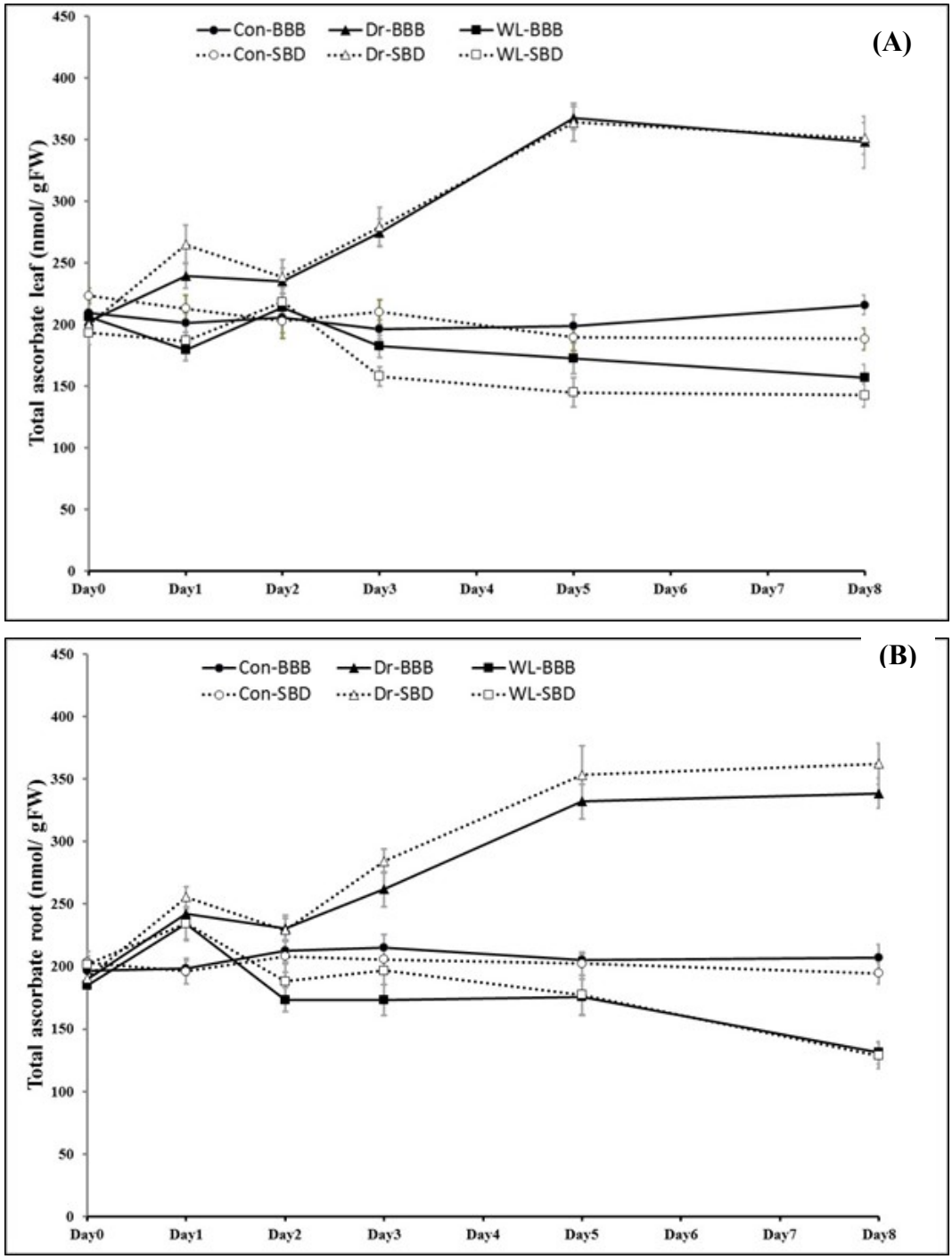


Figure 6.4.3.b Total ascorbate content in leaf tissues (A) and root tissues (B) of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf', during eight days of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

Levels of total ascorbate increased in both leaf and root tissues from Days 1 to 8 (16% - 88%) under drought stress, with one exception at Day 2 in the roots (Table 6.4.3.a, b and Figure 6.4.3.b). Waterlogging decreased total ascorbate level in the leaves by 12% - 26% from Days 1

to 8 (but not at Day 2). In roots, the levels of total ascorbate increased by 19% at Day 1 and then decreased from Days 2 to 8 (-12% to -35%). Levels of GSH decreased in the leaves by 9% to 29% in the early stages of water deficit (Table 6.4.3.a, b and Figure 6.4.3.c). GSH levels were lowered by 18% to 68% (Days 2 to 8) in leaves under waterlogging. In roots, GSH levels were increased under water deficit from Days 2 to 8 (20%-71%), while the levels of this antioxidant decreased by 18% (Day 2) and by 60% (Day 8) under waterlogging.

Total GSH levels increased from Days 3-8 (12-67%) in leaves and from Days 2-8 (62%-2.4-fold) in roots under drought stress. Increases in total GSH were also observed in both leaf and root tissues from 14% to 46% (Days 3-5) while they decreased by 39% at Day 8 in leaves (Table 6.4.3.a, b and Figure 6.4.3.d).

Plate 6. Harvesting activities of tomato fruits

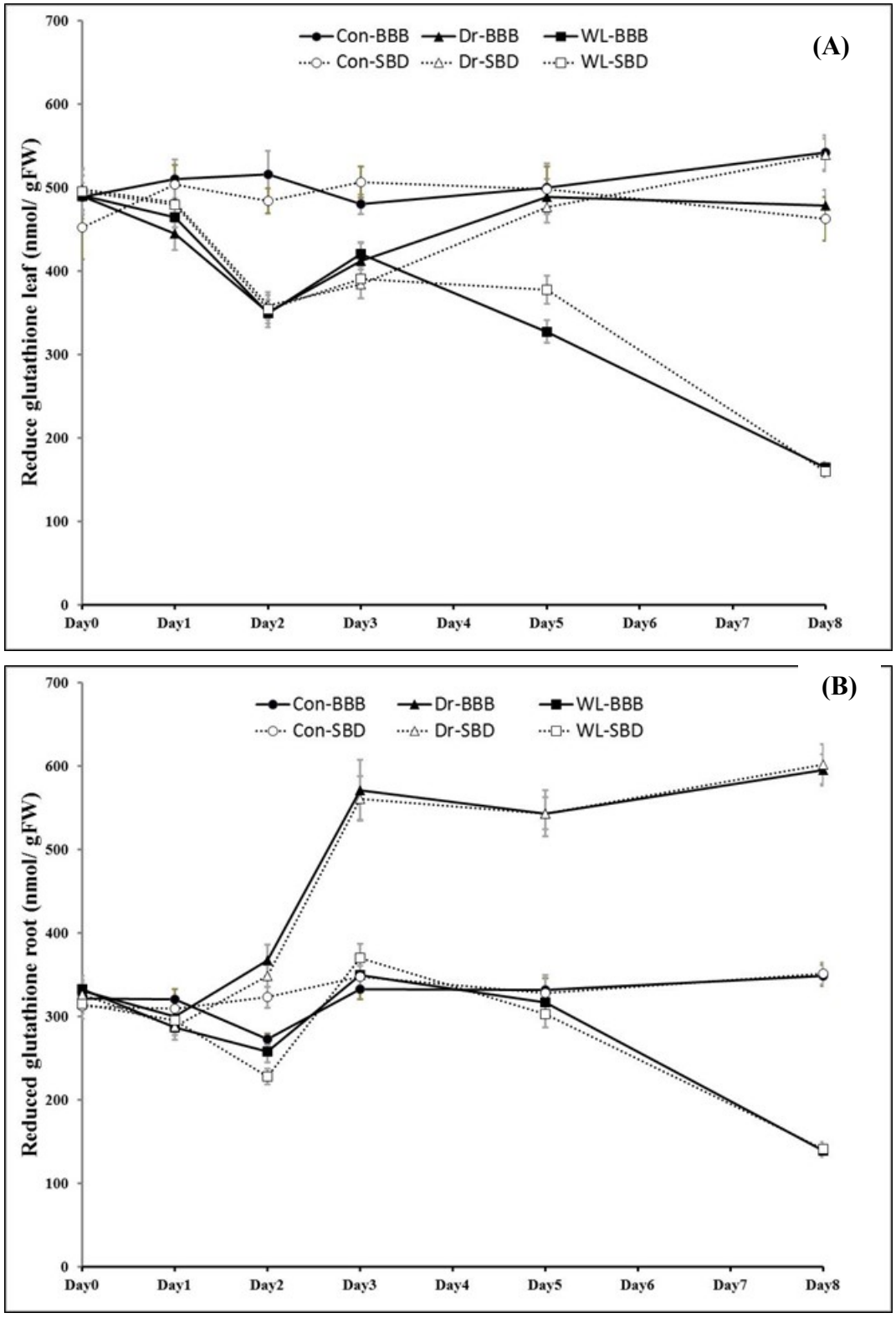


Figure 6.4.3.c GSH content in leaf tissues (A) and root tissues (B) of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf', during eight days of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

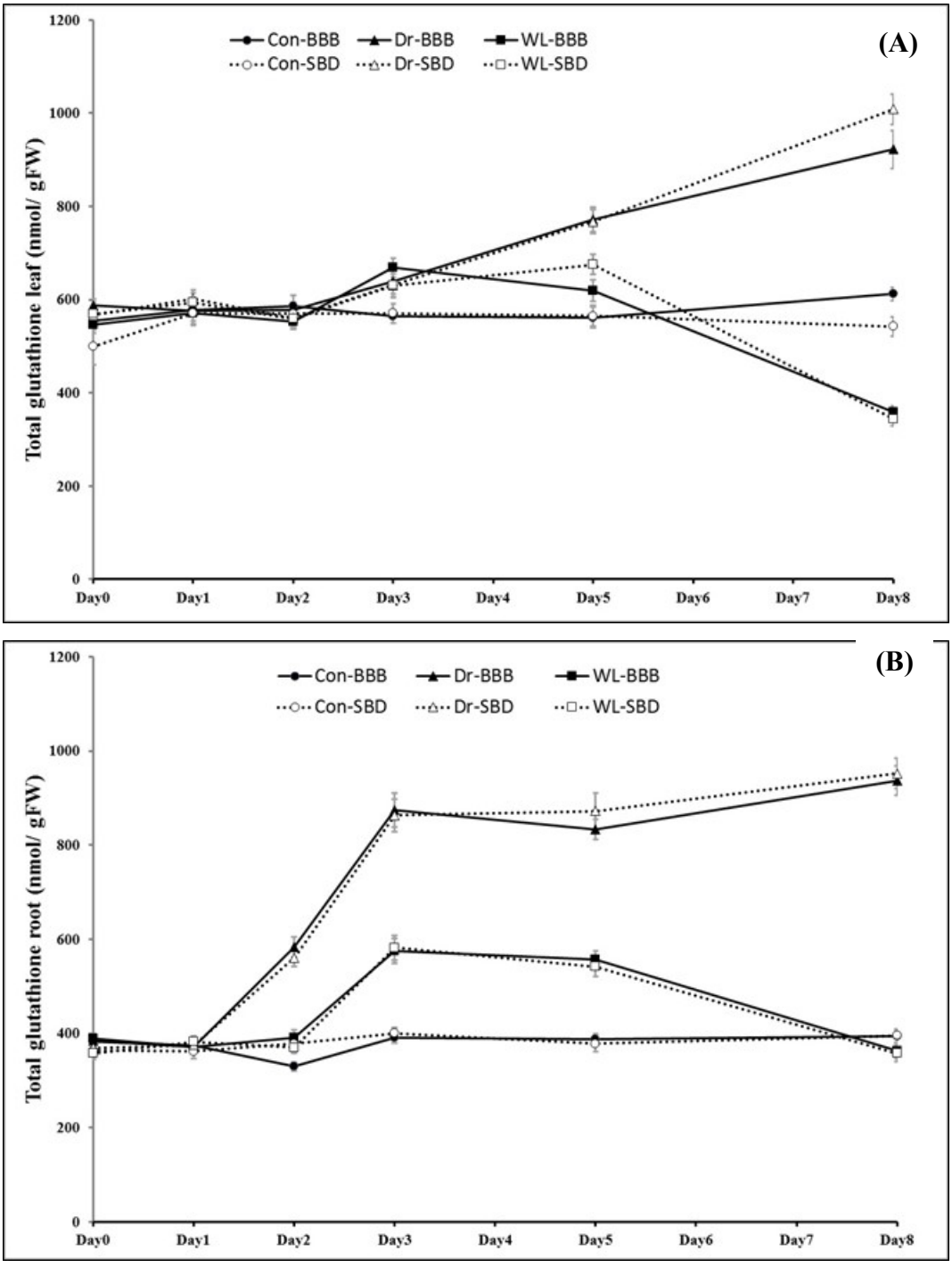


Figure 6.4.3.d Total glutathione content in leaf tissues (A) and root tissues (B) of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf', during eight days of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

6.4.3.3 Oxidised antioxidants: DHA and GSSG

Averaged across cultivars and harvesting time, water stress increased the oxidised antioxidant levels in both leaf and root tissues 3.4 - 4.1-fold under drought stress and 2.3- 3-fold under waterlogging (Table 6.4.3.c, d and Figure 6.4.3.e, f).

Table 6.4.3.c Summary of P values of the main effects and interactions with water treatments for oxidised antioxidant levels.

Traits	Water	Cultivar	Time	Water x Cultivar	Water x Time	Water x Cultivar x Time
DHA leaf	<0.001	0.804	<0.001	0.027	<0.001	0.985
DHA root	<0.001	0.066	<0.001	0.055	<0.001	0.552
GSSG leaf	<0.001	0.937	<0.001	0.916	<0.001	0.447
GSSG root	<0.001	0.965	<0.001	0.263	<0.001	0.911

Raw data are shown in Appendix B

Table 6.4.3.d Summary of percentage changes of the main effects and interactions with water treatments for oxidised antioxidant levels.

Traits	Water	Time	Water x Cultivar	Water x Time	
DHA leaf	Dr: 3.8x WL: 2.3x	T1: 60% T2: 2.6x T3: 2.7x T5: 3.1x T8: 3.7x	Dr BBB: 3.6x Dr SBD: 3.9x WL BBB: 2.4x WL SBD: 2.2x	Dr T1: 69% Dr T2: 4.3x Dr T3: 4.1x Dr T5: 5.5x Dr T8: 7.1x	WL T1: 49% WL T2: 4.1x WL T3: 2.5x WL T5: 2.9x WL T8: 2.8x
DHA root	Dr: 3.5x WL: 2.4x	T1: 73% T2: 2.4x T3: 2.8x T5: 3.2x T8: 3.2x	ns	Dr T1: 2x Dr T2: 3.8x Dr T3: 4.1x Dr T5: 5x Dr T8: 5.1x	WL T1: 2.2x WL T2: 3x WL T3: 2.8x WL T5: 3.1x WL T8: 2.5x
GSSG leaf	Dr: 3.4x WL: 2.7x	T1: 48% T2: 2.4x T3: 2.7x T5: 3.1x T8: 3.5x	ns	Dr T1: 87% Dr T2: 2.7x Dr T3: 3.2x Dr T5: 4.5x Dr T8: 6.1x	WL T1: 65% WLT2: 2.6x WLT3: 3.3x WL T5: 4.6x WL T8: 2.5x
GSSG root	Dr: 4.1x WL: 3x	T1: 41% T2: 2.6x T3: 3.7x T5: 3.9x T8: 3.9x	ns	Dr T1: 50% Dr T2: 3.8x Dr T3: 5.4x Dr T5: 5.8x Dr T8: 7.8x	WL T1: 60% WL T2: 2.5x WL T3: 3.9x WL T5: 4.5x WL T8: 4.9x

Drought (Dr), waterlogging (WL), harvesting time (T), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), fold (x)

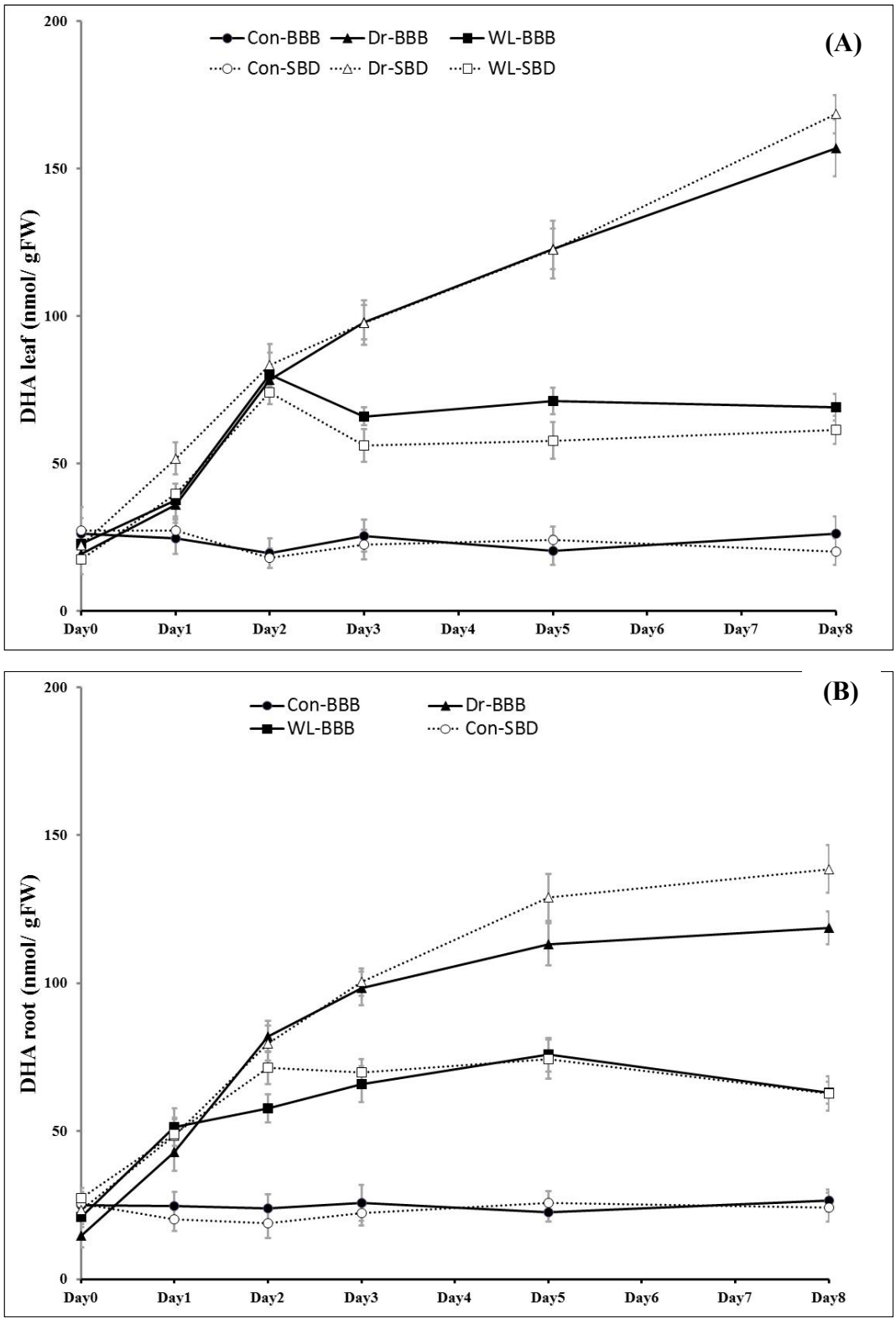


Figure 6.4.3.e Dehydroascorbate (DHA) content in leaf tissues (A) and root tissues (B) of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf', during eight days of exposure of well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

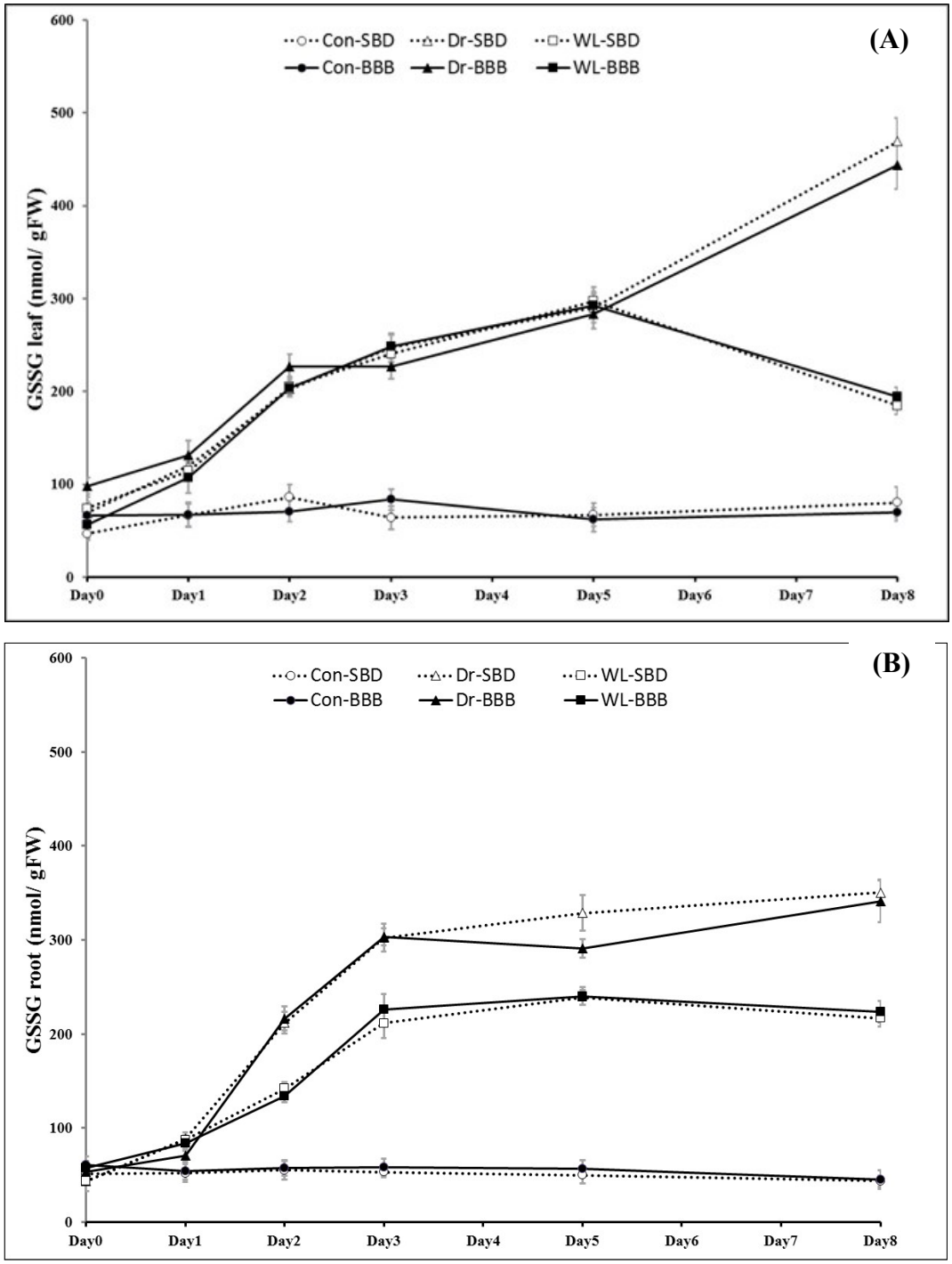


Figure 6.4.3.f GSSG content in leaf tissues (A) and root tissues (B) of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf', during eight days of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

Averaged across cultivars, levels of DHA and GSSG increased in leaves and roots from Days 1 to 8 under a water deficit (50% on Day 1 to 7.8-fold by Day 8) and waterlogging (49% on Day 1 to 4.9-fold by Day 8) (Table 6.4.3.c, d and Figure 6.4.3.e, f). A significant water x cultivar interaction indicated higher average increases in leaf DHA levels for 'Best Boy Bush', under waterlogging, and in 'Scoresby Dwarf', under drought.

6.4.3.4 Summary of the key findings

- Water deficit increased the levels of all antioxidants including reduced ascorbate, total ascorbate, total GSH from Days 1 to 8, in most cases, in both leaf and root tissues and GSH-leaf, while waterlogging caused reductions in the levels of these antioxidants (except for total GSH).
- In general, AsA traits decreased steadily over time under waterlogging, while they (and total GSH levels) increased steadily with time under drought.
- Water stress strongly increased levels of DHA and GSSG from Days 1 – 8 in both leaf and root tissues and these increases were highest under drought stress.

6.4.4 Methylglyoxal and glyoxalases

6.4.4.1 Main effects (Water treatment)

Averaged across cultivars and harvesting time, levels of methylglyoxal increased by 2.1-fold in both leaf and root tissues under drought stress and by 2.4 - 3-fold under waterlogging (Table 6.4.4.a, b and Figure 6.4.4.a). Levels of GLOX1 and GLOX2 were increased 2.1-2.2-fold under drought stress and by 2.4-2.9-fold under hypoxia (Table 6.4.4.a, b and Appendix F).

Table 6.4.4.a Summary of P values of the main effects and interactions with water treatments on methylgyloxal levels and the activities of glyoxalase enzymes

Traits	Water	Cultivar	Time	Water x Cultivar	Water x Time	Water x Cultivar x Time
MG leaf	<0.001	0.836	<0.001	0.799	<0.001	0.056
MG root	<0.001	0.383	<0.001	0.885	<0.001	0.991
GLOX1 leaf	<0.001	0.503	<0.001	0.644	<0.001	0.225
GLOX1 root	<0.001	0.650	<0.001	0.970	<0.001	0.986
GLOX2 leaf	<0.001	0.154	<0.001	0.422	<0.001	0.622
GLOX2 root	<0.001	0.145	<0.001	0.529	<0.001	0.656

Raw data are shown in Appendix B

6.4.4.2 Interaction effects (Water treatment x Time)

Averaged across cultivars, levels of MG increased substantially in both leaf and root tissues of plants subjected to water deficit and waterlogging relative to the control plants. Under water deficit, MG levels increased from Days 2- 8 in leaves twofold- 3.1-fold and in roots 2.5-2.8-fold (Table 6.4.4.a, b and Figure 6.4.4.a). Waterlogging also increased MG levels from Days 2-8 twofold to 3.9-fold in leaves and 2.1-6.1-fold in root tissues.

The activities of GLOX1 and GLOX2 increased steadily under water deficit from 2.2-fold on Day 2 to 3.2-fold on Day 8 in leaf tissues and between 2.4-fold and 2.7-fold in root tissues (Table 6.4.4.a, b and Appendix F). A similar picture was observed in waterlogged tissues: GLOX1 and GLOX2 activities increased steadily by 2.1-fold to 3.9-fold in leaf tissues and by 2.1-fold to 6-fold in root tissues under hypoxia.

Table 6.4.4.b Summary of P values of the main effects and interactions with water treatments for methylgyloxal levels and the activities of glyoxalase enzymes.

Traits	Water	Time	Water x Time	
MG leaf	Dr: 2.1x WL: 2.4x	T1: 24% T2: 85% T3: 2.1x T5: 2.5x T8: 2.8x	Dr T1: 24% Dr T2: 2x Dr T3: 2.3x Dr T5: 2.9x Dr T8: 3.1x	WL T2: 2x WL T3: 2.4x WL T5: 3.9x WL T8: 3.9x
MG root	Dr: 2.1x WL: 3x	T1: 24% T2: 2x T3: 2x T5: 3.1x T8: 3x	Dr T2: 2.5x Dr T3: 2.5x Dr T5: 2.8x Dr T8: 2.7x	WL T2: 2.1x WL T3: 2.4x WL T5: 5.3x WL T8: 6.1x
GLOX1 leaf	Dr: 2.2x WL: 2.5x	T1: 21% T2: 84% T3: 2.1x T5: 2.5x T8: 2.9x	Dr T2: 2.2x Dr T3: 2.4x Dr T5: 2.9x Dr T8: 3.2x	WL T2: 2.2x WL T3: 2.3x WL T5: 3.9x WL T8: 3.9x
GLOX1 root	Dr: 2.1x WL: 2.9x	T1: 25% T2: 2x T3: 2x T5: 3.1x T8: 3.1x	Dr T2: 2.6x Dr T3: 2.5x Dr T5: 2.6x Dr T8: 2.6x	WL T2: 2.1x WL T3: 2.3x WL T5: 5x WL T8: 6x
GLOX2 leaf	Dr: 2.2x WL: 2.4x	T1: 19% T2: 81% T3: 2.2x T5: 2.5x T8: 2.9x	Dr T2: 2.2x Dr T3: 2.5x Dr T5: 2.9x Dr T8: 3.2x	WL T2: 2.1x WL T3: 2.3x WL T5: 3.7x WL T8: 3.7x
GLOX2 root	Dr: 2.1x WL: 2.9x	T2: 88% T3: 93% T5: 2.9x T8: 2.9x	Dr T2: 2.4x Dr T3: 2.3x Dr T5: 2.6x Dr T8: 2.7x	WL T2: 2x WL T3: 2.2x WL T5: 5.1x WL T8: 5.9x

Drought (Dr), waterlogging (WL), harvesting time (T), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), fold (x)

6.4.4.3 Summary of the key findings

- Water stress extremes increased the production of methylglyoxal.
- The activity of GLOX1 and GLOX2 increased in parallel with rising MG in tissues of plants subjected to water stress.
- Levels of MG and activities of GLOX1 and GLOX2 were greater in hypoxic plants.

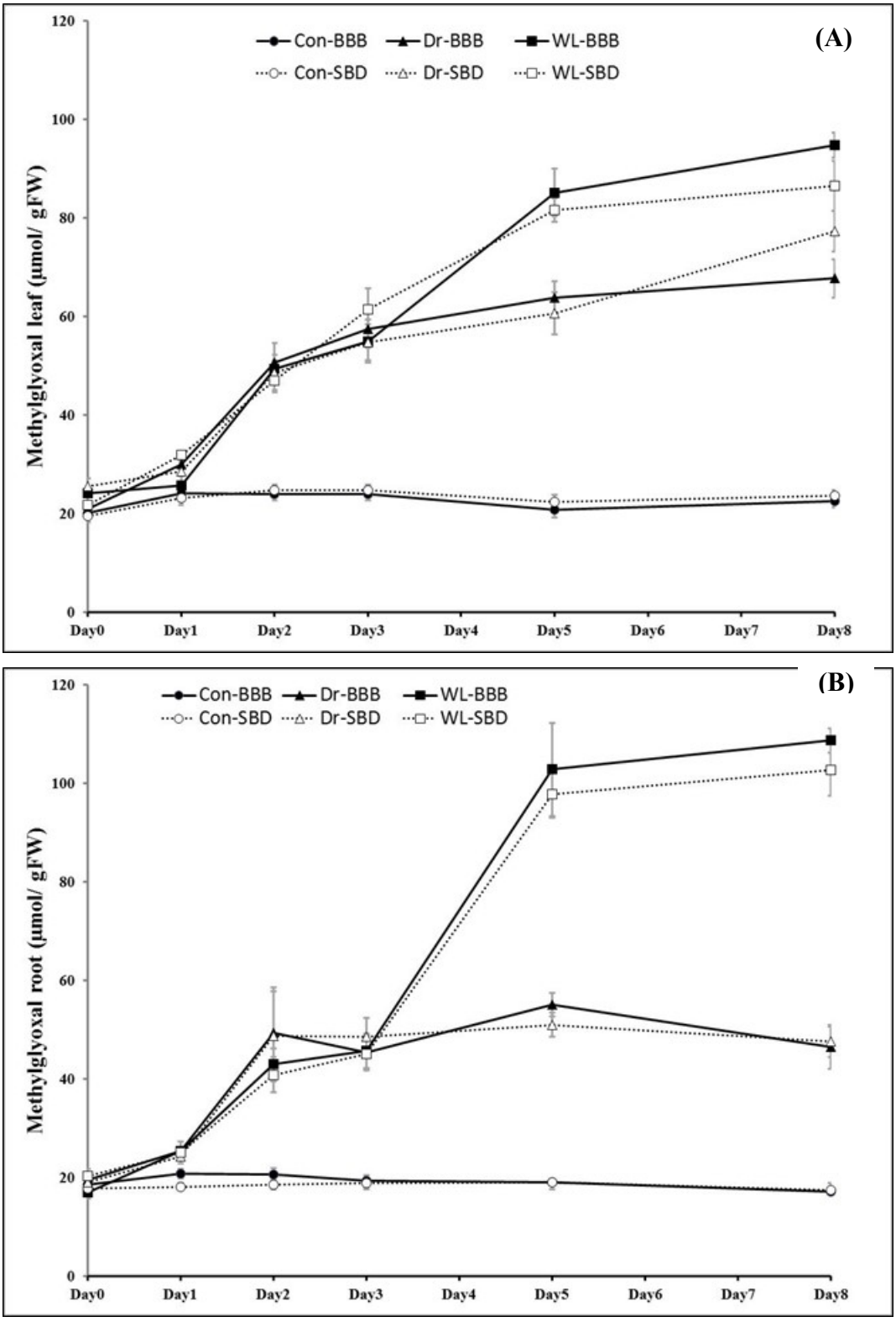


Figure 6.4.4.a Methylglyoxal in leaves (A) and roots (B) of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf', during eight days of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

6.4.5 Nutritional quality and bioactivity of tomato fruit

6.4.5.1 Main effects

Water treatment

Averaged across cultivars, the levels of total carotenoids in tomato fruit pericarp were reduced under drought (-23%) and waterlogging (-32%) (Table 6.4.5.a and Figure 6.5.4.a). Levels of total antioxidant capacity (TAC) in tomato fruits sampled from drought-exposed plants increased by 25% in the pericarp. Tomato fruits grown under waterlogging had decreased TAC levels in the fruit pericarp of 16%.

The viability of Caco-2 cells pre-incubated with the digest of drought-exposed tomato fruits increased by 13% after exposure to the exogenous application of 200 μmol of hydrogen peroxide (Table 6.4.5.a and Figure 6.4.5.b).

Table 6.4.5.a Summary of P values of the main effects and interactions with water treatments on nutritional quality and viability of Caco-2 cells.

Traits	Water	Cultivar	Time	Water x Cultivar	Water x Time
Total carotenoids skin (μg/mL)	0.733	0.886	-	0.133	-
Total carotenoids pericarp (μg/mL)	0.020	0.091	-	0.193	-
TAC _Skin (μm TE/ 100 g FW)	0.177	0.337	-	0.357	-
TAC_pericarp (μm TE/ 100 g FW)	<0.001	0.780	-	0.917	-
Cell viability with H_2O_2 (0μm) (%)	0.339	-	0.094	-	0.041
Cell viability with H_2O_2 (200μm) (%)	<0.001	-	0.004	-	<0.001

Raw data are shown in Appendix B

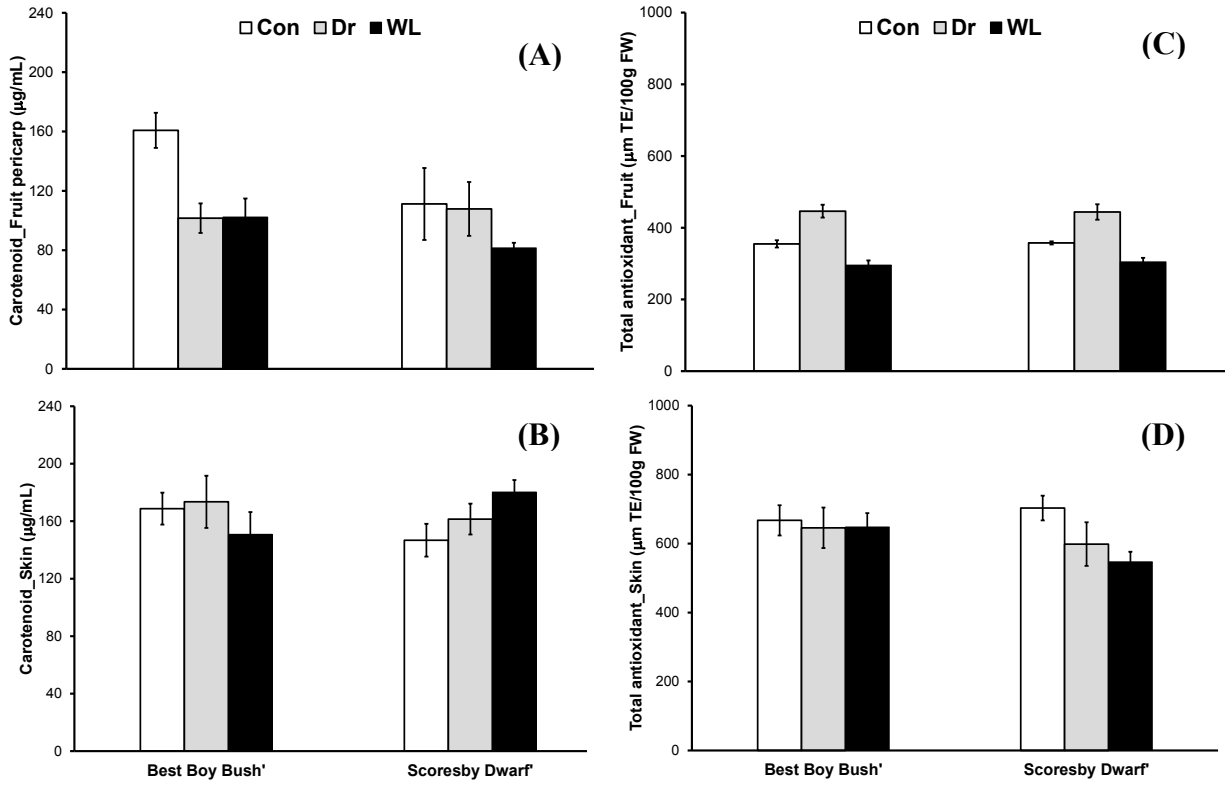


Figure 6.4.5.a Total carotenoid content in fruit pericarp (A) and in tomato skin (B), and total antioxidant capacity in fruit pericarp (C) and in tomato skin (D) of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf', after eight days (Day 8) of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

Time

Averaged across water treatments and cultivars, Caco-2 cells supplemented with the digest of tomato fruits increased their viability under H_2O_2 (200 µmol) application by 8% and by 10% on Day 3 and Day 8, respectively (Table 6.4.5.a and Figure 6.4.5.b).

6.4.5.2 Interaction effects

Water x cultivar

Total carotenoid content in the pericarp of 'Best Boy Bush' tomato fruits decreased by 36-37% under drought and waterlogging, whereas no significant treatment-induced change was observed in 'Scoresby Dwarf' (based on LSDP<0.05) (Table 6.4.5.a and Figure 6.4.5.a).

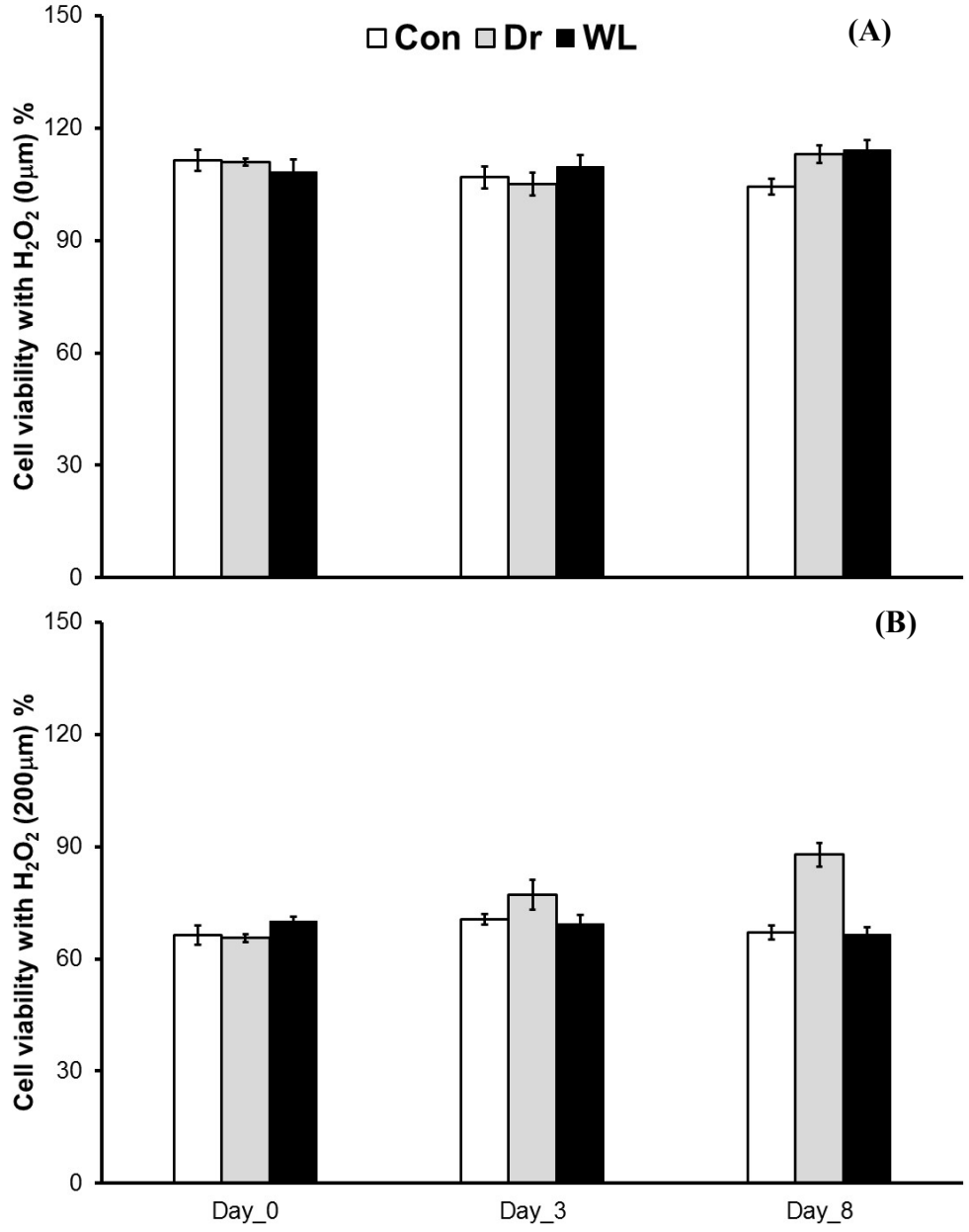


Figure 6.4.5.b Viability of Caco-2 cells following supplementation of tomato digests before treatment with 0 μmol (A) and 200 μmol (B) of H_2O_2. The digests were taken from tomato fruits sampled before (Day 0), after three days (Day 3) and eight days (Day 8) of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

Water x Time

The viability of Caco-2 cells increased after incubation with the digest of tomato fruits sampled eight days after exposure to either a water deficit (8%) or waterlogging (9%) (Table 6.4.5.a and Figrue 6.4.5.b). The viability of Caco-2 cells to the exogenous application of 200 μmol of hydrogen peroxide increased by 31% in Caco-2 cells that had been pre-incubated with the digest of drought-exposed tomato fruits.

6.4.5.3 Summary of the key findings

- Water stress extremes caused a reduction in total carotenoid content in 'Best Boy Bush', but not in 'Scoresby Dwarf' fruits.
- Drought increased total antioxidant capacity in the pericarp of tomato fruit, while waterlogging decreased it.
- Digests of water-stressed tomato fruits improved Caco-2 cell viability. Moreover, cell viability under oxidative stress induced by exogenous application of H_2O_2 was improved by digests of drought-stressed tomato fruits.

6.5 Principal component analysis and heatmap clustering analysis

6.5.1 Multivariate biochemical responses to the effect of water stress

6.5.1.1 Plant trait responses to water stress in the first principal component

The PCA biplot revealed clear oxidative stress response patterns. PC1 accounted for 35% of the variance in the dataset. This axis was largely characterised by increases in all enzymatic antioxidant activities and levels of GSSG in both leaf and root tissues (Figure 6.5.1.a). Changes in the levels of non-enzymatic antioxidants (total ascorbate, total glutathione and proline in leaves and roots, and GSH in root tissue) were positively associated with both PC1 and PC2, whereas increases in stress markers were associated positively with PC1 and inversely with PC2

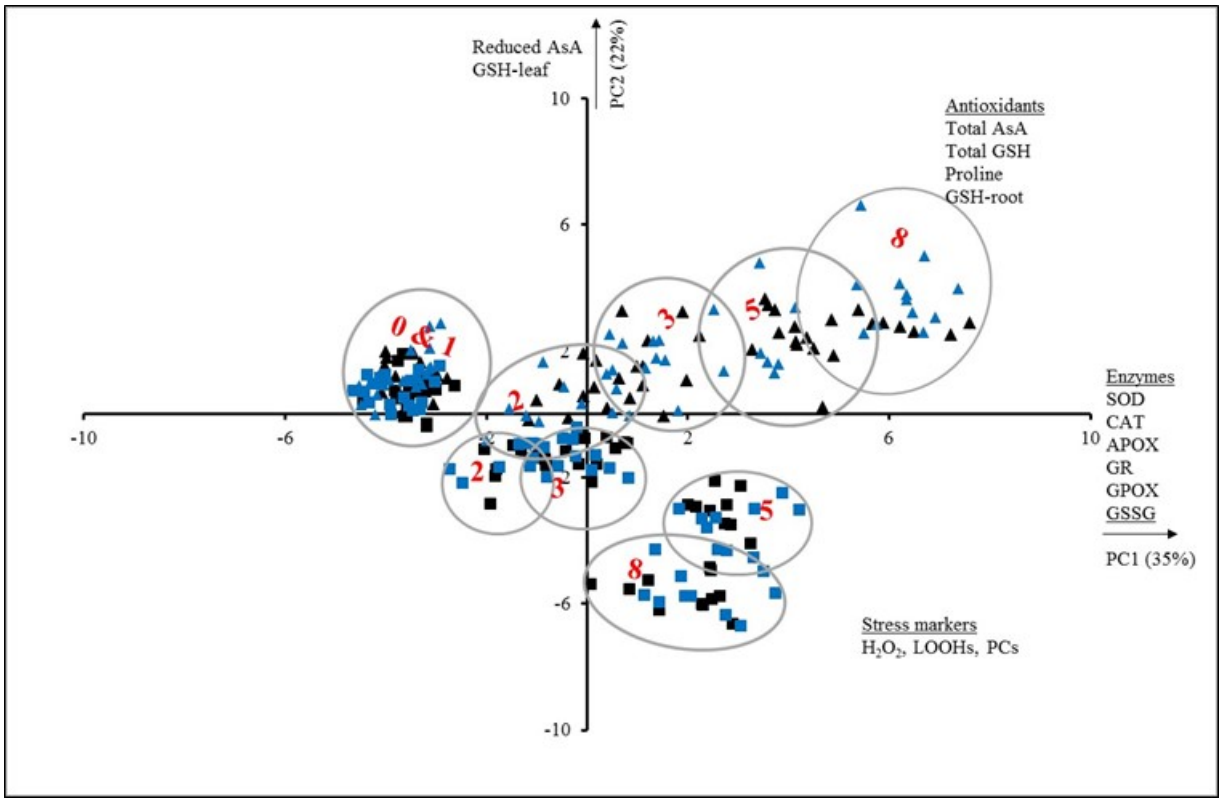


Figure 6.5.1.a Biplot of treatment responses (ratios of treatment/control) in two tomato cultivars at the fruiting stage measured during eight days of exposure to water stress extremes. The data was grouped by time: numerals 0-8 represent Day 0 – Day 8 of the stress period. Symbols: 'Best Boy Bush': drought (▲), waterlogging (■). 'Scoresby Dwarf': drought (▲), waterlogging (■).

6.5.1.2 Cultivar and time responses to water stress in the first principal component

Both cultivars, 'Best Boy Bush' and 'Scoresby Dwarf', responded similarly to the water stress treatments (Figure 6.5.1.a). The intensity of drought stress responses was characterised in PC1 by a continuous progression of scores with harvesting time, with the highest positive scores at Day 8 and the lowest scores (negative PC1 scores) for measurements recorded at the beginning of the stress treatment. The latter starting point was also observed for waterlogged plants, whereas samples taken at Day 5 and Day 8 had similar positive PC1 scores.

6.5.1.3 Plant trait responses to water stress in the second principal component

The second principal component (PC2) explained 22% of the variance in the dataset. Stress-induced changes in non-enzymatic antioxidant accumulation in leaf and root tissues had positive scores in PC2 (Figure 6.5.1.a).

6.5.1.4 Cultivar and time responses to water stress in the second principal component

There was no cultivar difference in response to the change in PC2 (Figure 6.5.1.a). The waterlogging responses were characterised in PC2 by the most negative scores at Day 8, less negative scores at Days 2 and 3 and positive scores at the beginning of the stress phase. A time-dependent pattern for drought responses was not as clearly separated in PC2 but showed the highest PC2 scores at Day 8 and the lowest score at the beginning of the stress treatment.

6.5.2 Similarities of biochemical responses

The HCA identified three distinctive stress response clusters among the biochemical traits (Figure 6.5.2.a). The first can be described as the "oxidative effects" cluster as it consisted of oxidative load, H_2O_2 and the oxidative damage parameters (LOOHs, PCs and DNA oxidation) as well as MG and glyoxalase enzymes (both GLOX1 and GLOX2). This cluster was subdivided by HCA into two groups. The first group comprised all the above-mentioned traits in the root tissues, whereas Group 2 comprised all these traits in the leaves. The heatmap of this first cluster analysis revealed more pronounced increases in these stress markers under waterlogging (Day 5 and Day 8) than under water deficit.

The second cluster was subdivided by HCA into a main group and a small group. The main group was characterised by increases in enzymatic antioxidants including SOD, CAT, APOX, GR and GPOX in both leaf and root tissues. The activities of all antioxidant enzymes were mostly increased from Day 2 to Day 8 in leaves and roots under both treatments. The small group comprised of the oxidised antioxidants, DHA and GSSG in leaves and roots. DHA and GSSG increased from the first day of water stress, and the increases were particularly strong for Day 5 and Day 8 under a water deficit.

The third cluster was mainly characterised by stress-induced changes in non-enzymatic antioxidants, such as reduced and total ascorbate, reduced and total glutathione and proline in both leaves and roots. Levels of these antioxidants mostly increased from Days 2 to 8 under drought stress, while they were mostly decreased by waterlogging, with the largest reductions at Day 8.

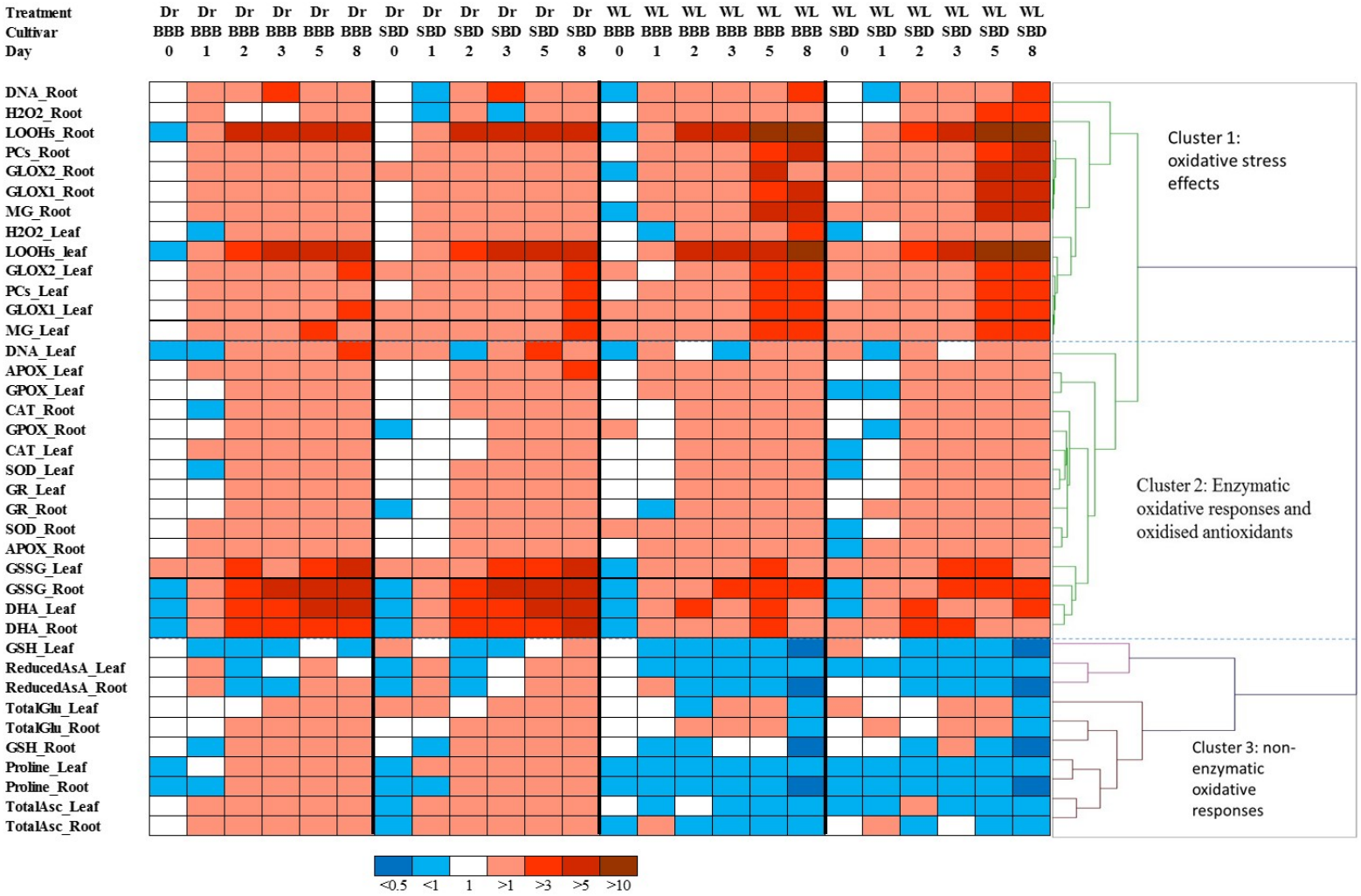


Figure 6.5.2.a Heat map and agglomerative hierarchical clustering of average biochemical changes in two tomato cultivars, 'Best Boy Bush' (BBB) and 'Scoresby Dwarf' (SBD), at the fruiting stage, before (Day 0) and after Day 1, Day 2, Day 3, Day 5 and Day 8 of drought stress (Dr) and waterlogging (WL). The list of biochemical traits is shown on the left and the similarity clustering is shown on the right.

6.5.3 Multivariate analysis of morphological, physiological and biochemical traits

6.5.3.1 Plant traits in the first principal component

The first principal component (PC1) accounted for 37% of the variance in the dataset, double to the next principal component (Figure 6.5.3.a). High scores on this axis were associated with elevated activities of antioxidant enzymes and high levels in the % leaf damage, leaf senescence and dry matter percentage. In addition, PC1 was characterised by elevated levels in the oxidised forms of antioxidants (DHA and GSSG) in both the leaf and root tissues. High levels of total ascorbate, total glutathione and proline in the leaves and roots were associated positively with both PC1 and PC2. Negative PC1 scores were correlated with high plant water status, such as high levels of leaf relative water content, water potential and osmotic potential, as well as high gas exchange parameters such as photosynthetic rate, stomatal conductance and transpiration rates. Plants subjected to water deficit had the highest PC1 scores, waterlogged plants intermediate scores and well-watered control plants the lowest PC1 scores. Both cultivars responded similarly to water stress (Figure 6.5.3.a).

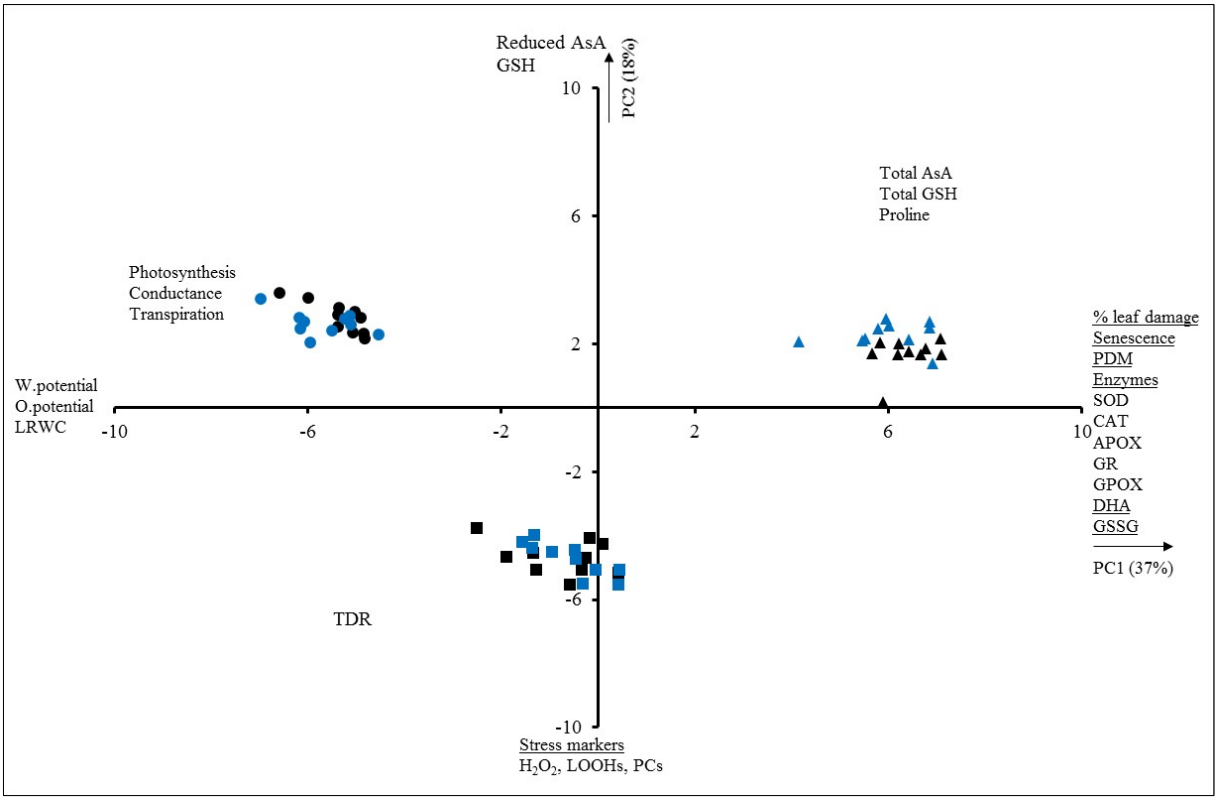


Figure 6.5.3.a Biplot of plant traits in two tomato cultivars, 'Best Boy Bush' (black) and 'Scoresby Dwarf' (blue) at the fruiting stage, measured after eight days exposure to control conditions (circles), drought (triangles) and waterlogging (squares).

6.5.3.2 Plant traits in the second principal component

PC2 accounted for 18% of all the variance in the dataset. High PC2 scores were associated with the reduced forms of ascorbate and glutathione (Figure 6.5.3.a). This was inversely associated in PC2 with high levels of stress markers, including H_2O_2, LOOHs and PCs in both leaf and root tissues. Plants subjected to waterlogging had the lowest PC2 scores and both cultivars responded similarly to the water stress treatments (Figure 6.5.3.a).

6.6 Discussion

This investigation of oxidative responses under field conditions was conducted for two main purposes. First, the field trial was performed to examine oxidative stress and antioxidant responses in the natural environment. The second purpose was the inclusion of new parameters and of kinetic studies to examine the antioxidant defence responses under water stress.
Tomato fruits contain vitamins, minerals and phytochemicals which are known to provide significant health benefits for consumers. However, information on the influence of environmental stress on bioactive properties of tomato fruits is limited. This study is the first to investigate the bioactivity of tomato fruits grown under water stress conditions using *in vitro* digestion and a Caco-2 cell culture system.

6.6.1 Influence of water deficit and waterlogging on production of hydrogen peroxide and oxidative damage

The results of the field study confirmed similar oxidative stress responses as in the glasshouse study. Production of H_2O_2 and oxidative damage parameters (LOOHs, PCs and DNA oxidation) mostly increased after two days of exposure to the treatments (Figure 6.4.1.a, b, see Chapter 5 for a detailed discussion). However, production of H_2O_2 in the hypoxic roots in the field trial differed from the glasshouse study. In the field trial, H_2O_2 levels increased significantly from Day 2 to Day 8 after exposure to waterlogging, while production of H_2O_2 slightly decreased in the hypoxic roots of tomatoes grown under glasshouse conditions. The field trial result suggested for H_2O_2 to reach a plateau after five days of waterlogging. However, the results could have been more similar if H_2O_2 from the field experiment would have been measured after 14 days of prolonged stress as in the glasshouse, which investigated developmental responses as an endpoint study. Water stress symptoms developed faster in the field and thus the harvest had to be conducted after eight days.

Oxidative stress can cause metabolic dysfunctions including lipid peroxidation, protein oxidation and also DNA damage (Aherne et al., 2007; Sharma et al., 2012). DNA damage increased in parallel to rising ROS levels in the present study (Figure 6.4.1.b). Enzymatic antioxidants play an important role in DNA damage reactions (Lin et al., 2007). For example, an increase in SOD activity leads to an increase in H_2O_2 production (Lin et al., 2007). When H_2O_2 cannot be eliminated from the tissues, DNA damage will increase (Lin et al., 2007) as - due to the Haber-Weiss reaction - H_2O_2 can react with $O_2^{\bullet-}$ in the presence of ions (Fe^{2+} and Fe^{3+}) resulting in in $OH^{\bullet-}$ formation (Burritt & Mackenzie, 2003; Wahid et al., 2007). Hydroxyl

radicals attack DNA by reacting with DNA bases and the deoxyribose backbone of DNA (Gill & Tuteja, 2010; Sharma et al., 2012). Gill and Tuteja (2010) suggested that the consequences of DNA oxidation can include a reduction in protein synthesis, a loss of cellular membrane function and destruction of photosynthetic proteins, and that this results in a reduction in plant growth and development (Gill & Tuteja, 2010).

A primary effect of waterlogging in plants is a reduction in the oxygen concentration in the root zone, as the air spaces are filled with water (Sairam et al., 2008). In accordance with the findings in this present study (Figure 6.4.2.b), ADH activities are known to increase during the transition from aerobic conditions to hypoxic conditions (Peng et al., 2001; Wei et al., 2013). ADH catalyses the conversion of acetaldehyde to ethanol and produces NAD^+ (Wei et al., 2013). Wei et al. (2013) reported that the activity of alcohol dehydrogenase increased in sesame plant roots. A time-course study reported that the presence of ADH was observed two days after the onset of waterlogging and the highest activity was seen at Day 6 (Wei et al., 2013), similar to the findings in the present study.

6.6.2 Changes in enzymes and antioxidants in response to oxidative stress

Compared to the glasshouse studies, the antioxidant response of the tomato plants to water stress was similar under drought but differed under waterlogging. In contrast to the glasshouse study, activities of antioxidant enzymes increased in hypoxic plants subjected in the time-course field experiment. There are two possible explanations for this result. First, there was a trend in the field experiment that most of these enzymatic antioxidants in both leaf and root tissues reached a peak after five days of waterlogging, with less pronounced increases at Day 8 (10% to 20% lower) than at Day 5 (Figure 6.4.2.a and Appendix F). It is possible that the activities of these enzymes could have dropped further if the waterlogging treatment in the field had been prolonged to the 14 days of the glasshouse experiment. The findings from PC1 of the field results further highlighted the clear time-dependency of stress-induced antioxidant enzyme activity (Figure 6.5.1.a). The distribution of PCA scores emphasised that this progressive effect was more pronounced in drought-exposed plants compared to plants exposed to hypoxia, where antioxidant enzyme activities had, by and large, peaked by Day 5. In parallel with a downward trend in the activity of antioxidant enzymes, levels of MG and of oxidatively damaged macromolecules were building up in tissues of the waterlogged plants (Figure 6.4.1.a, b and Figure 6.4.4.a). Levels of H_2O_2 and MG, as well as oxidative damage parameters (LOOHs, PCs and DNA oxidation), were all at high levels at Day 8 compared with the previous sampling days and were between 2 to 8-fold that of the control plants. Therefore, if the waterlogging treatment

in the field would have been 14 days, as in the glasshouse, the antioxidant defence system could have been inactivated.

The production of non-enzymatic antioxidants increased in plants subjected to water deficit. Waterlogged plants, on the other hand, showed a decline in reduced ascorbate, total ascorbate and GSH levels (Figure 6.4.3.a, b, c). The decline in reduced ascorbate and GSH levels could contribute to the high levels of ROS recorded in the plant tissues. Both reduced ascorbate and GSH have the ability to directly detoxify ROS and are indispensable co-factors of many enzymes, including APOX and GPOX. Foyer and Noctor (2011) suggested that the shift from the reduced form of either ascorbate or glutathione to the oxidised form, DHA or GSSG, was a sign of increased intercellular reactive oxygen species. The PCA also highlighted these differences in drought and waterlogging responses in terms of non-enzymatic antioxidants. The PC2 trait scores showed the clear distinction into general increases of non-enzymatic antioxidant levels under drought and decreases of these levels under waterlogging (Figure 6.5.1.a). Again, a time component was identified by PC2 for non-enzymatic antioxidants, showing the strongest increases and decreases at the end of the drought and waterlogging periods, respectively. The co-location of stress markers with waterlogging responses emphasised that oxidative stress effects were generally more severe under hypoxia compared to drought. This was also demonstrated in the third cluster of HCA (Figure 6.5.2.a), where the heatmap clearly highlighted the increases in all non-enzymatic antioxidant levels under water deficit and decreases of the non-enzymatic antioxidant levels under waterlogging. Details of enzymatic and non-enzymatic antioxidant responses to the accumulation of H_2O_2 and oxidative stress have already been discussed in Chapter 5.

6.6.3 The relationship between methylglyoxal and glyoxalases and the oxidative stress response

Methylglyoxal levels increased in tissues of plants subjected to water stress (Figure 6.4.4.a). Methylglyoxal levels are typically increased under stress (Viveros et al., 2013). MG is a highly toxic compound that can inhibit cell growth and react with DNA and proteins (Hoque et al., 2008; Viveros et al., 2013). In both leaf and root tissues levels of methylglyoxal were found to increase from Day 2 to Day 8 under both drought and waterlogging. However, the increase was more pronounced in plants subjected to waterlogging. It is possible that methylglyoxal is the cause of the reduced enzymatic antioxidant activities. This is in line with findings by Yadav et al. (2005b), showing that high levels of methylglyoxal can inactivate antioxidant defence

systems. In the present study, methylglyoxal levels increased in conjunction with the falling activities of enzymatic antioxidants during waterlogging.

The activity of the glyoxalase enzymes GLOX1 and GLOX2 increased in parallel with the methylglyoxal increases under stress (Appendix F). GLOX1 and GLOX2 are thought to help confer tolerance to oxidative stress by detoxifying methylglyoxal (Hoque et al., 2008; Yadav et al., 2005b). GLOX1 catalyses the formation of S-D-lactoylglutathione (SDL) from methylglyoxal using GSH as a co-factor. GLOX2 converts, by hydrolysis, SDL to D-lactic acid and regenerates GSH (Hoque et al., 2008; Viveros et al., 2013). High GLOX1 activity was reported in tolerant groundnut species that were subject to salt stress (Hoque et al., 2008). Mung bean seedlings, after 24 h of salt stress, also had high levels of GLOX1 activity (Hossain & Fujita, 2010). In the present study, the activity of GLOX1 was mirrored by the accumulation of MG in both leaf and root tissues for plants under drought and waterlogging (Appendix F). This finding supports the role of GLOX1 as a detoxification response to MG accumulation. High activity of GLOX2 may be accompanied by high levels of GSH as the GLOX2 enzyme plays a significant role in the regeneration of GSH (Hoque et al., 2008; Hossain & Fujita, 2010). In this study, a significant reduction in GSH was observed in both leaf and root tissues taken from plants that had been subjected to waterlogging (Figure 6.4.3.c), despite there being high GLOX2 activity (Appendix F). Hossain and Fujita (2010) suggested that low levels of GSH might be caused by low activity of the enzyme responsible for generating GSH from GSSG, glutathione reductase, and also by an increase in GSH degradation. GR, in this field study, was found to increase in both leaf and root tissues when the plants had been subjected waterlogging (Appendix F). Therefore, an increase in GSH degradation might be a possible reason for declining GSH levels.

6.6.4 Influence of water stress on the nutritional quality and bioactivity of tomato fruits

Beneficial effect of the dietary intake of tomato fruits on human health is linked to the antioxidant activity of tomato phytochemicals, specifically the ability to detoxify reactive oxygen species (Frusciante et al., 2007; Panthee et al., 2013).

Water stress extremes caused a reduction in total carotenoid content in ‘Best Boy Bush’, but not in ‘Scoresby Dwarf’ fruits (Figure 6.4.5.a). The total carotenoid content of tomato fruit has been largely attributed to lycopene (about 80- 90%) and β-carotene (7-10%) (Frusciante et al., 2007; George et al., 2004). Lycopene exhibits high antioxidant activities particularly in the detoxification of singlet oxygen, while β-carotene is known for its provitamin A activity. The

provitamin A carotenoids have the ability to maintain healthy epithelial cells, normal reproductive performance and visual functions (Kopsell & Kopsell, 2006). In addition, lycopene, which is predominantly found in red tomato fruits, has the ability to suppress cell proliferation and restrict the growth of some cancer cells (Dumas et al., 2003). Carotenoid levels in tomato fruits generally increase during the ripening process, when the colour of the fruits turns from green to red (Horchani et al., 2010b). However, the biosynthesis of carotenoids can be affected by several factors including physiological, genetic and biochemical attributes as well as environmental factors (e.g. temperature, light and fertilisers) (Chandra et al., 2012; George et al., 2004; Kopsell & Kopsell, 2006). Dumas et al. (2003) suggested that the total carotenoid content in fully ripe tomato fruit increased under a water deficit. The water stress-induced reductions in total carotenoid content in 'Best Boy Bush' are similar to observations by Horchani et al. (2010b). These authors suggested that waterlogging was unlikely to induce fruit hypoxia and did not affect all the aspects of fruit ripening including the accumulation of sugars, organic acids and amino acids. However, the authors proposed that a limitation to carotenoid synthesis in tomato fruits at the ripening stage was caused by the disruption of gene expression under waterlogging. Specifically, the expression of genes that control carbon entering the carotenoid biosynthesis pathway decreased (Horchani et al., 2010b).

To the best of the author's knowledge, there have thus far been no studies that investigated the effect of abiotic stress on the total antioxidant capacity in the fruits of either tomatoes or other crops. TAC in tomato leaves subjected to drought stress was reported to increase (Sanchez-Rodriguez et al., 2010a). In this study, TAC in tomato fruits increased under drought. Total antioxidant capacity was previously reported to increase during fruit ripening, which was mainly attributed to changes carotenoid accumulation (Cano et la., 2003). However it has been suggested that lipophilic antioxidants such as carotenoids contributed less than 8% of total antioxidant capacity (Toor et al., 2006). The authors, therefore, proposed that the most important contributors to the total antioxidant capacity of tomato fruits were soluble phenolic compounds. Alternatively, a reduction in TAC in the tomato fruit pericarp grown under waterlogging might also be caused by the decreases in the reduced form of ascorbate or ascorbic acid, as observed in the glasshouse study (Chapter 5).

The results of the present study suggest that the viability of oxidatively-stressed (H_2O_2 treated) Caco-2 cells can be improved following supplementation of digested tomato fruits harvested from water stressed plants. Thus, digested tomato fruits from plants grown under water deficit showed bioprotective capacity (Figure 6.4.5.b). Oxidants are produced in the human body during normal metabolism and as immune responses against diseases (Kopsell & Kopsell,

2006). Accumulation of oxidants results in oxidative stress in the body, which is known to cause cellular damage. Cellular damage has been associated with the development of many diseases including certain types of cancer and cardiovascular disease (Aherne et al., 2007). Such effects of oxidative stress might be preventable with an enhanced antioxidant defence system. However, humans are unable to synthesise certain antioxidant compounds, and since tomatoes are reservoirs of antioxidant molecules (ascorbic acid, carotenoids, vitamin E and phenolics), regular consumption of tomato fruits can contribute to a lower risk of disease. The findings from the present study suggest that water stress could induce an accumulation of beneficial phytochemicals, due to an increase in total antioxidant capacity in tomato fruits. Therefore, diets rich with tomatoes grown under water stress could be beneficial to human health through a potential reduction in oxidative stress.

The improved Caco-2 viability under stress observed here could be attributed to the antioxidant activity contained in the tomato digest. In this study, tomato fruits grown under drought stress contained more ascorbic acid (Chapter 5: 5.4.3) and had a higher total antioxidant capacity (Figure 6.4.5.a). A recent study reported that berry homogenates could also increase the viability of Caco-2 cells under oxidative stress induced by an exogenous challenge of H_2O_2 (Slemmer et al., 2013). The author suggested that this increase in cell viability was due to an increase in metabolic activity. The study showed that digested berry homogenates did not cause any cell death but were found to enhance cell metabolism (Slemmer et al., 2013). Aherne et al. (2007) reported that supplementation of Caco-2 cells with herbal extracts, oregano, sage and rosemary protected Caco-2 cells against H_2O_2-induced DNA damage. This protective capacity was due to free radical scavenging capacity in the plant extracts (Aherne et al., 2007).

6.6.5 Conclusions

Oxidative effect of water deficit and waterlogging were observable from 1-2 days after the imposition of the water stress treatment and became more pronounced with prolonged stress. Water stress extremes induced the accumulation of hydrogen peroxide in plant tissues and resulted in oxidative damage to plants, as measured by the production of lipid hydroperoxides, protein carbonyls, DNA oxidation and oxidised antioxidant levels. Tomato plants responded to oxidative stress by the activation of enzymatic antioxidant activities and (under drought) with increased accumulation of non-enzymatic antioxidants. Oxygen deprivation reduced levels of the latter compounds whilst it increased ADH activity in hypoxic roots. Water stress also induced production of methylglyoxal and, concomitantly, stimulated activity of glyoxalase enzymes. Tomato fruits grown under water deficit had large total antioxidant capacity and, therefore, improved the viability of Caco-2 cells under oxidative stress, suggesting merit for using drought as a phytostimulatory treatment before harvest of tomato fruits to enhance its antioxidant potential. In general, there were no significant intraspecific differences in the treatment responses under field conditions.

Chapter 7

Effects of high temperature stress on tomato depend on plant development and cultivar

Abstract

Thus far there had been limited studies examining the effects of elevated heat stress on tomato (*Lycopersicon esculentum* Mill.). This study investigated such effects at the morphological and physiological levels in three tomato cultivars at various developmental stages. Tomato plants were grown in two growth chambers under optimal conditions (25°C/20°C, day/night) and under elevated heat stress (40°C/30°C, day/night). Stem-based traits were in general heat stress-tolerant (internode length, node numbers, stem diameter) whilst there were several heat stress effects on leaf morphology, which included reductions in leaf length (-20%), leaf area (-52%) and leaf angle (-44%). This meant that the leaves grew with a more vertical orientation under heat stress. Reproductive plant attributes were most susceptible to heat stress, with strong reductions in tomato fruit set as a response to 53% increases in flower abscission and of stigma tube elongation. The latter was most pronounced in the cultivar 'Best Boy Bush' and was linked to reductions in flower numbers by 28% in this cultivar. The findings indicate intraspecific differences in the responses to elevated heat stress in tomato and that these are predominantly expressed in aspects of plant reproduction.

7.1 Introduction

Tomato productivity decreases significantly once the growing temperature exceeds 35°C (Wahid et al., 2007). Temperatures in excess of 30°C are common in the warmest months in many places in the world - particularly in tropical and subtropical regions (Heuvelink, 2005; Preedy & Watson, 2008; Sato et al., 2000). In these regions, which are major producers of the world's tomatoes, tomatoes are mainly cultivated outdoors, in the field without any protective structures (*Food and Agricultural Commodities Production*, 2010). It is well-established that temperatures will continue to rise due to global climate change and therefore, high temperatures can pose a threat to world tomato production.

Heat stress induces several negative effects on the morphology and physiology of tomato plants (Wahid et al., 2007). Heat stress (or thermal stress) can cause considerable damage to plant growth and morphology, including reductions in plant height and leaf size, resulting in a more vertical leaf orientation (Wahid et al., 2007). The reproductive stage of plant growth is also known to be critically sensitive to high temperature stress. Fruit set in tomatoes is poor if the temperature exceeds 30°C (Sato et al., 2000). Poor fruit set under high temperatures is mainly caused by stigma tube elongation, abscission of flowers and may also be linked with low

carbohydrate levels (Saeed et al., 2007; Wahid et al., 2007). Temperatures of 26°C or more can alter dry matter partitioning in shoot and tomato fruits, as expressed by the dry matter percentage (Adams et al., 2001).

In addition, high temperature stress can decrease chlorophyll content and modify chlorophyll fluorescence. These changes can be associated with the functional and structural damage to photosystem II (Camejo et al., 2005). Most research on high temperature stress in tomatoes has investigated tomato responses to temperatures well below 40°C and mostly for relatively short periods of time. For example, a heat stress study of Sato et al. (2000) was conducted at 32°C/26°C for day/night time and Adams et al., (2001) investigated the effect of a temperature of 26°C on fruit set in tomatoes. However, these temperatures are relatively low compared to the current trend of global warming scenarios. Even in temperate regions such as in Korea, the temperature could approach 40°C during summer in both the open field and in glasshouses (Kang et al., 2009). With this trend of global climate change it is important to conduct high temperature studies. There has been little research on tomato responses to high temperatures, and for durations that extend through all the plant's growth and development stages from the vegetative stage through to the time when fruit harvesting takes place.

The general objective of the work reported in this chapter was the examination of possible genotypic differences and above ground responses in tomato plants subjected to stress from high temperature exposure. Specifically, the aim of this chapter was to investigate how three tomato cultivars ('Best Boy Bush', 'Scoresby Dwarf' and 'Soprano') responded morphologically and physiologically to high temperature exposure (40°C) in their vegetative and reproductive phases of growth and development. The rationale for cultivar selection of 'Best Boy Bush' and 'Scoresby Dwarf' has already been mentioned in Chapter 3. 'Soprano' is an F1 hybrid and a determinate tomato cultivar used for open field production in Spain (Gragera et al., 2003) and it was hypothesised to possess heat tolerance. Thus, it was expected that these cultivars would respond differently in terms of their response to high temperature stress.

7.2 Material and methods

7.2.1 Experimental design

The experiment was conducted in two walk-in growth chambers (1.37 m x 2.45 m, Conviron, PGV36, Canada) at Lincoln University, Canterbury, New Zealand from 18th February to 08th July 2011. The light intensity was set at 400 µmol photons m^{-2} s^{-1} and with a relative humidity of 60%. The experiment was laid out as a randomised complete block design within each growth environmental chamber. One growth chamber was set to provide a day time temperature of 40°C and a 30°C temperature for the dark period, which was set to last for 10 hours. Another growth chamber was set to provide a 25°C/20°C regime for day/night time temperatures, respectively. Temperature treatments were shifted together with the corresponding plant material between, and plants re-randomised, within the chambers every two weeks to address possible room effects. Each pot contained one plant and there were 15 replicated pots per cultivar per treatment, giving a total of 45 plant pots for each growth chamber.

7.2.2 Plant materials and growth conditions

Three determinate tomato (*Lycopersicon esculentum* Mill.) cultivars from different origins were selected with different levels of thermo-tolerance. It was hypothesised that the cultivar 'Scoresby Dwarf', which was of Australian origin, would be most heat tolerant. 'Scoresby Dwarf' has been used commercially in Australia for about 30 years for field tomato production (Morley-Bunker, 2010 pers. Com). Similarly, there was a presumption of heat tolerance in the tomato cultivar 'Soprano' due to its origin in Spain (Gragera et al., 2003). The tomato cultivar 'Best Boy Bush' is used as a New Zealand home garden cultivar and was expected to be heat sensitive. 'Soprano' and 'Best Boy Bush' are F1 hybrids and determinate plants used mostly for open field tomato production.

The tomato seed was germinated in a glasshouse using polystyrene trays and a potting mix (80% bark, 20% pumice and osmocote 16-35-0, with horticultural lime and hydraflo as wetting agent). Four weeks after germination, the seedlings were transplanted into 15 cm diameter pots (2.5 litre pots) using potting mix (80% bark, 20% pumice and osmocote 15-4-7.5, horticultural lime and hydraflo). Sixty days after germination (beginning of anthesis) the plants were moved to the growth chambers.

The details of the analytical procedures for most parameters shown here have already been described in Chapter 3, with the exception of the following:

The leaf angle was measured between the petiole of the second fully unfolded leaf and the stem in relation to the 90 degree perpendicular. Leaf area was measured using a LI-Cor (model 3100) area meter (Li-COR Biosciences, Lincoln Nebraska). The fresh leaf of the second fully unfolded leaf of each plant was measured. In addition, counts were made of the numbers of nodes and the numbers of flowers abscised after anthesis. The internode length was measured using a ruler. Stigma tube elongation was determined by visual observation of flowers where the stigma tubes (styles) exceeded the antheridial cones (Saeed, Hayat, Khan, & Iqbal, 2007), using a ruler, a headlamp and magnifying glass. Some traits, including leaf area and traits related to dry matter and the percentage of dry matter (total leaf, total stem, total flowers and fruits and total shoots), were only measured at the final harvest (fruiting stage) because these traits were harvested destructively. Percentage of dry matter, PDM was calculated as (dry matter/fresh matter) x 100 (Hofmann & Campbell, 2011).

7.3 Statistical analyses

7.3.1 Analysis

Statistical analyses of traits in this heat stress experiment at final harvest were conducted using the General Analysis of Variance (ANOVA) procedure in Genstat 14 to examine the main effects and their interactions. In addition, traits measured over time were analysed using the repeated measures function in Genstat 14. In addition to the overall interaction probabilities from ANOVA, comparisons between interaction means were based on Tukey's 95% confidence intervals calculated in Genstat 14. Raw data are shown in Appendix G.

All multiple comparisons were based on Tukey's 95% confidence intervals. For conciseness, only the significant results are presented.

The methodology for principal component analysis has already been outlined in chapter 3. The biplot of the effects of heat stress were based on the PCA scores of heat treatment in the three cultivars at the final harvest (fruiting stage) to allow inclusion of all measured traits in the analysis (Ballizany et al., 2012). Only traits that showed significant correlations with either PC1 and/or PC2 are presented in the PCAs.

7.3.2 Data presentation

The P values from each set of plant traits are summarised in tables at the beginning of each results section. Unless stated otherwise, results are expressed as percentage change of heat treatment (40°/30°C, day/night) compared to the control (25°/20°C) plants.
For conciseness, tables are used to show the statistical summaries of main effects and their interactions with temperature treatments, followed by the corresponding tables showing significant treatment-induced changes. These responses are then summarised in the text to assist readability.

7.4 Results

7.4.1 Plant growth and morphology

7.4.1.1 Main effects

Averaged across cultivars and developmental stages, heat stress caused a reduction in leaf length (-20%), leaf angle (-34%) and leaf area (-52%) (Table 7.4.1.a, b and Figure 7.4.1.a, b, c, d).

Averaged across temperature treatments and developmental stages, 'Scoresby Dwarf' was smaller than 'Best Boy Bush' and 'Soprano' in terms of plant height (-22% to -58%), leaf length (-3% to -10%) and leaf area (-18% to -30%) (Table 7.4.1.a, b and Figure 7.4.1.a, b, c, d).

'Scoresby Dwarf' however had higher leaf number (about 30%), node number (about 20%) and stem diameter (7% to 18%) than 'Best Boy Bush' and 'Soprano'. 'Scoresby Dwarf' was 34% shorter than 'Soprano' in terms of its internode length (Table 7.4.1.a, b and Figure 7.4.1.a, b, c, d).

Table 7.4.1.a Summary of P values of the main effects and interactions with heat treatment on tomato growth and morphology attributes

Traits	Heat	Cultivar	Devel. stage	Heat x cultviar	Heat x Devel. stage	Heat x cultivar x Devel .stage
Height (cm)	0.137	<.001	<.001	0.050	<.001	0.964
Leaf Number	0.893	<.001	<.001	0.321	0.117	0.448
Node Number	0.387	<.001	<.001	0.133	0.153	0.061
Leaf Length (mm)	<.001	0.002	0.004	0.073	<.001	0.081
Leaf Angle (°)	<.001	0.242	<.001	0.042	<.001	0.826
Stem Diameter (mm)	0.731	<.001	<.001	0.875	0.056	0.634
Leaf area (cm^2)	<.001	0.008	-	0.606	-	-
Internode length	0.109	0.005	-	0.786	-	-

Raw data are shown in Appendix G

Table 7.4.1.b Summary of percentage changes of the main effects and interactions with heat treatment on tomato growth and morphology attributes

Traits	Heat	Cultivar	Devel. stage	Heat x Cultviar	Heat x Devel. stage
Height (cm)	ns	BBB: 22% SPN: 58%	T2: 5% T3: 18%	ns	H T3: -6%
Leaf Number	ns	BBB: -29% SPN: -31%	T2: 35% T3: 93%	ns	ns
Node Number	ns	BBB: -21% SPN: -23%	T2: 29% T3: 77%	ns	ns
Leaf Length (mm)	H: -20%	BBB: 10% SPN: 3%	T2: -5%	ns	H T1: -13% H T2: -15% H T3: -30%
Leaf Angle (°)	H: -44%	ns	T3: 37%	H BBB: -48% H SBD: -38% H SPN: -46%	H T1: -45% H T2: -39% H T3: -47%
Stem Diameter (mm)	ns	BBB: -18% SPN: -7%	T2: 6% T3: 11%	ns	ns
Leaf area (cm^2)	H: -52%	BBB: 30% SPN: 18%	-	ns	-
Internode length	ns	SPN: 34%	-	ns	-

Heat (H), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), 'Soprano' (SPN), developmental stage (T, Time of harvest 1: vegetative, 2: flowering, 3: fruiting), non-significant (ns), fold (x)

Averaged across heat treatments and cultivars, relative to vegetative stage plant height, leaf number, node number and stem diameter all increased by 5-35% at flowering stage and by 11-93% at fruiting stage (except for leaf length) (Table 7.4.1.a, b and Figure 7.4.1.a, b, c, d). Leaf angle was also increased by 37% at the fruiting stage.

7.4.1.2 Interaction effects

Heat x Cultivar

There were no significant two way interactions between heat treatment and cultivar except for leaf angle. Heat stress caused subtle yet differential reductions in leaf angle among the three cultivars, with smaller decreases in 'Scoresby Dwarf' (-38%) compared to 'Best Boy Bush' (-48%) and 'Soprano' (-46%) (Table 7.4.1.a, b and Figure 7.4.1.a, b, c, d).

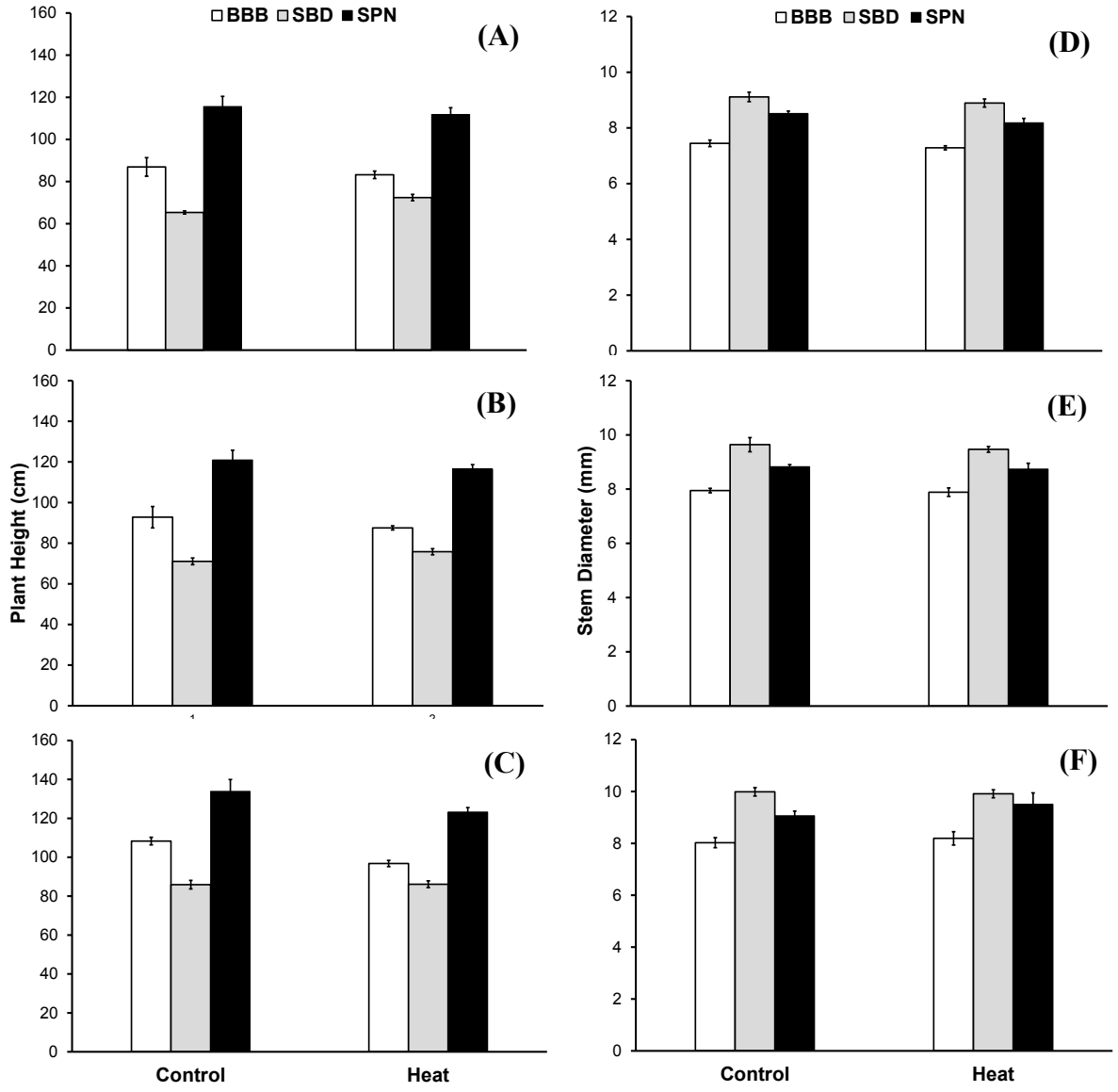


Figure 7.4.1.a Plant height at the vegetative (A), flowering (B) and fruiting (C) stages; and stem diameter at the vegetative (D), flowering (E) and fruiting (F) stages of three tomato cultivars grown under heat stress and control conditions; values are means (n=15) ± standard error.

Heat x Developmental stage

Averaged across cultivars, heat stress reduced leaf length progressively with developmental stages (from -13% at the vegetative stage to -30% at fruiting), and it also decreased leaf angle by 39% to 47% throughout plant development (Table 7.4.1.a, b and Figure 7.4.1.a, b, c, d). Heat treatment also decreased plant height by 6% at fruiting.

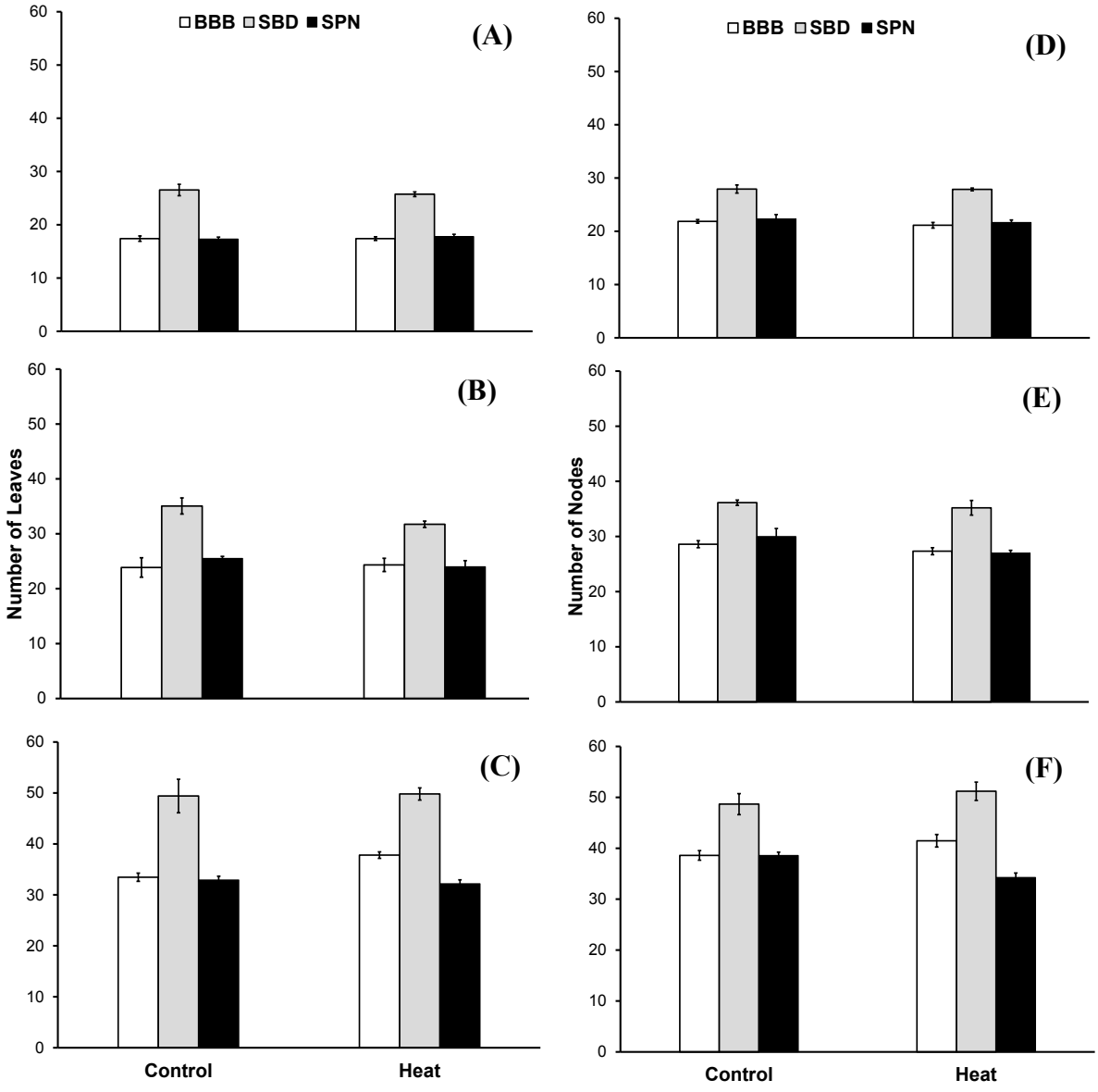


Figure 7.4.1.b Leaf number at the vegetative (A), flowering (B), fruiting (C) stages; and node number at the vegetative (D), flowering (E) and fruiting (F) stages of three tomato cultivars grown under heat stress and control conditions; values are means (n=15) ± standard error.

7.4.1.3 Summary of the key findings

- ❖ Among the vegetative attributes, heat stress decreased leaf traits such as leaf length, leaf area and leaf angle.
- ❖ The reductions in leaf angle under heat stress were more pronounced in 'Best Boy Bush' and 'Soprano' relative to 'Scoresby Dwarf'.
- ❖ Leaf length decreased progressively under heat stress with increasing plant maturity.

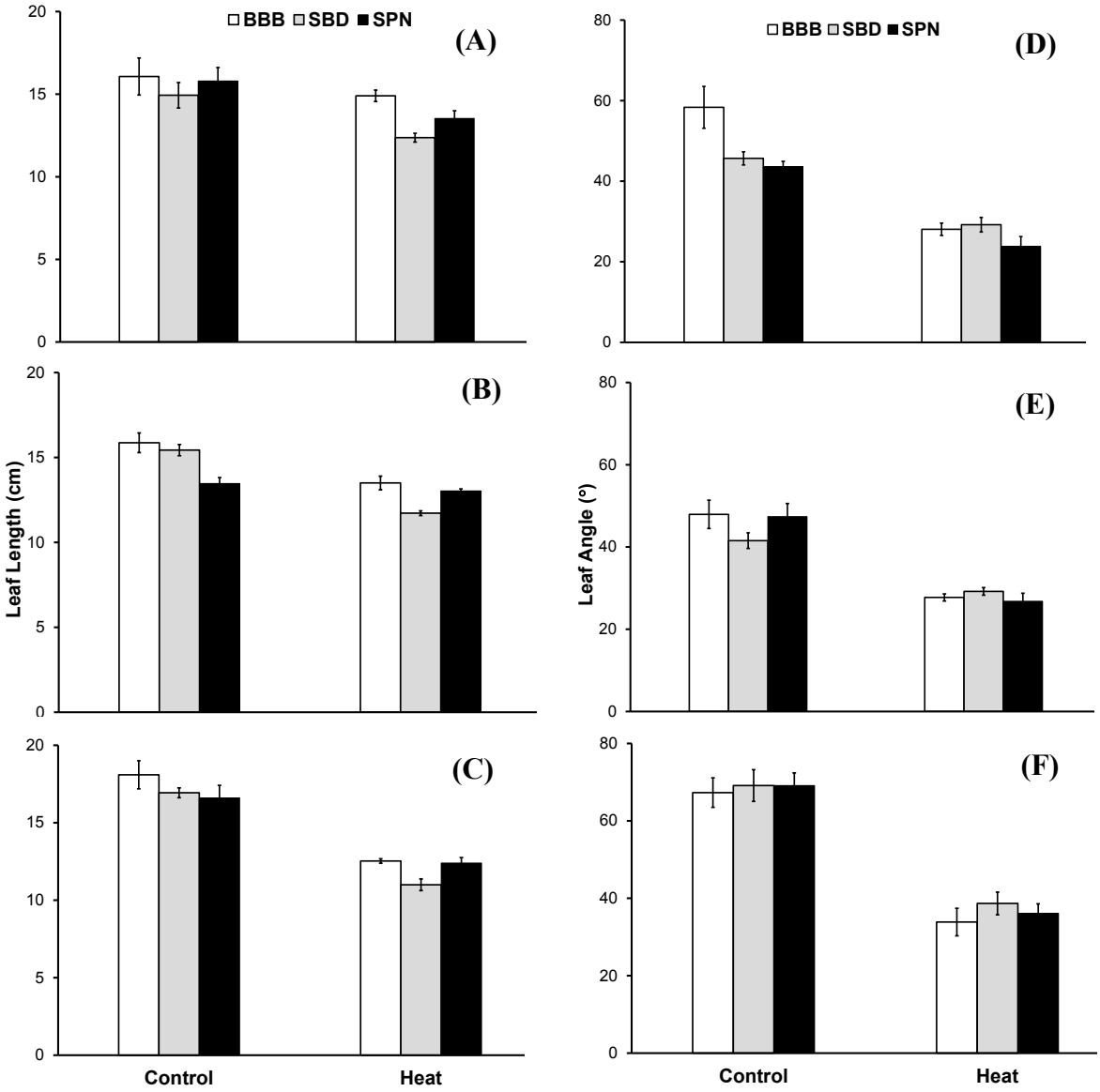


Figure 7.4.1.c Leaf length at the vegetative (A), flowering (B), fruiting (B) stages; and leaf angle at the vegetative (D), flowering (E) and fruiting (F) stages of three tomato cultivars grown under heat stress and control conditions; values are means (n=15) ± standard error.

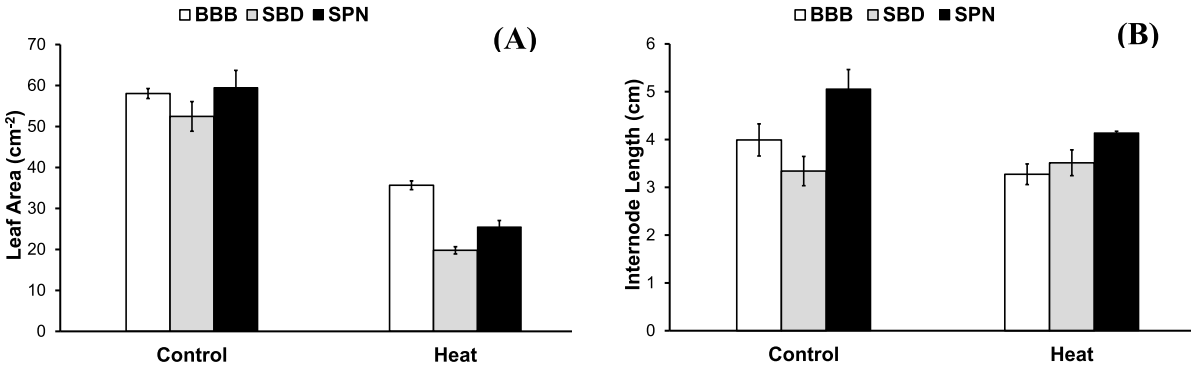


Figure 7.4.1.d Leaf area (A) and internode length (B) at the fruiting stage of three tomato cultivars grown under heat stress and control conditions; values are means (n=15) ± standard error.

7.4.2 Plant dry matter traits

7.4.2.1 Main effects

Averaged across cultivars, heat stress increased both the dry matter and the percentage of dry matter of leaves by about 10% (Table 7.4.2.a, b and Figure 7.4.2.a).

Table 7.4.2.a Summary of P values of the main effects and interactions with heat treatment on plant dry matter production and % dry matter attributes

Traits	Heat	Cultivar	Heat x Cultivar
Leaf DM	0.009	<.001	0.181
Stem DM	0.101	<.001	0.168
Leaf PDM	<.001	0.162	0.360
Stem PDM	0.062	<.001	0.168

Raw data are shown in Appendix G

Averaged across heat treatments and developmental stages, 'Scoresby Dwarf' had lower leaf DM (about 20%), stem DM (31-80%) and stem PDM (12-42%) relative to 'Best Boy Bush' and 'Soprano' (Table 7.4.2.a, b and Figure 7.4.2.a).

7.4.2.2 Interaction effects

There were no significant two-way interactions for the plant dry matter related traits (Table 7.4.2.a, b and Figure 7.4.2.a). However, using Tukey's 95% confidence intervals, there was a heat-induced cultivar-specific increase in 'Soprano' leaf DM by 19%.

Table 7.4.2.b Summary of percentage change of the main effects and interactions with heat treatment on plant dry matter production and % dry matter attributes

Traits	Heat	Cultivar	Heat x Cultivar
Leaf DM	H: 10%	BBB: 16% SPN: 24%	ns
Stem DM	ns	BBB: 31% SPN: 80%	ns
Leaf PDM	H: 9%	ns	ns
Stem PDM	ns	BBB: 12% SPN: 42%	ns

Heat (H), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), 'Soprano' (SPN), developmental stage (T, 1: vegetative, 2: flowering, 3: fruiting), non-significant (ns), fold (x)

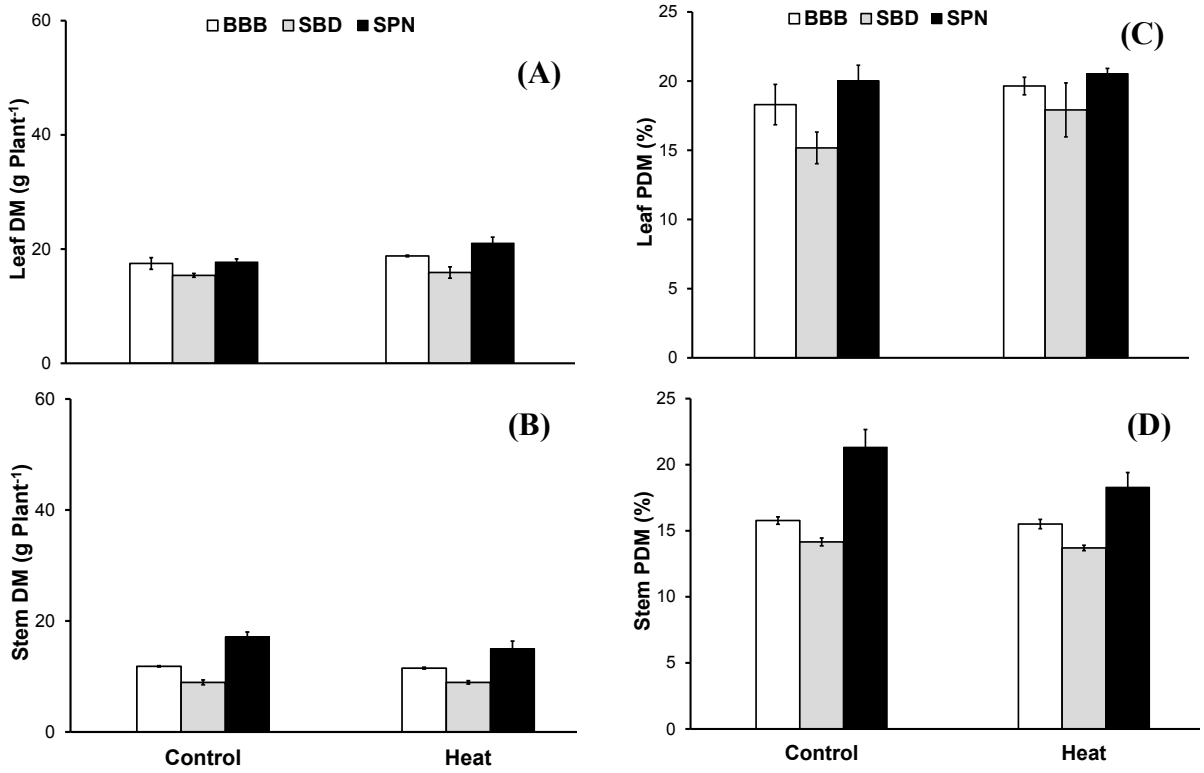


Figure 7.4.2.a Leaf DM (A), stem DM (B), leaf PDM (C) and stem PDM (D) measured at the fruiting stage of three tomato cultivars grown under heat stress and control conditions; values are means (n=15) ± standard error.

7.4.2.3 Summary of the key findings

- ❖ Heat stress increased leaf dry matter production. 'Scoresby Dwarf' had lower dry matter accumulation relative to 'Best Boy Bush' and 'Soprano'.
- ❖ There was an indication of a heat-induced cultivar-specific leaf DM increase in 'Soprano', but not the other two cultivars.

7.4.3 Reproductive components

7.4.3.1 Main effects

Averaged across all cultivars and developmental stages, heat stress affected most reproductive components including the number of abscised flowers (-53%), the number of flowers with elongated stigma tubes (11.7, on average compared to none in the control), flower and fruit DM (-97%) and flower and fruit PDM (2.4-fold) (Table 7.4.3.a, b and Figure 7.4.3.a, b, c).

Averaged across heat treatment and developmental stages, compared with 'Scoresby Dwarf', the numbers of abscised flowers for ‘Best Boy Bush' was 23% higher, but for 'Soprano' was 33% lower (Table 7.4.3.a, b and Figure 7.4.3.a, b, c). The number of flowers with elongated stigma tubes were 73% higher for 'Soprano' relative to 'Scoresby Dwarf', whilst compared to the latter, the greatest values of the flowers with elongated stigma tubes were observed in 'Best Boy Bush' (2.6-times higher). Compared to 'Scoresby Dwarf', flower and fruit PDM for 'Soprano' was 87% higher.

Table 7.4.3.a Summary of P values of the main effects and interactions of heat treatment, cultivar and developmental stage on plant reproductive components

Traits	Heat	Cultivar	Devel. Stage	Heat x Cultivar	Heat x Devel. stage	Heat x Cultivar x Devel. stage
Flower Number	0.200	0.059	<.001	0.007	0.007	0.079
Number of Abscised Flowers	<.001	<.001	<.001	0.558	0.040	0.204
Number of Flowers with Elongated Stigma Tubes	<.001	<.001	<.001	<.001	<.001	<.001
Flower and Fruit DM	<.001	0.083	-	0.109	-	-
Flower and Fruit PDM	<.001	0.020	-	0.021	-	-

Raw data are shown in Appendix G

Averaged across heat treatments and cultivars and compared to the flowering stage, when plants reached the fruiting stage, there were still flowers being produced (3.3-fold) and there was an increase in the numbers of flowers that abscised (5.4-fold) and an increase in the numbers of flowers with elongated stigma tubes (3.5-fold) (Table 7.4.3.a, b and Figure 7.4.3.a, b, c).

Table 7.4.3.b Summary of percentage changes of the main effects and interactions with heat treatment on plant reproductive components

Traits	Heat	Cultivar	Devel. Stage	Heat x Cultivar	Heat x Devel. stage	Heat x Cultivar x Devel. stage
Flower Number	ns	ns	T3: 3.3x	H BBB: -28%	H T2: -32%	ns
Number of Abscised Flowers	H: 53%	BBB: 23% SPN: -33%	T3: 5.4x	ns	H T2: 21.4x H T3: 18%	ns
Number of Flowers with Elongated Stigma Tubes	H: 11.7a	BBB: 2.6x SPN: 73%	T3: 3.5x	H BBB: 16.3a H SBD: 6.3a H SPN: 10.9a	H T2: 5a H T3: 17.3a	H T2 BBB: 7a H T2 SBD: 2.2a H T2 SPN: 5.9x H T3 BBB: 25.6a H T3 SBD: 10.4a H T3 SPN: 15.9a
Flower and Fruit DM	H: -97%	ns	-	ns	-	-
Flower and Fruit PDM	H: 2.4x	SPN: 87%	-	H SPN: 3.7x	-	-

Heat (H), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), 'Soprano' (SPN), developmental stage (T, 1: vegetative, 2: flowering, 3: fruiting), non-significant (ns), fold (x), absolute differences (a)

7.4.3.2 Interaction effects

Heat x Cultivar

Averaged across developmental stages, heat stress caused a reduction in the number of flowers of 'Best Boy Bush' by 28% (Table 7.4.3.a, b and Figure 7.4.3.a, b, c). There were absolute differences in the numbers of flowers with elongated stigma tubes among the three cultivars grown under heat stress, which were highest in 'Best Boy Bush' (16.3 on average, compared to none in the control), followed by 'Soprano' (10.9 on average) and lowest in 'Scoresby Dwarf' (6.3 on average). Flower and fruit PDM of 'Soprano' grown under heat stress was 3.7 times that of plants grown under optimal conditions.

Heat x Developmental stage

Averaged across cultivars, heat stress caused a reduction in number of flowers (by 32%) at the flowering stage but increased the number of abscised flowers (21.4-fold at the flowering stage and 18% at fruiting) and the number of flowers with elongated stigma tubes (with absolute numbers of 5-17.3, compared to none in the control) at both the flowering and fruiting stages (Table 7.4.3.a, b and Figure 7.4.3.a, b, c).

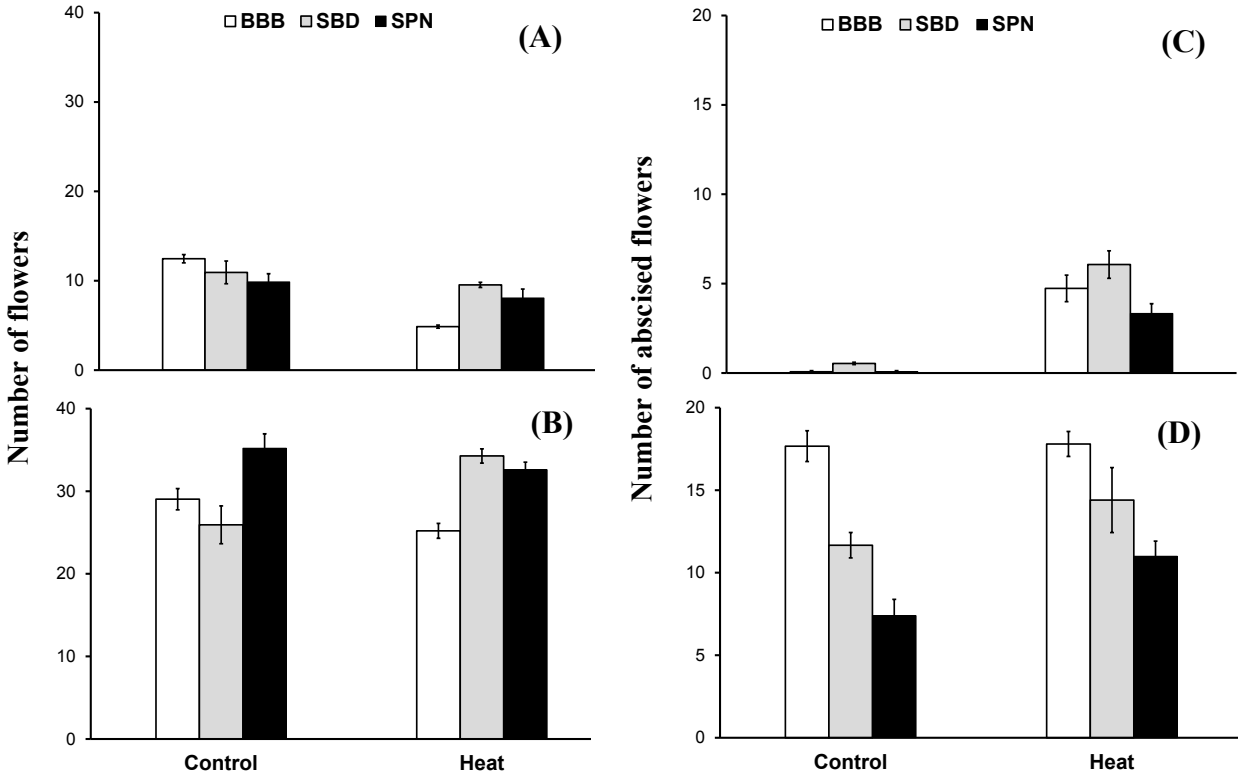


Figure 7.4.3.a Flower number per plant at the flowering (A) and fruiting (B) stages; and number of abscised flowers per plant at the flowering (C) and fruiting (D) stages of three tomato cultivars grown under heat stress and control conditions; values are means (n=15) ± standard error.

Heat x Cultivar x Developmental stage

There was one significant three-way interaction for the numbers of flowers with elongated stigma tubes. Compared with optimum growing conditions, there were significant heat-induced differences in the numbers of flowers with elongated tubes among the three cultivars at both the flowering and fruiting stages: 'Best Boy Bush' (7 and 25.6, compared to none in the control), 'Soprano' (5.9 and 15.9, compared to none in the control) and 'Scoresby Dwarf' (2.2 and 10.4, compared to none in the control) (Table 7.4.3.a, b and Figure 7.4.3.a, b, c).

7.4.3.3 Summary of the key findings

- ❖ Heat stress increased the number of abscised flowers and flowers with elongated stigma tubes, strongly decreased flower and fruit DM and increased flower and fruit PDM.
- ❖ Under the heat treatment, 'Best Boy Bush' reduced the number of flowers and had the highest number of flowers with elongated stigma tubes, whilst 'Scoresby Dwarf' had the lowest.

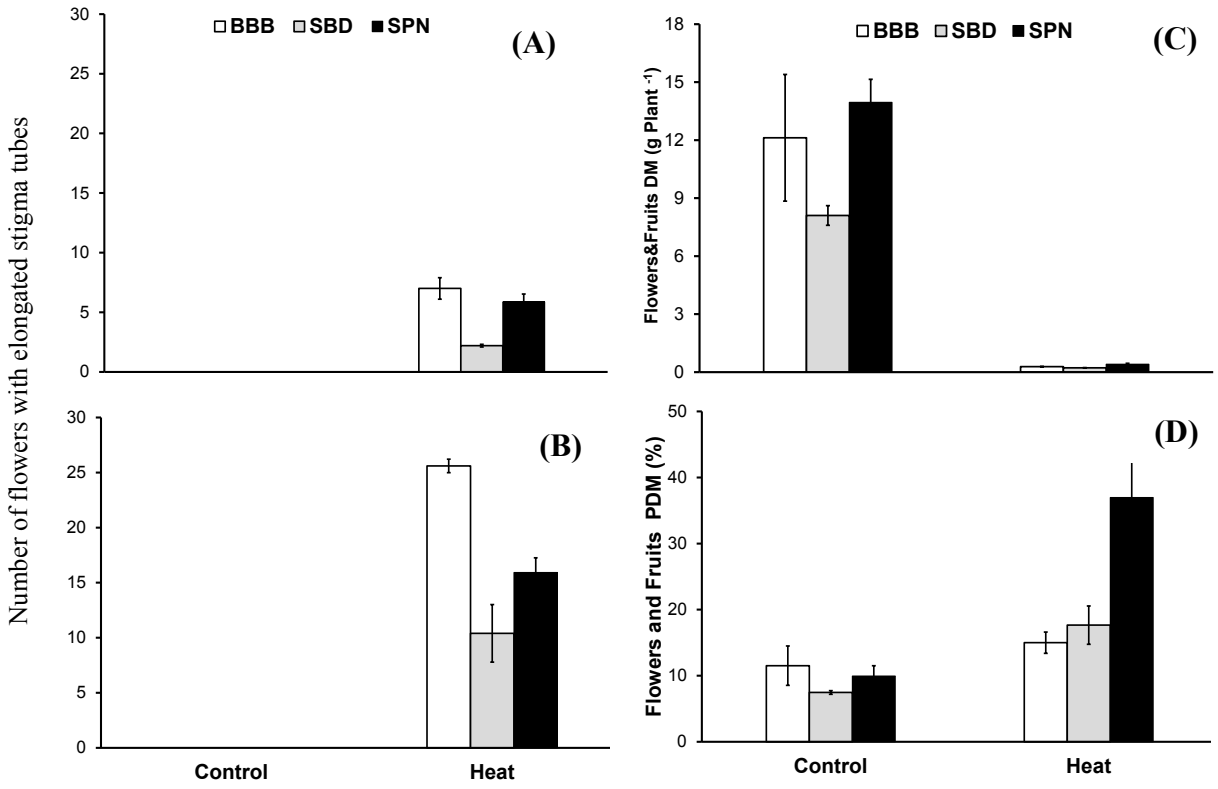


Figure 7.4.3.b Numbers of flowers with elongated stigma tubes at the flowering (A) and fruiting (B) stages; flower and fruit DM at the fruiting stage (C); and PDM of flowers and fruits at the fruiting stage (D) of three tomato cultivars grown under heat stress and control conditions; values are means (n=15) ± standard error.

Figure 7.4.3.c Pictures of tomato flowers from plants grown under control conditions (25°C/20°C) (A) and under heat stress (40°C/30°C) (B). In picture (B) the stigma tube of the flower elongated to exceed the anther cone of the flower.

7.4.4 Chlorophyll fluorescence and SPAD levels

7.4.4.1 Main effects

Averaged across developmental stages and cultivars, heat stress increased SPAD levels by 19% but decreased chlorophyll fluorescence by 2% (Table 7.4.4.a, b and Figure 7.4.4.a).

Table 7.4.4.a Summary of P values of the main effects and interactions with heat treatment on SPAD levels and fluorescence

Traits	Heat	Cultivar	Devel. stage	Heat x Cultivar	Heat x Devel. stage	Heat x Cultivar x Devel. stage
SPAD levels	<.001	0.009	<.001	0.143	<.001	<.001
Chlorophyll fluorescence	<.001	0.018	<.001	0.074	<.001	0.002

Raw data are shown in Appendix G

Compared to 'Scoresby Dwarf', the SPAD level of 'Best Boy Bush' was 4% higher whilst leaf chlorophyll fluorescence for 'Soprano' was 2% lower (Table 7.4.4.a, b and Figure 7.4.4.a).

Table 7.4.4.b Summary of percentage changes of the main effects and interactions with heat treatment on SPAD levels and fluorescence

Traits	Heat	Cultivar	Devel. stage	Heat x Cultivar	Heat x Devel. stage	Heat x Cultivar x Devel. stage
SPAD levels	H: 19%	BBB: 4%	T2: 5% T3: 4%	ns	H T1: 13% H T2: 24% H T3: 21%	H T1 BBB: 9% H T1 SBD: 12% H T1 SPN: 18% H T2 BBB: 24% H T2 SBD: 22% H T2 SPN: 25% H T3 BBB: 25% H T3 SBD: 36% H T3 SPN: 7%
Chlorophyll fluorescence	H: -2%	SPN: -2%	T2: -2% T3: -5%	ns	H T1: 2% H T2: -1% H T3: -5%	H T1 BBB: 2% H T2 BBB: -2% H T3 BBB: -3% H T3 SBD: -6% H T3 SPN: -6%

Heat (H), 'Best Boy Bush' (BBB), 'Scoresby Dwarf' (SBD), 'Soprano' (SPN), developmental stage (T, 1: vegetative, 2: flowering, 3: fruiting), non-significant (ns), fold (x)

Averaged across heat treatments and cultivars and relative to the vegetative stage, SPAD levels increased by 5% at flowering and by 4% at fruiting, whilst leaf chlorophyll fluorescence decreased by 2% at flowering and by 5% fruiting (Table 7.4.4.a, b and Figure 7.4.4.a).

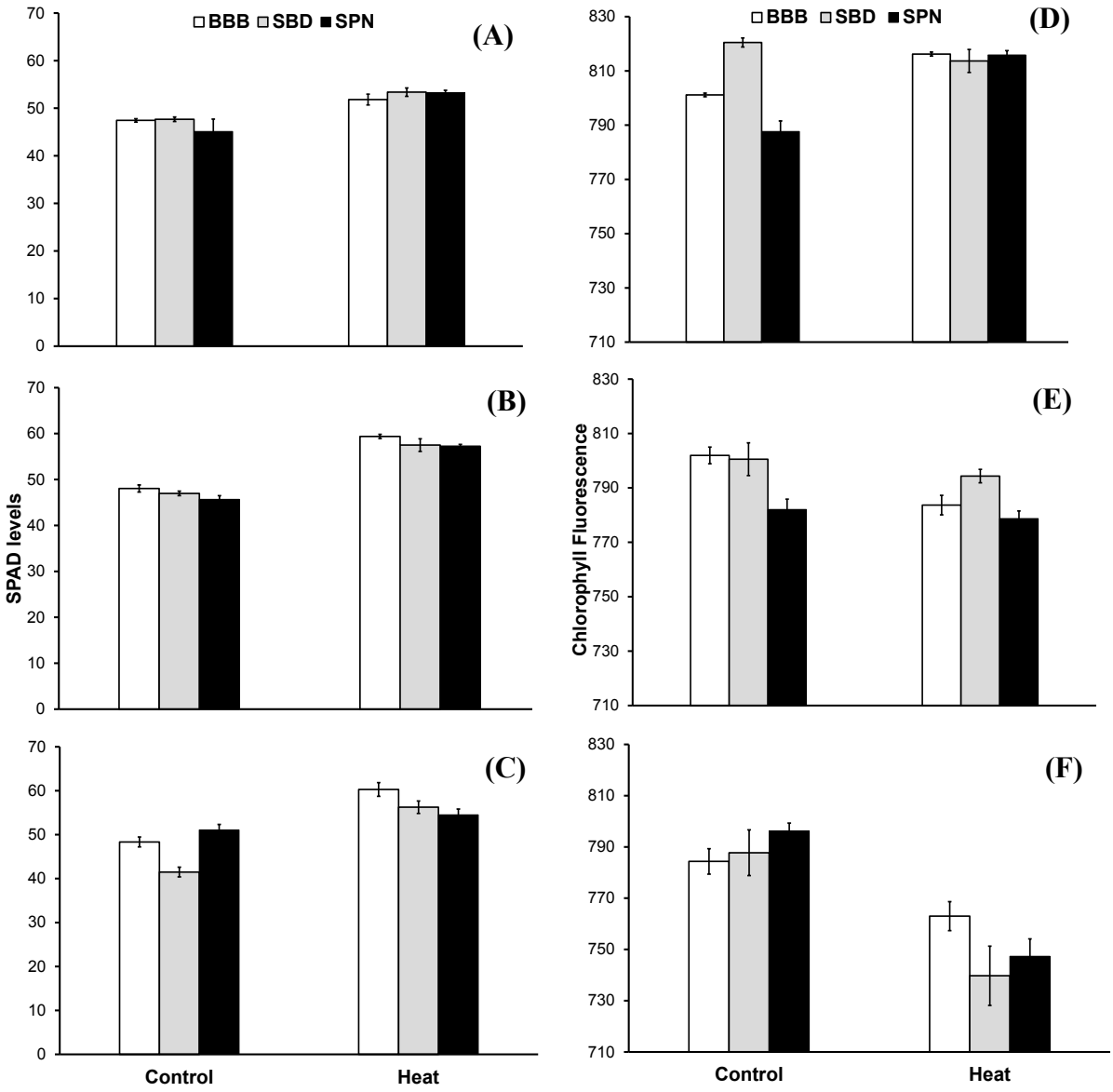


Figure 7.4.4.a SPAD levels at the vegetative (A), flowering (B), fruiting (C) stages; and leaf chlorophyll fluorescence at the vegetative (D), flowering (E) and fruiting (F) stages of three tomato cultivars grown under heat stress and control conditions; values are means (n=15) ± standard error.

7.4.4.2 Interaction effects

Heat x Developmental stage

Averaged across cultivars, heat increased SPAD levels during plant development from 13% at the vegetative stage to 21%-24% at flowering/fruiting (Table 7.4.4.a, b and Figure 7.4.4.a). Chlorophyll fluorescence, on the other hand, decreased from 2% at the vegetative stage to -5% at fruiting.

Heat x Cultivar x Developmental stage

Heat stress increased SPAD levels throughout plant development in 'Best Boy Bush' (9-25%) and 'Scoresby Dwarf' (12-36%), whilst SPAD levels peaked at the flowering stage in 'Soprano' (25%). Leaf chlorophyll fluorescence, on the other hand, decreased from the vegetative (2%) to the fruiting stage (-3%) in 'Best Boy Bush'. Reductions of chlorophyll fluorescence by 6% were also found in 'Scoresby Dwarf' and 'Soprano' at the fruiting stage.

7.4.4.3 Summary of the key findings

- ❖ Heat increased SPAD levels in all three cultivars but more and progressively with plant development in 'Scoresby Dwarf' relative to 'Best Boy Bush' and 'Soprano'.
- ❖ Chlorophyll fluorescence was slightly reduced at the fruiting stage in all three cultivars.

Plate 7. Tomato plants grown in the heat stress chamber

7.5 Multivariate traits response to the principal component analysis (PCA)

7.5.1 Plant traits response to the effects of heat stress

7.5.1.1 Plant trait responses to heat stress in the first principal component

The first principal component (PC1) explained 33% of the variance in the dataset (Figure 7.5.1.a). PC1 characterised the heat responses on the basis of vegetative attributes, with growth traits (plant height, DM and PDM) associating at the positive end of PC1 and high scores for leaf angle changes as well as in the number of leaves and of nodes at the negative PC1 end.

7.5.1.2 Cultivar responses to heat stress in the first principal component

'Soprano' responses showed positive scores on PC1, whilst 'Scoresby Dwarf' scores were associated with the negative end of PC1 and ‘Best Boy Bush’ responses were intermediate between these two positions (Figure 7.5.1.a).

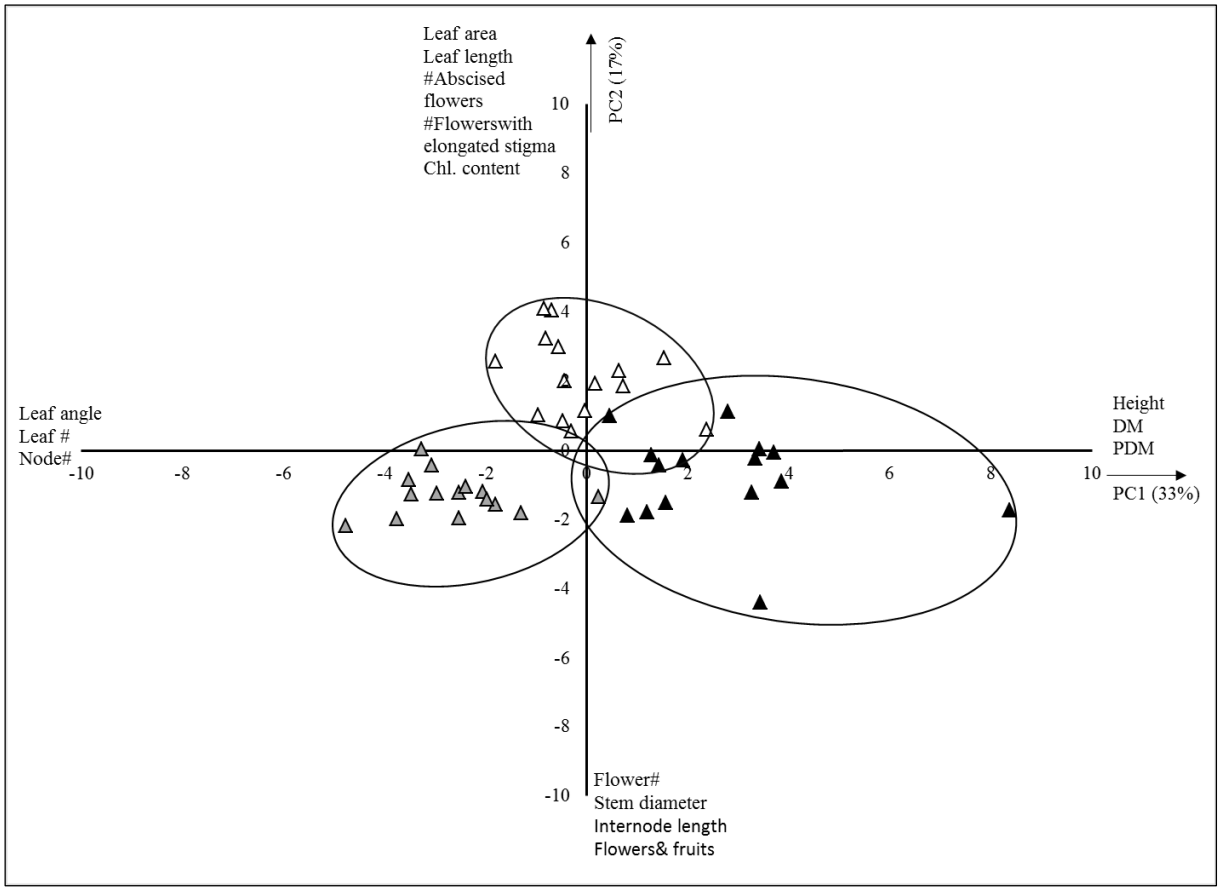


Figure 7.5.1.a Biplot of heat treatment PCA scores of three tomato cultivars, 'Best Boy Bush', 'Scoresby Dwarf' and 'Soprano' at the final harvest (fruiting stage). Symbols: 'Best Boy Bush' (△), 'Scoresby Dwarf' (◬) and 'Soprano' (▲).

7.5.1.3 Plant trait responses to heat stress in the second principal component

The second principal component (PC2) accounted for 17% of all the variation in the dataset (Figure 7.5.1.a). PC2 contained the reproductive responses, with high positive PC2 scores for heat damage characteristics in flowers (numbers of abscised flowers and of flowers with elongated stigma), and flower number and flower and fruit PDM on the negative end. PC2 also associated with the remaining vegetative and physiological responses in the dataset.

7.5.1.4 Cultivar responses to heat stress in the second principal component

PC2 separated 'Best Boy Bush' responses from the other cultivars. 'Best Boy Bush' had positive PC2 scores and 'Scoresby Dwarf' and 'Soprano' predominantly negative scores. (Figure 7.5.1.a).

7.6 Discussion

Most heat stress studies in tomato have, thus far, been conducted at temperatures below 40°C, and for relatively short periods of time (e.g. Sato et al., 2000; Wahid et al., 2007). To the best of author's knowledge, there has been no research in this crop where the day/night temperatures were elevated to 40/30°C and conducted during the period from vegetative development through to fruit harvesting.

7.6.1 Plant growth and morphology

Several growth-related traits were altered by heat stress in this study, including reductions in leaf length, leaf area and leaf angle (Figure 7.4.1.c). Although high temperature stress is known to cause reductions in plant growth and thereby a reduction in crop yield (Wahid et al., 2007), thus far there had been little information with regard to responses in these growth traits in tomato plants subjected to high temperature stress. Previous studies reported a reduction in plant height when tomatoes were grown under temperature stress (Heuvelink, 1989; Morgan & Clarke, 1975). However, in this study, the plant height of all three cultivars was not altered by heat stress (Figure 7.4.1.a). EL-Abd et al. (1999) suggested that a reduction in plant height can only observed in heat-sensitive cultivars. In this study heat stress also had very little effect on the numbers of leaves and nodes. Heuvelink (1989) reported a slight reduction in numbers of leaves under heat stress, but other findings have reported increased leaf numbers under heat stress (El-Abd et al., 1999). The heat-induced reduction in leaf area observed in the present study has also been reported by Heuvelink (1989). It has been suggested that a smaller leaf area is associated with higher fruit yields in heat tolerance genotypes (Lu et al., 1994). In this study, the stress tolerant cultivar, 'Scoresby Dwarf' had the smallest leaf area relative to 'Best Boy Bush' and 'Soprano' (Figure 7.4.1.d). Lu et al. (1994) suggested that a smaller leaf area under high temperature stress is an advantage due to its smaller boundary layer and higher heat dissipation. The reduction of leaf length in this study was observed at later developmental stages, suggesting that leaf growth was only compromised by heat stress when carbon flow was diverted towards fruit development.

Plants can avoid damaging and excessive light levels by preventing light absorption through decreasing the amount of chlorophyll pigments in cells and diverting some of the absorbed light energy from photochemistry towards other processes (Chaves et al., 2003). Adjusting the leaf angle is another heat stress prevention strategy. A steeper leaf angle, or as it is termed 'paraheliotropism', mainly occurs if there is inadequate water supply to a plant suffering under

heat stress, and changes in leaf angle are part of a leaf's cooling system (Chaves et al., 2003). In this present study, the leaf angle was increased throughout plant development (i.e. leaf orientation became more horizontal) and it decreased in response to heat treatment (i.e. the leaf orientation became more vertical) (Figure 7.4.1.c). PC1 in the PCA indicated a relationship between heat-induced changes in leaf angle and plant DM responses (Figure 7.5.2.a). In this, reductions in leaf angle (more vertically arranged leaves) were associated with maintenance of growth parameters. The more vertical leaf position is likely to reduce heat stress effects by turning the leaf away from exposure to direct light and air movement which both contribute to transpiration via light-induced stomatal opening and reduction of the boundary layer resistance via air movements. The fact that 'Scoresby Dwarf' showed lower heat-induced leaf angle reductions could suggest higher heat stress tolerance in this cultivar.

There are not many plant species that possess effective leaf cooling systems capable of cooling their leaves to lower than air temperature under heat stress (Chaves et al., 2003). Heat tolerant plants have an enhanced capacity for leaf cooling (Lu et al., 1994; Salvucci & Crafts-Brandner, 2004). Camejo et al. (2006) reported that leaf temperature in tomato plants subjected to heat shock treatment can be reduced by 5°C, and 20-30% of the observed heat energy can be dissipated via transpiration. This heat dissipation is the result of an efficient transpiration system, even when stomatal conductance is not altered. Plants grown under high temperatures and provided with an adequate supply of water may keep their stomata open, thereby creating an evapotranspiration cooling effect to reduce leaf temperatures. The efficiency of this cooling system can be decreased with either a high relative humidity or a reduction in transpiration rate under a water deficit (Salvucci & Crafts-Brandner, 2004).

7.6.2 Plant dry matter traits

The effect of heat stress on the biomass of tomato plants remains equivocal. Some researchers have reported a significant reduction in leaf DM and plant DM (Heuvelink, 1989), whereas others have found that stem DM and plant DM can be increased by heat (Morgan & Clarke, 1975). In this research, stem DM was not affected by heat stress, whilst leaf DM and also PDM were increased by heat stress (Figure 7.4.2.a). The increase in the dry matter of leaves could be due to an alteration of leaf structure: heat-induced reductions in the expansion of leaf cells results in leaves that are more compact and have reduced leaf area, while the leaf itself is thicker and denser (Calcagno et al., 2011). Similarly, the present study found heat-induced decreases in leaf area, concomitant with leaf DM increases (Table 7.4.1.b). The latter occurred particularly in the cultivar 'Soprano', suggesting heat tolerance in this cultivar. Heat-induced reductions in

shoot growth are due to a severe reduction in internode length (Wahid et al., 2007). In the present study, heat stress did not cause any significant change to internode lengths, which explains the unchanged stem DM under heat stress.

7.6.3 Plant reproductive traits

Tomato plants that have become reproductive might be more susceptible to elevated heat treatments in comparison with plants that are physiologically less mature. Tomato plant reproductive components such as flowers and fruits can be significantly affected by high temperature stress (Saeed et al., 2007; Sato et al., 2000). In the present study, heat stress decreased flower numbers only in 'Best Boy Bush', indicating intraspecific differences in heat stress sensitivity in tomato plants (Figure 7.4.3.a). Flower abscission was markedly higher under heat stress (Figure 7.4.3.b). A consequence of increased flower abscission was a significant reduction in fruit set, as expressed by the large decreases in flower and fruit DM in this study (Figure 7.4.3.a), which is in line with other findings (Abdul-Baki, 1991; Lohar & Peat, 1998; Rudich et al., 1997). Dinar and Rudich (1985) suggested that this could be caused by heat-induced carbohydrate stress in the earliest stages of flower development. Heat-induced reductions in carbohydrate supply and carbon import by flower buds was closely associated with the plant's ability to set fruit (Dinar & Rudich, 1985). The sensitivity of 'Best Boy Bush' to heat-induced changes in reproductive components was also revealed in the PCA. PC2 proved useful in separating cultivar responses on the basis of a multivariate trait combination that included reproductive attributes (Figure 7.5.2.a). For example, compared to the other cultivars, PC2 associated 'Best Boy Bush' with the stronger increases in reproductive damage characteristics such as the number of flowers with elongated stigma tubes, which came at a cost for other reproductive features, exemplified by heat-induced reductions in flower numbers in this cultivar. This suggests a trade-off in reproductive growth, where a heat-induced over-investment into style formation in individual flowers comes at a cost for the production of total flower number. In contrast, 'Scoresby Dwarf' had the lowest number of flowers with heat-induced stigma tube elongation (Figure 7.4.3.b).

7.6.4 Chlorophyll fluorescence and SPAD levels

Environmental stress, including high temperature stress, can limit a plant's ability to utilise light energy. It has been suggested that the photochemical reactions in the thylakoid lamellae and carbon metabolism in the stroma of chloroplasts are the primary site of injury under high temperature stress (Wahid et al. 2007). Several studies have reported modifications in chlorophyll content and chlorophyll fluorescence when plants were subjected to high

temperatures. Alterations of chlorophyll fluorescence reflect changes in photochemical efficiency of photosystem II (PSII) (Camejo et al., 2005). Decreases of chlorophyll fluorescence suggest that the PSII reaction centre has been damaged and the leaf mesophyll tissues have been affected by heat stress. However, Camejo et al. (2006) reported that heat stress only induced an alteration of chlorophyll fluorescence in some tomato cultivars. In this study, only slight reductions in chlorophyll fluorescence were observed in all three cultivars at later stages of plant development (Figure 7.4.4.a). The subtle nature of these changes shows that photochemical efficiency was not strongly affected in this study.

Camejo et al. (2005) and Kang et al. (2009) observed a redcution in chlorophyll content when tomato plants were subjected to heat stress. In contrast, chlorophyll content (SPAD levels) increased in response to heat stress, with the greatest rise in 'Scoresby Dwarf' (Figure 7.4.4.a). This can be explained by the higher density of leaves under heat stress discussed above, resulting in a concentration effect: heat-induced reductions in plant cell volumes and relative maintenance of chlorophyll production effectively concentrates the pigmentation of the cell and increases the ‘green-ness’ of the smaller leaves under heat stress.

7.6.5 Conclusions

Heat stress resulted in changes of leaf morphology, decreasing leaf area but not leaf weight, thus resulting in a more compact leaves that were more vertically arranged to withstand heat stress. Stem-based parameters such as internode length, node numbers and stem diameter were not altered by heat stress. Compared to the vegetative morphological responses, reproductive development was particularly strongly affected by heat stress, resulting in pronounced reductions in flower DM, flower abscission and stigma tube elongation. This was most pronounced in ‘Best Boy Bush’ and related to the reduction of flower numbers in this cultivar, providing evidence of intraspecific differences in stress tolerance also under elevated temperature stress in tomato.

Chapter 8
General Discussion and Conclusion

Environmental stress is of increasing concern worldwide. Among environmental stress factors, water supply and temperature stress pose the greatest threats to global crop production. Limitations in the availability of water are responsible for about 50% reductions of global production (Mahajan & Tuteja, 2005), whereas flooding and waterlogging affect approximately 13% of the land area and 16% of the production area worldwide (Ahsan et al., 2007; Cramer et al., 2011). In the field, drought is closely associated with heat stress and together, drought and heat stress affect about 64% of the global land area (Cramer et al., 2011). This situation is likely to worsen in the future under global climate change which is also predicted to increase the incidence of extreme climatic events such as extremes in water supply to plants. The understanding of how plants tolerate these adverse environmental conditions is therefore of crucial importance for sustainable agricultural production into the future.

The studies reported here examined various responses of tomato plants to the two water stress extremes of drought and waterlogging, as well as to elevated heat stress in the form of a temperature regime of 40/30°C for day and night temperatures. This chapter discusses the similarities and differences in plant responses recorded in the previous five chapters.

Generally, both heat stress and water stress caused significant reductions in plant growth and typical morphological attributes. These included lower numbers of leaves, shorter leaf lengths, smaller leaf areas and lower levels in dry matter traits (Figure 8.a) and are in line with previous reports of plant responses to heat stress and water stress (e.g: Mishra et al., 2011; Selim & El-Nady, 2011; Wahid et al., 2007). Mahajan and Tuteja (2005) suggested that a reduction in plant growth under drought stress can be caused by the decreasing activity of cyclin-dependent kinases. The kinase proteins are responsible for the cell cycle and a reduction of the activity of these proteins results in slower cell division and, eventually, termination of growth (Mahajan & Tuteja, 2005). In addition, a reduction of leaf gas exchange, particularly photosynthesis as observed in the water stress studies, may also contribute to the inhibition of plant growth due to lower carbohydrate supply.

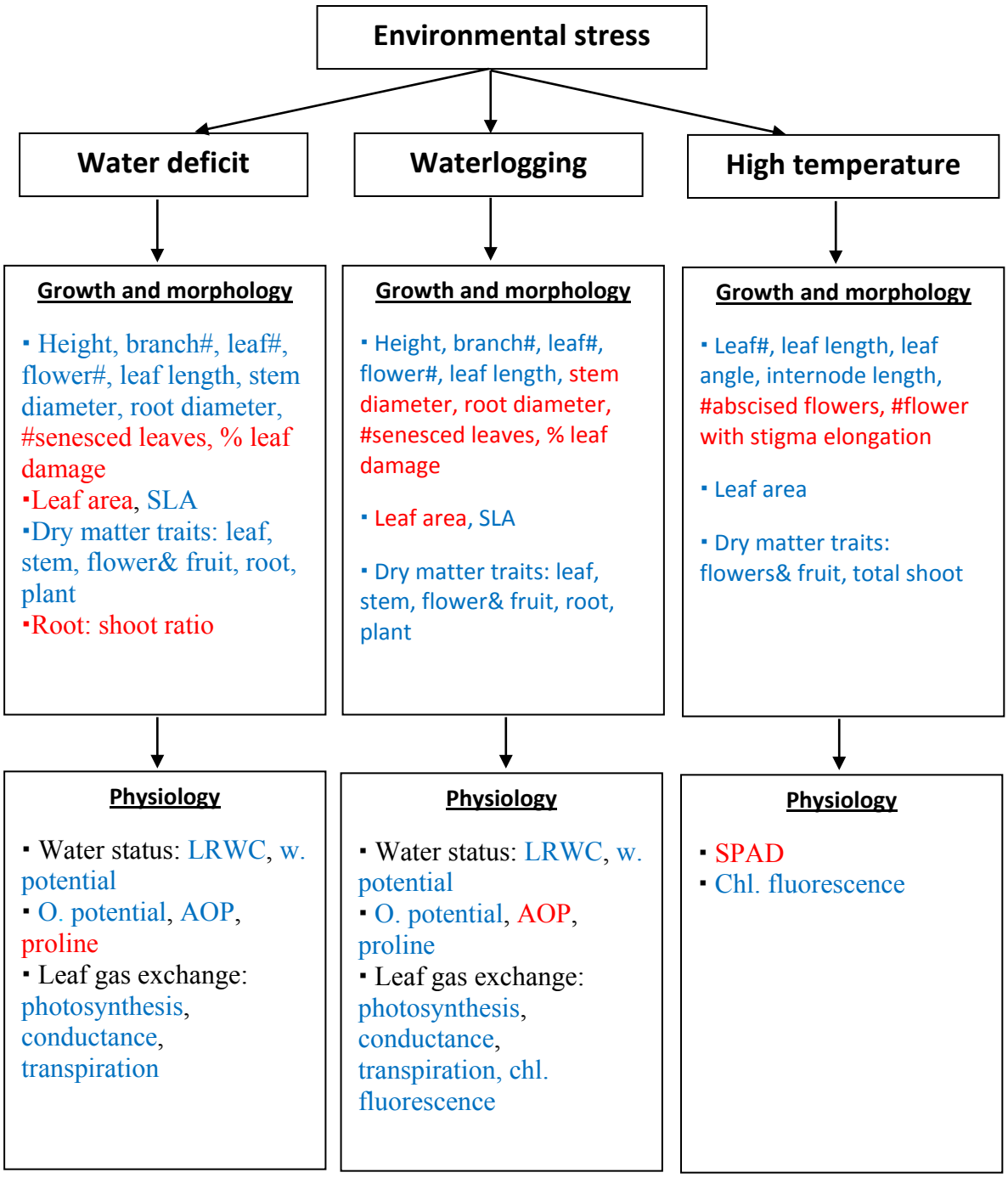


Figure 8.a Summary of the significant findings for the response to water deficit, waterlogging and high temperature on tomato morphological and physiological traits. Changes in plant traits are marked: blue font, reduction and red font, increase.

The reduction in plant height for plants subjected to water stress was not found in plants subjected to a high temperature (Figure 8.a). A difference in stem diameter, as a stress response, was also found in plants subjected to drought stress and there was an increase in diameters of the stems of waterlogged plants (Figure 8.a). There was no difference in stem diameter for plants subjected to high temperature stress relative to the control plants. It is possible that the increase in stem diameter for waterlogged plants was a result of lateral meristem activity and the noticeable formation of additional adventitious roots.

Heat stressed plants experienced reductions in leaf angle, high numbers of abscised flowers and of flowers with stigma tube elongation. Such changes were not observed under water stress. The more vertical leaf arrangement is likely to decrease transpirational water loss (Chaves et al., 2003). The increase in flower abscission can be attributed to a lack of ovule fertilization, which can be caused by stigma tube elongation (Levy et al., 1978). Furthermore under conditions of extreme heat there are likely to be floral meristem malformations.
Plants can respond to water deficit with leaf area adjustment in the form of accelerated senescence and abscission of older leaves as a common water saving method (Chaves et al., 2003; Mahajan & Tuteja, 2005). In this study, a greater number of senesced leaves was observed in plants subjected to both water deficit and waterlogging.

Water deficit and waterlogging caused similar physiological responses both in the glasshouse and under field conditions (Figure 8.a). These similarities included reductions in plant water status, as measured by leaf relative water content, leaf water potential, and leaf gas exchange including photosynthesis, stomatal conductance and transpiration. Aloni and Rosenshtein (1982) suggested that waterlogging induces a water deficit in the rhizophere despite plants being completely submerged in water. This suggestion might explain the similarities in terms of the physiological responses of these two stress factors. However, there was evidence from the study here (glasshouse and field trials) that osmotic adjustment occurred only in plants grown under drought stress, together with significant increases in proline levels. The reduction in the adjusted osmotic potential was not observed in plants experiencing waterlogging (and therefore there was no osmotic adjustment), despite a reduction in osmotic potential. This increase in cellular solute concentration was therefore merely a result of water loss from cells and was not caused by the active production of compatible solutes as would be the case under osmotic adjustment. In addition, proline levels in hypoxic leaf and root tissues all significantly decreased in both the end-point glasshouse study and in the kinetic investigations in the field trial.

The overall similarities and differences of the oxidative biochemical responses between water deficit and waterlogging have already been discussed in Chapters 5 and 6 using PCA biplots and heatmaps. The relationships of the biochemical attributes to morphological and physiological traits have also been presented in Chapter 5 (Figure 5.5.3.a) and Chapter 6 (Figure 6.5.3.a) and are discussed here as they relate to all water stress chapters in this thesis.

The first principal component of the overall glasshouse PCA (Figure 5.5.3.a) demonstrated that the main drivers for treatment, cultivar and developmental differences in the dataset were attributes linked to oxidative metabolism, namely, oxidative damage parameters that were countered by enzyme antioxidant activities. This showed (i) more pronounced oxidative damage under waterlogging than under drought, (ii) higher oxidative stress tolerance for 'Scoresby Dwarf' than for 'Best Boy Bush' under both water stress extremes, and (iii) more pronounced oxidative damage with increasing plant development, especially under waterlogging. It was of particular interest to note the close relationship of stress-protective antioxidant enzyme activity with osmotic potential in PC1. The findings suggest the value of the latter trait as a convenient measure of oxidative stress tolerance in glasshouse-grown tomato plants. Recent findings have suggested that plant tolerance against oxidative stress is associated with high enzymatic antioxidant activity (Hameed et al., 2014) and high levels of reduced ascorbate, GSH and proline (Hossain et al., 2014). The glasshouse study supported the hypothesis that 'Scoresby Dwarf' is more tolerant to oxidative stress than 'Best Boy Bush'.
The second principal component separated the plant developmental stages under drought (Figure 5.5.3.a), showing that tomato plants were most affected by water limitation at the vegetative stage, with pronounced reductions in plant growth attributes, plant water status and leaf gas exchange. It also demonstrated that protective responses against these effects included higher root:shoot ratios and GSH accumulation. Foyer and Noctor (2005) suggested that root growth was predominantly controlled by reactive oxygen species and glutathione. These authors also reported that cell division of the root meristem was regulated by GSH, which could explain the linkage between high levels of GSH accumulation and root: shoot ratios in PC2.

The overall PCA of the field experimental data revealed a clear distinction in the key attributes of drought-exposed versus waterlogged plants (Figure 6.5.3.a). PC1 was the main multivariate measure of drought attributes, while PC2 provided the main multivariate waterlogging dimension. PC1, the ‘drought axis’, was characterised by biochemical and morpho-physiological attributes. The latter comprised the expected low levels of plant water status and gas exchange, together with the expected high levels of leaf damage and senescence in drought-

exposed plants. The biochemical component of PC1 clearly distinguished the high enzymatic antioxidant levels in drought-exposed plants as the key antioxidant response to oxidative stress, signified by high levels of oxidised antioxidants (DHA and GSSG). High levels of DHA and GSSG in plants indicate high levels of intercellular ROS accumulation and growth restriction (Foyer & Noctor, 2011). On the other hand, 'the waterlogging axis' in PC2 pointed to the accumulation of the oxidative stress markers H_2O_2, LOOHs and PCs, as the main symptoms of damage under hypoxia and that high levels of these were, in particular, due to the decreases in the reduced forms of ascorbate and glutathione under waterlogging. Both ascorbate and glutathione are highly abundant in their reduced forms under control conditions. Reduced glutathione, GSH is known to control shoot and root apical meristem development (Foyer & Noctor, 2011), whereas ascorbate is closely associated with photosynthesis and exposure to light (Foyer & Noctor, 2005). Therefore, maintaining the redox states of both ascorbate and glutathione, as demonstrated by 'Scoresby Dwarf' under water stress in the glasshouse studies, is essential for plant stress resistance via ROS scavenging and via their roles as indispensable co-factors of enzymes.

8.1 Conclusions

The key findings, conclusions and recommendations from this thesis can be summarised as follows. The experimental approach of exposing plants to water stress extremes in a concurrent layout allowed the direct comparison of intraspecific drought and waterlogging effects, as all other environmental variables were kept the same, such as variations in light and temperature. Multiple comparison procedures, including principal component analysis and heatmap clustering analysis provided an overall picture for the various biochemical responses under stress and showed clear patterns of stress effects and of protective responses.

Both stress factors induced the accumulation of reactive oxygen species (exemplified by H_2O_2) in both leaf and root tissues, showing most pronounced effects under waterlogging, with membrane damage in the form of increased levels of lipid hydroperoxides, protein oxidation as shown by the accumulation of protein carbonyls, as well as DNA oxidation. This oxidative damage was countered by both enzymatic and non-enzymatic antioxidants. However, enzymatic antioxidants such as SOD, CAT, APOX, GR and GPOX increased their activities in both the leaf and root tissues of plants subjected to water deficit, whilst these enzymes plateaued or decreased in activity under waterlogging. These findings clearly highlight a specific role of enzymatic antioxidants in water stress protection. Future studies could substantiate whether longer waterlogging periods under outdoor conditions would also result in decreases of antioxidant enzyme activities in the field, as was evident in the glasshouse.

Non-enzymatic antioxidant levels increased in both leaf and root tissues of plants subjected to water deficit, whilst levels of the reduced forms of ascorbate and glutathione decreased in tissues of hypoxic plants. This is important, because the reduced forms of ascorbate and glutathione are crucial for plant survival under oxidative stress as radical scavengers. For instance, reduced ascorbate can remove many forms of ROS including $^1O_2$, $O_2{\cdot}^-$, OH^- and also lipid hydroperoxides. Both reduced ascorbate and glutathione also have an indispensable role as co-enzyme factors. Therefore under water logging, enzymatic antioxidants such APOX, GPOX and GLOX1 are unable to catalyse ROS or MG in the absence of these co-factors.

There were significant cultivar differences in these responses under glasshouse conditions, with lower levels of oxidative damage and increased protective responses of the antioxidant apparatus in ‘Scoresby Dwarf’ under both stress factors. This suggests merit for studies that investigate these stress responses across a wide range of tomato germplasm for future plant improvement. The identification of key compounds associated with intraspecific differences in

oxidative stress responsiveness can be used as markers by plant breeders for the generation of new cultivars with improved stress resistance in the face of global change.

Similar to ROS, methylglyoxal is highly toxic to plant cells when it accumulates at excess levels, as was found in this present study after prolonged water stress. This suggests the value of MG as a useful alternative to ROS as a measure of oxidative stress damage. The activities of GLOX1 and GLOX2 increased in parallel with rising MG, suggesting an effective detoxification system. Further study on the role of glyoxalase enzymes in plant water stress tolerance is recommended, as these enzymes can contribute to the recycling of the glutathione pool.

Alcohol dehydrogenase activity increased strongly under hypoxia and the activity of this enzyme was further enhanced with prolonged stress. This expected effect demonstrated that the respiratory system was inhibited under waterlogging conditions. Future stress studies in plans should also measure the activity of alternative oxidase as a measure of hypoxic stress, because it provides an alternative route for electron transport that diverges from the main respiratory pathway under waterlogging.

Oxidative stress decreased the viability of Caco-2 cells, and this was improved when cells were pre-treated with digests from tomato fruits that had been exposed to drought stress, but not in fruits of plants that had experienced waterlogging. This protective outcome on Caco-2 cells might be attributed to the accumulation of ascorbic acid and of total antioxidant capacity in tomato fruits grown under drought stress. The findings strongly suggest merit for the application of a targeted drought period before harvesting tomato fruits to boost the production of phytochemicals of benefit for human health. Future studies should therefore investigate the importance of timing and of frequency of targeted drought application for enhanced antioxidant production in tomato.

Most of the osmotic adjustment occurred when plants were subjected to water deficit, but not under waterlogging. Evidence for this was seen in the reduction of adjusted osmotic potential and increases of proline content in leaf and root tissues under water deficit. An inverse picture was seen in waterlogged plants and there were some concerns that plant roots were already damaged under hypoxic conditions. In addition, the findings from overall multiple comparison analysis suggest value for using osmotic potential as a convenient measure of oxidative stress tolerance.

The final thesis chapter was the first long term study that examined extreme day and night temperatures in tomato. The three cultivars did not set fruit when temperatures were as high as 40/30°C (day/night). Such temperatures are common in tropical areas and are likely to increase

under climate change. ‘Scoresby Dwarf’ showed tolerance to heat stress compared to 'Best Boy Bush'. This further supports the view of utilizing germplasm differences in future breeding programmes to develop heat-resistant tomato cultivars.

Appendix A

Raw data of the water stress experiment in the glasshouse

In the attached CD

Appendix B

Raw data of the water stress experiment in the field

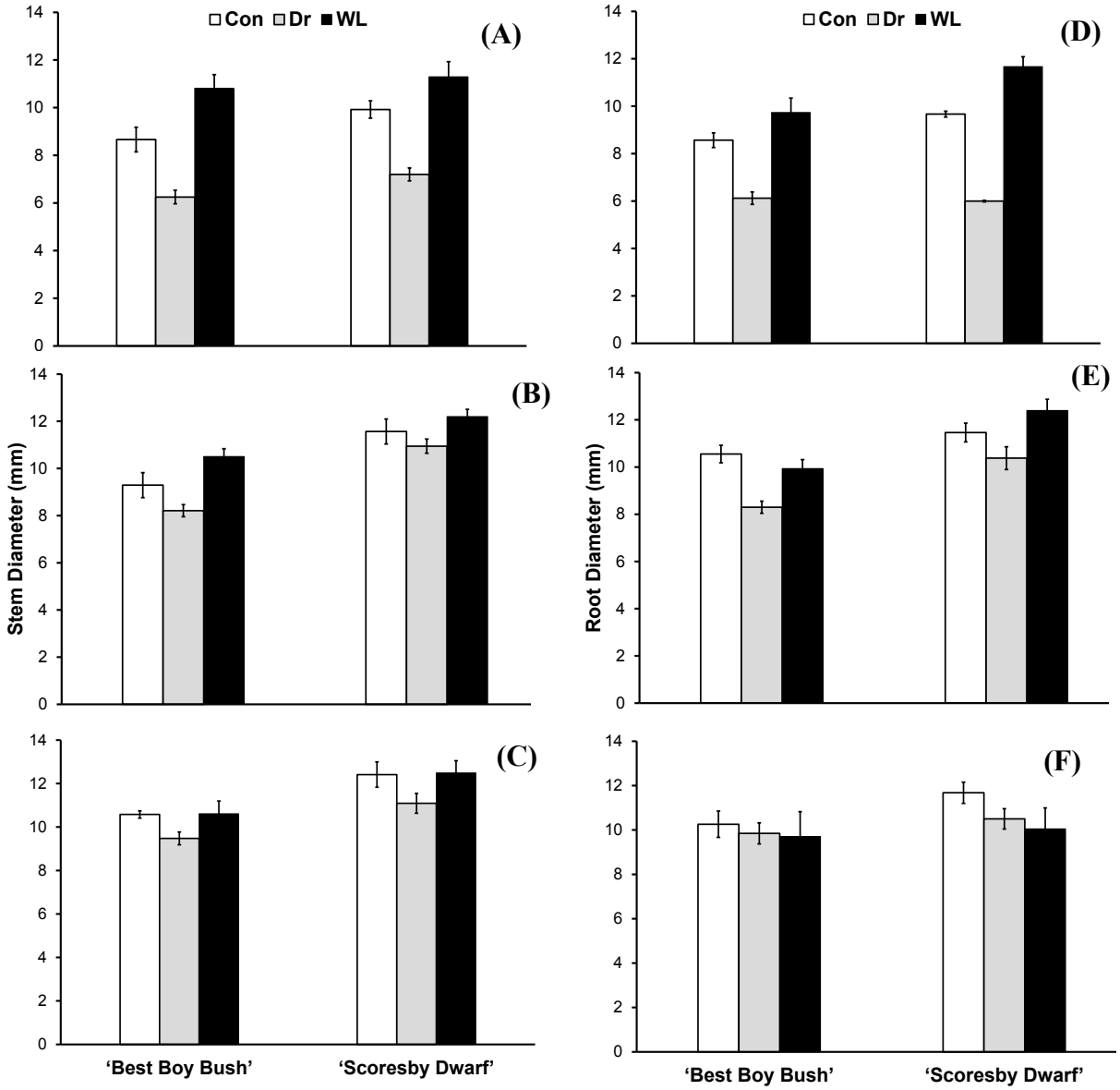
In the attached CD

Appendix C

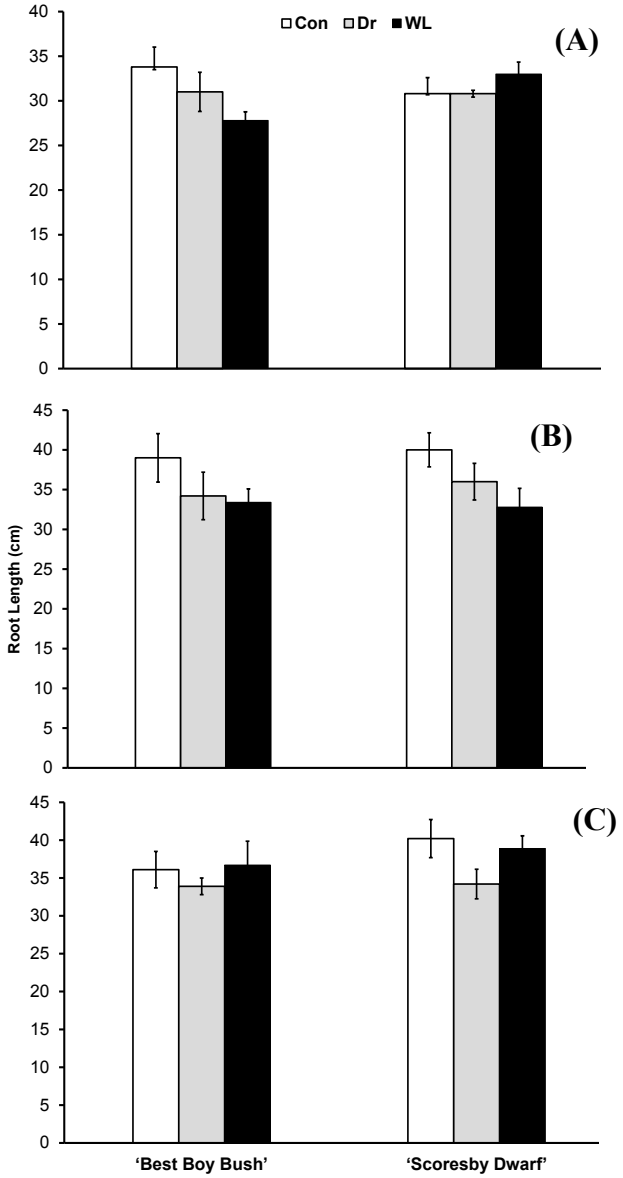
Supplementary information for Chapter 3

C.1 Glasshouse experiment

C.1.1 Plant growth and morphology

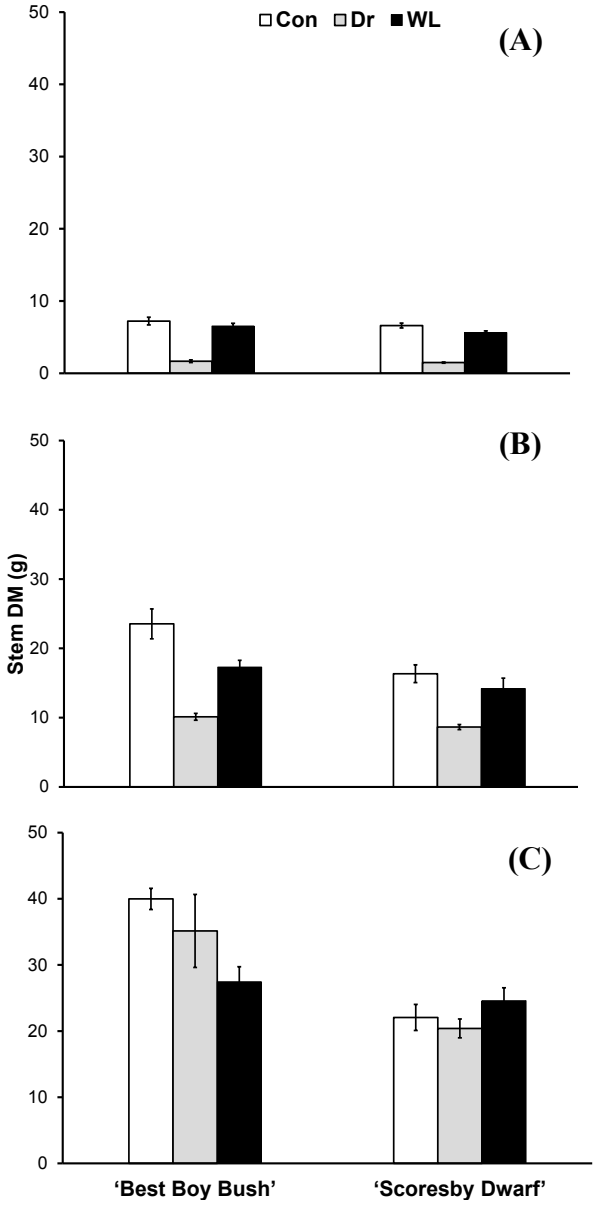


Stem diameter at the vegetative (A), flowering (B), fruiting (C) stages and root diameter at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-water control conditions (Con), drought (Dr) and waterlogging (WL); values are mean (n=5) ± standard error.

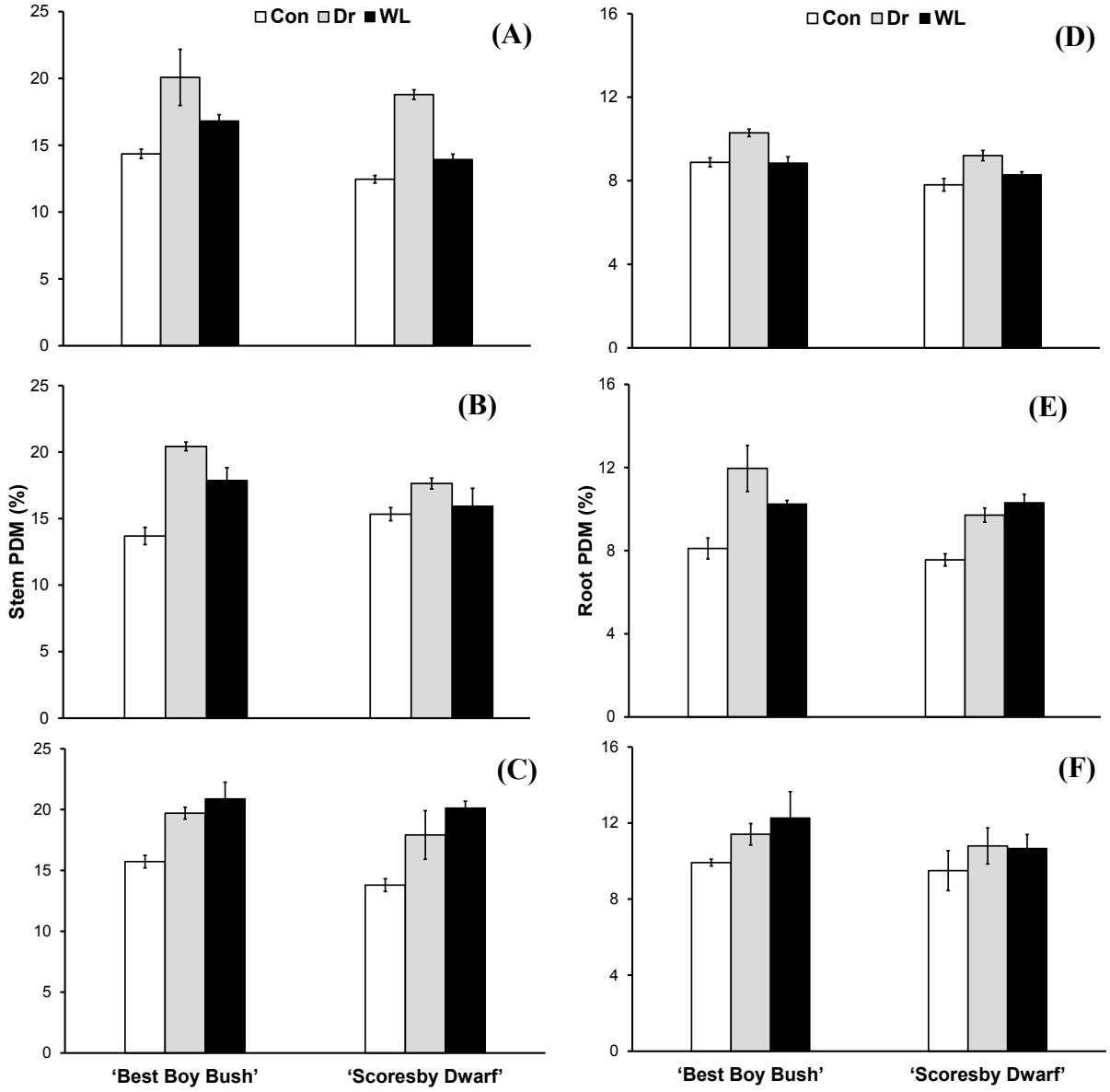


Root length at the vegetative (A), flowering (B), fruiting (C) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

C.1.2 Plant dry matter traits



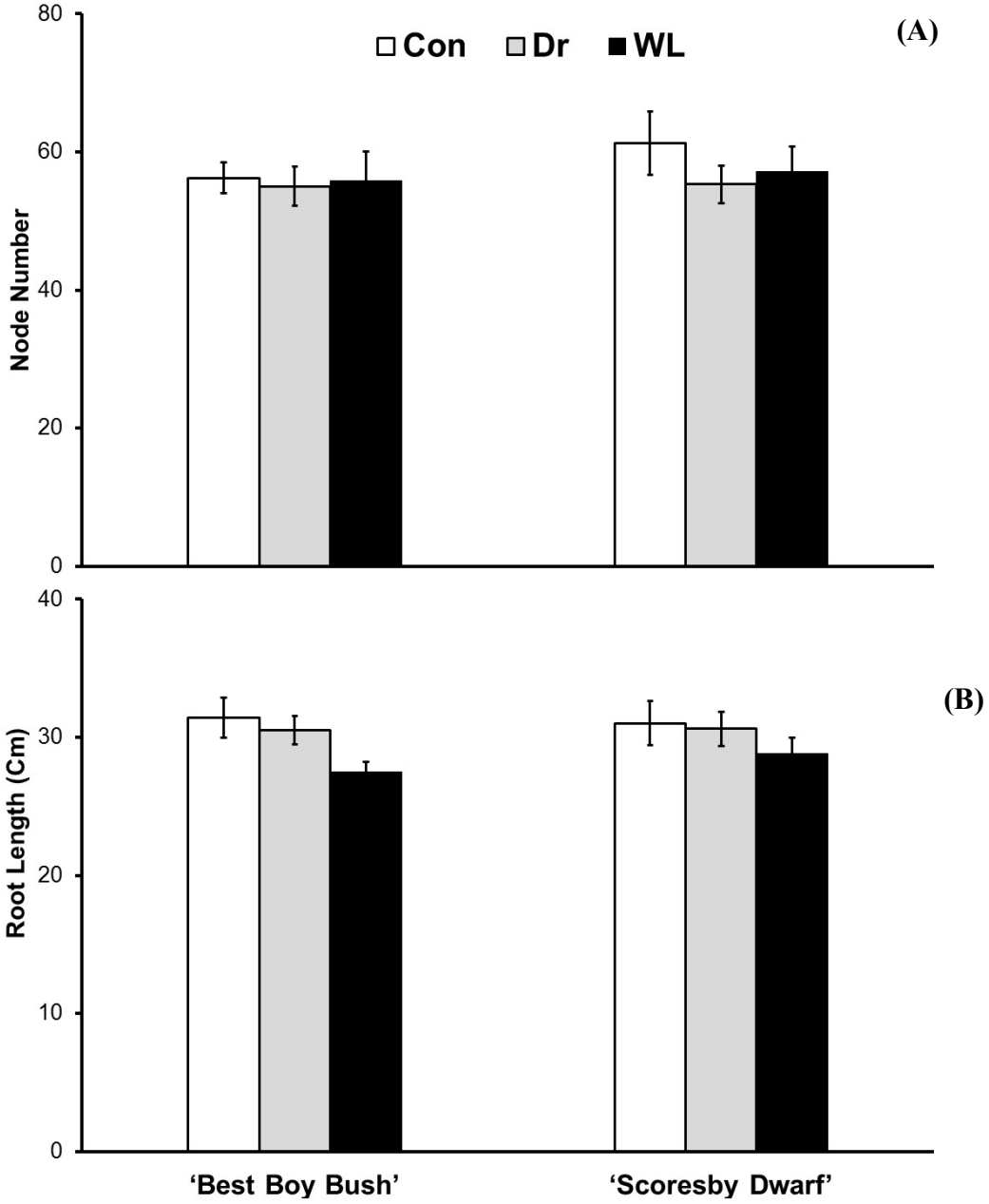
Stem dry matter at the vegetative (A), flowering (B), fruiting (C) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.



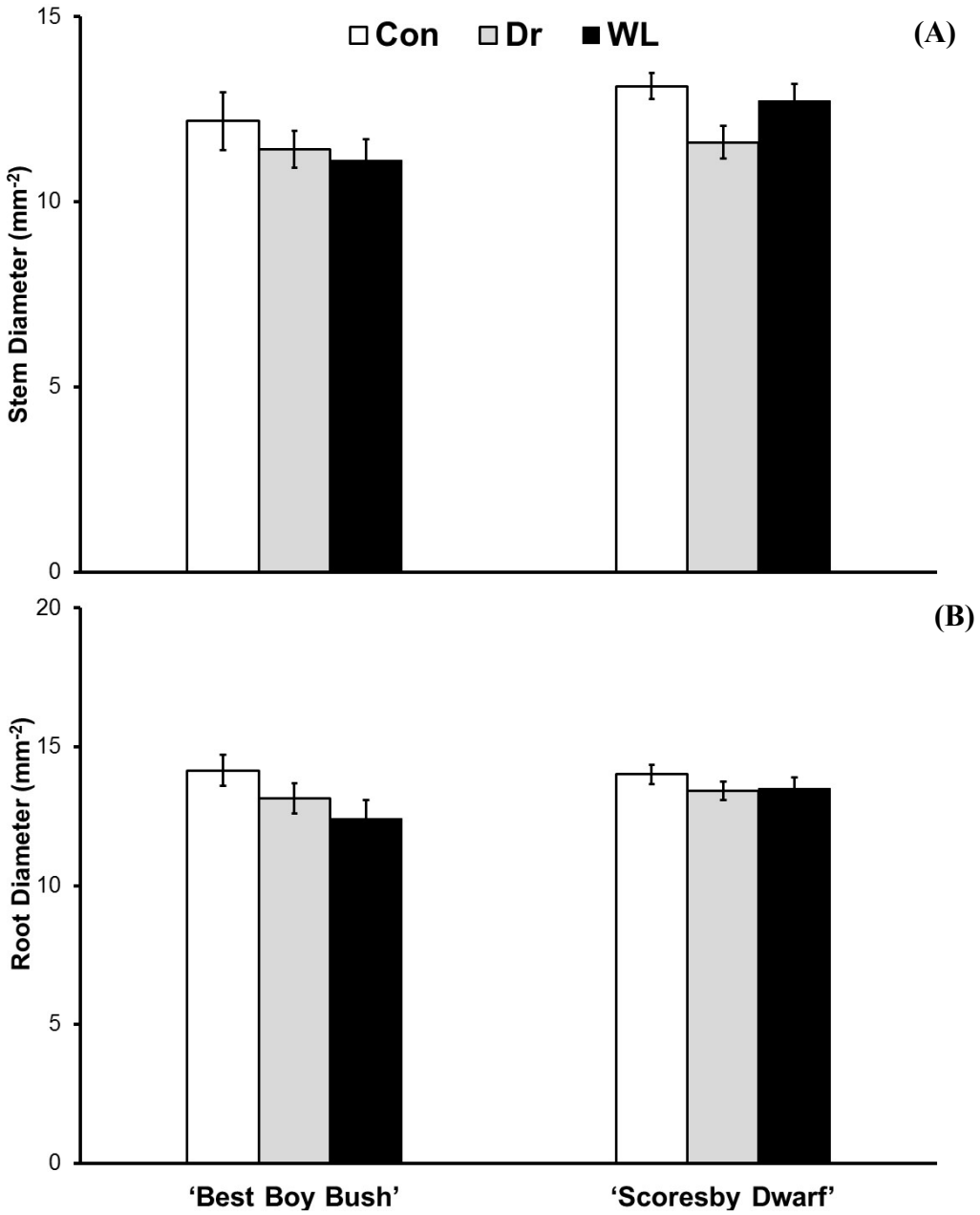
Stem PDM at the vegetative (A), flowering (B), fruiting (C) stages and root PDM at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control (Con), drought (Dr) and waterlogging (WL) conditions; values are means (n=5) ± standard error.

C.2 Field experiment

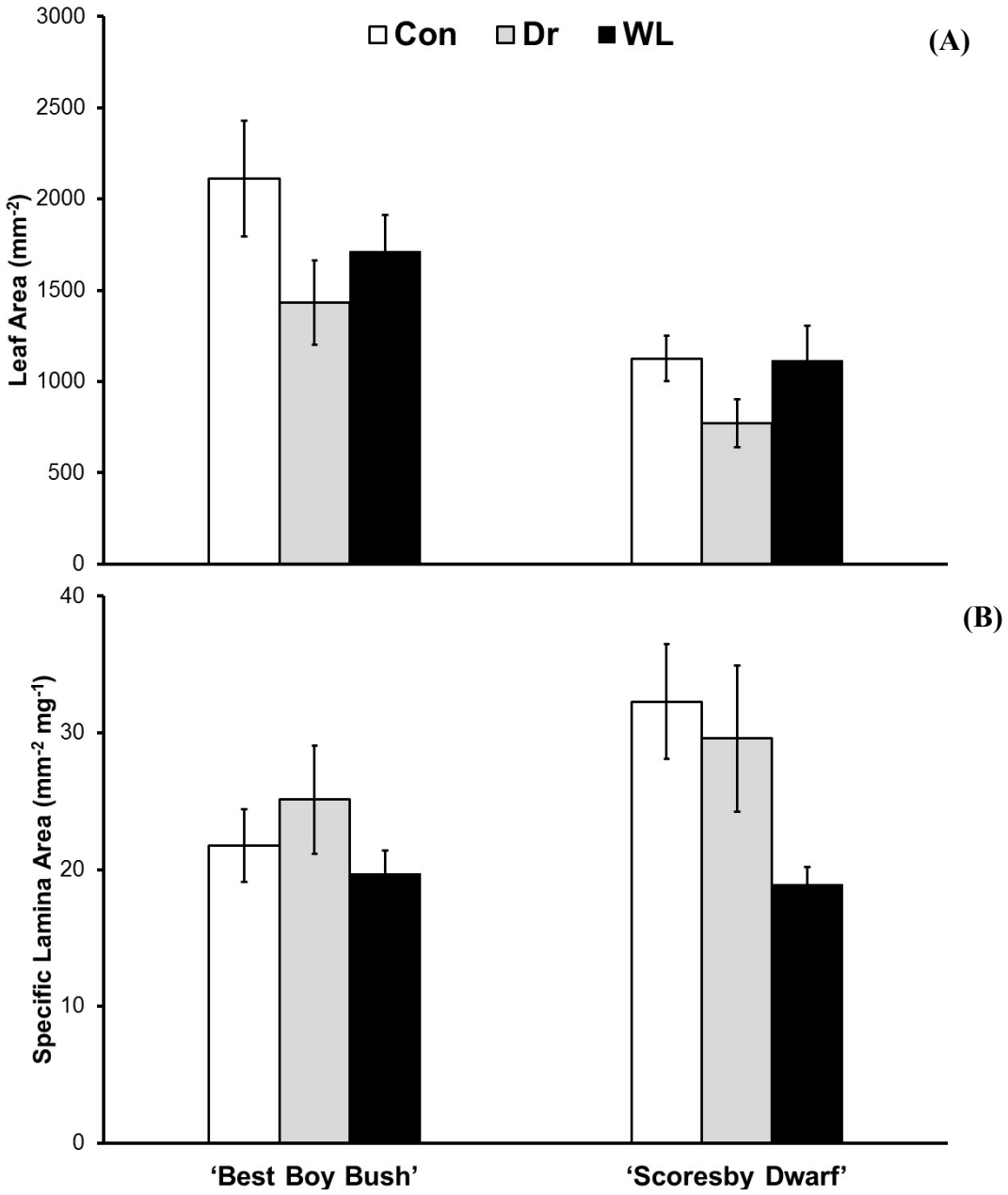
C.2.1 Plant growth and morphology



Node number (A) and root length (B) of two tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' after eight days (Day8) of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

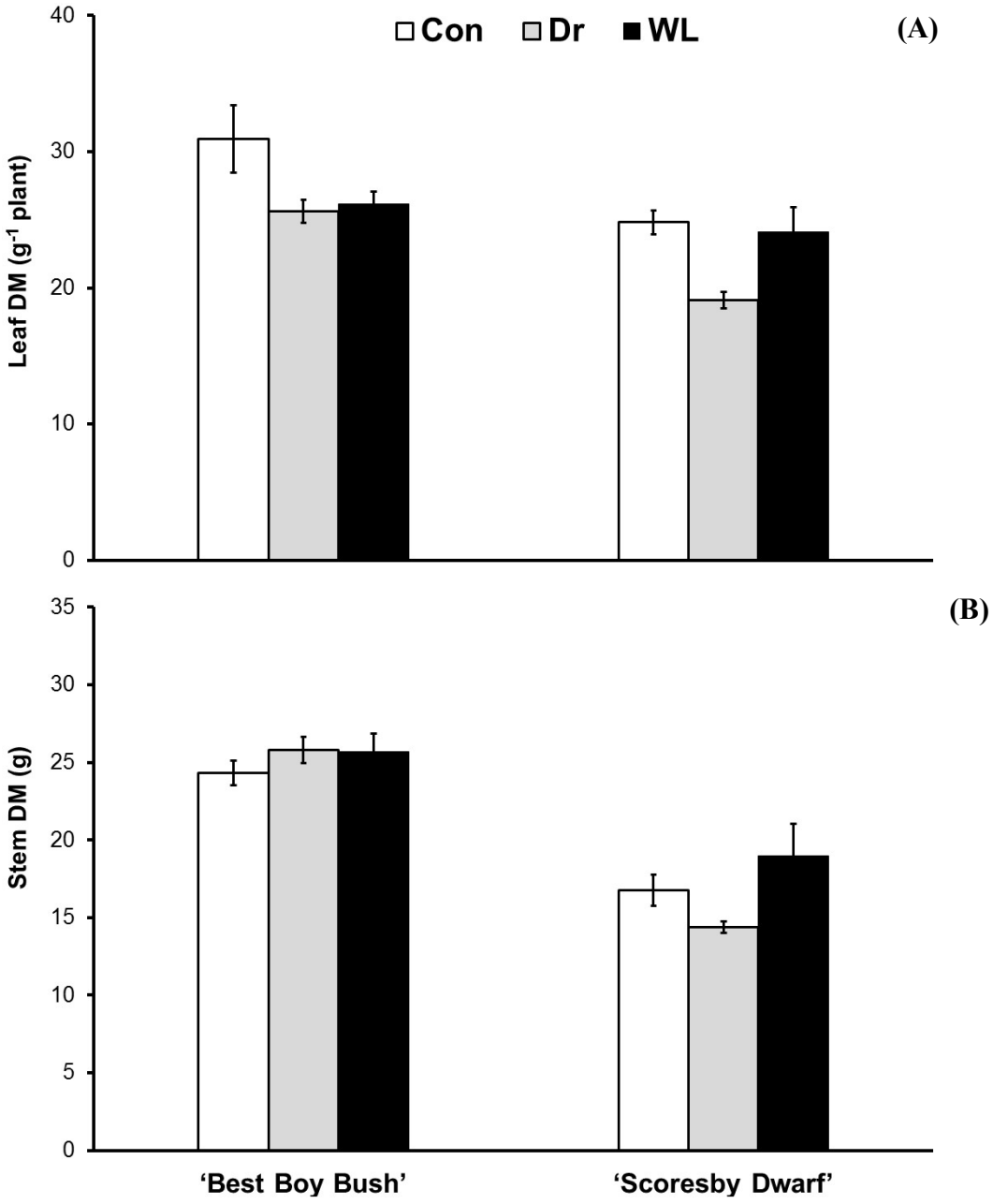


Stem diameter (A) and root diameter (B) of two tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' of eight days (Day8) after exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

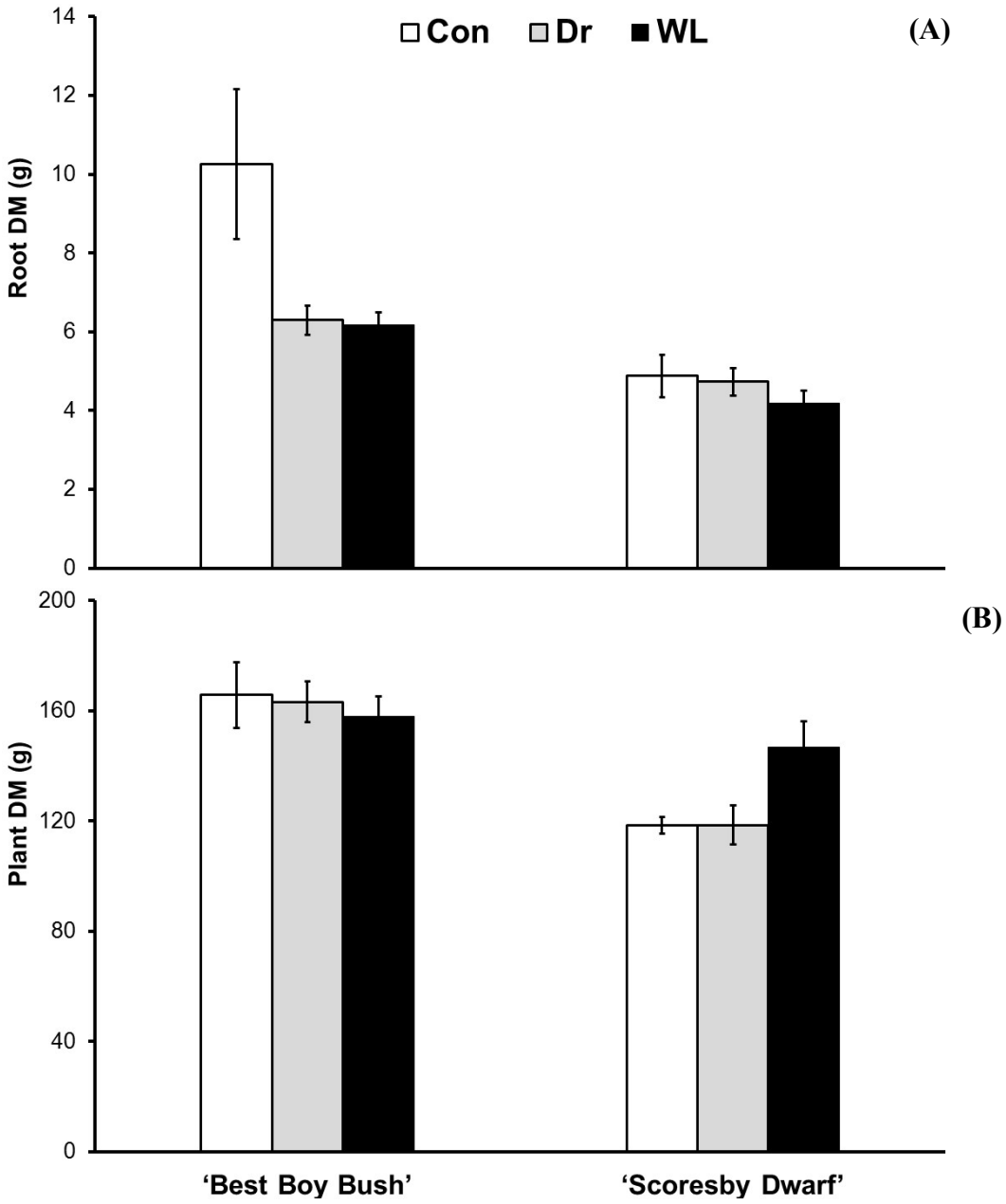


Leaf area (A) and SLA (B) of two tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' after eight days (Day8) of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

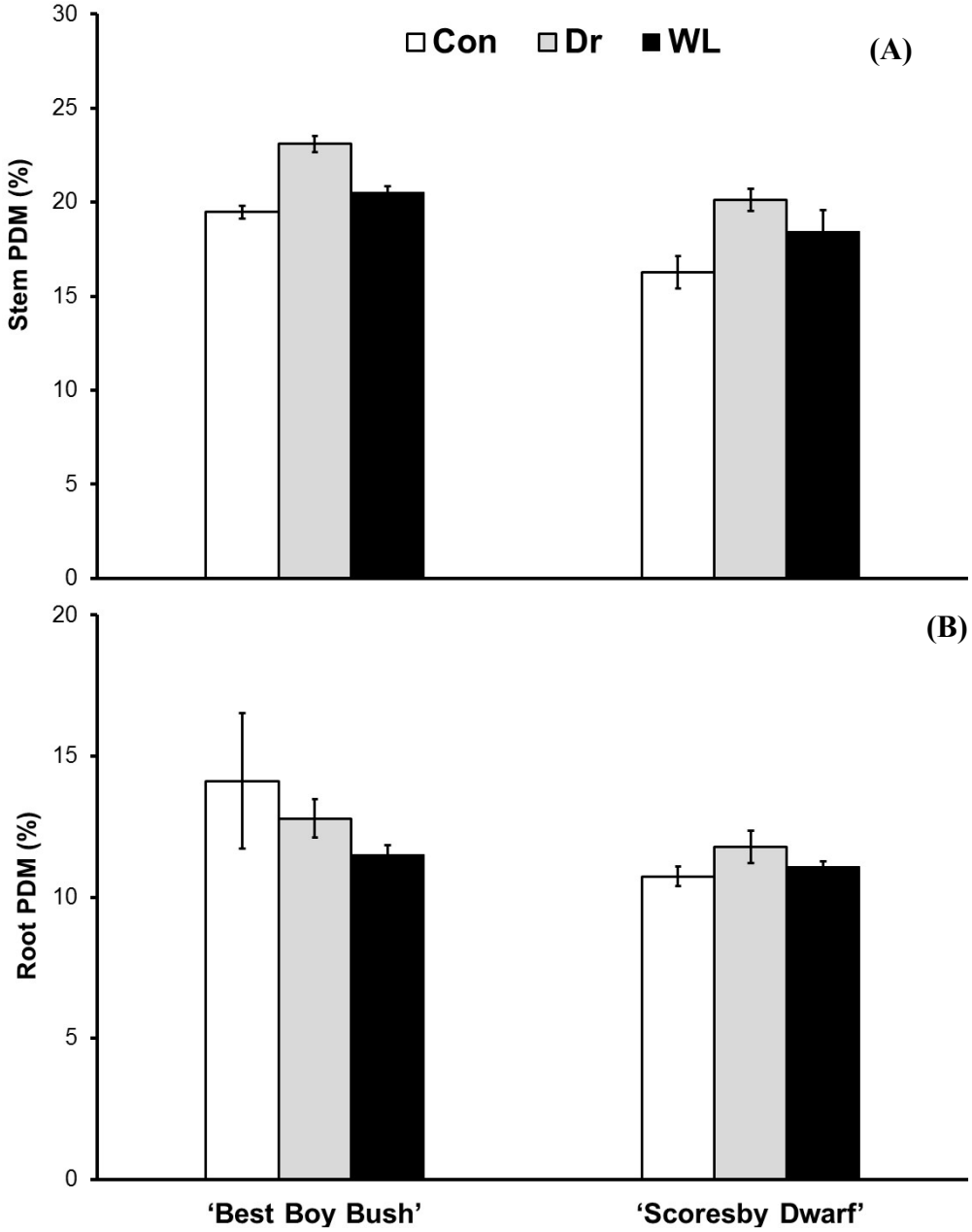
C.2.2 Plant dry matter traits



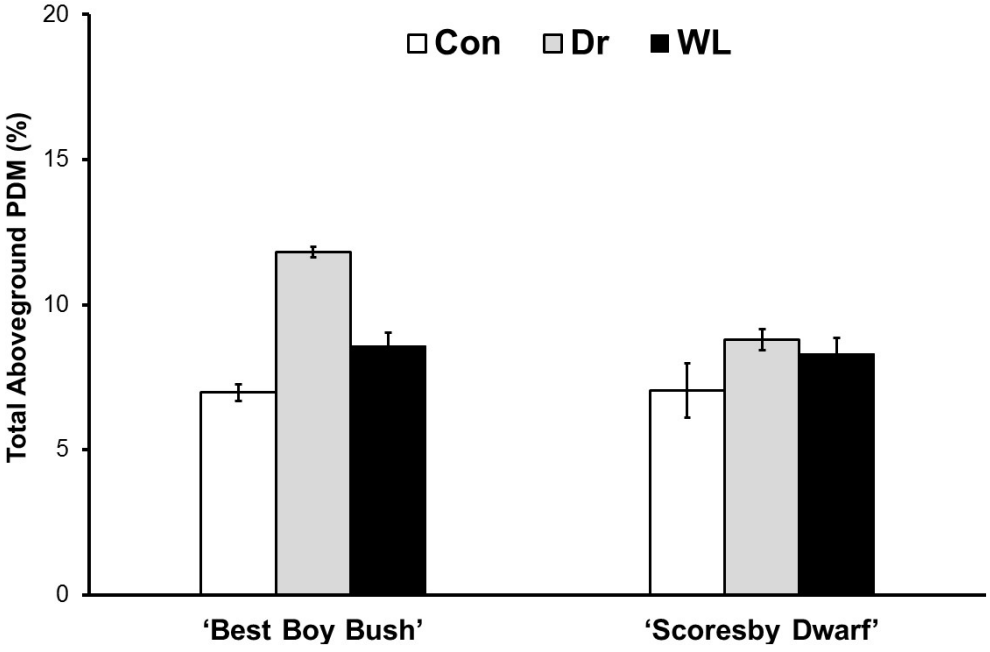
Leaf DM (A) and stem DM (B) of two tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' after eight days (Day8) of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.



Root DM (A) and plant DM (B) of two tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' after eight days (Day8) of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

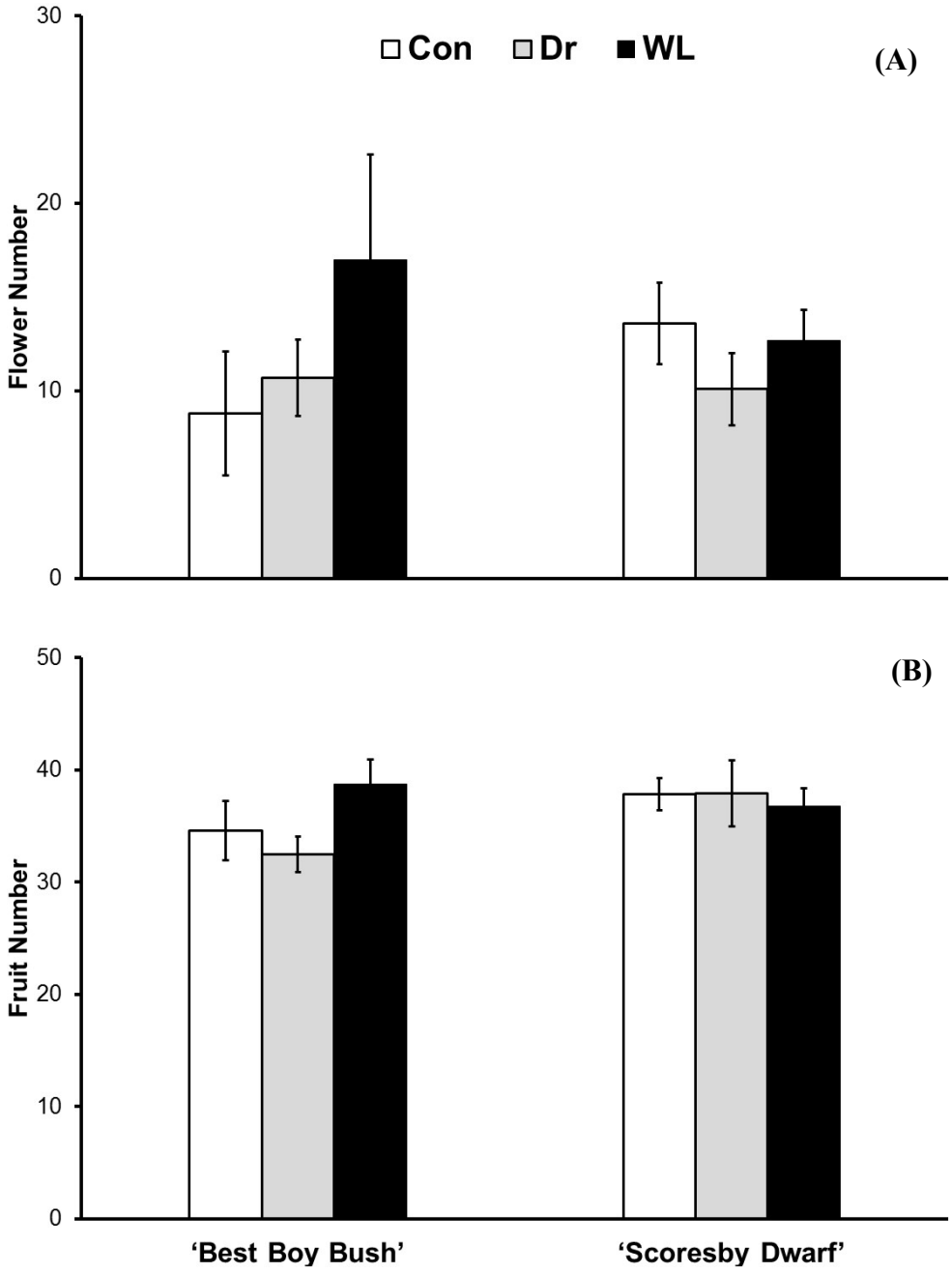


Stem PDM (A) and root PDM (B) of two tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' after eight days (Day8) of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.



Aboveground PDM of two tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' after eight days (Day8) of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

C.2.3 Reproductive components



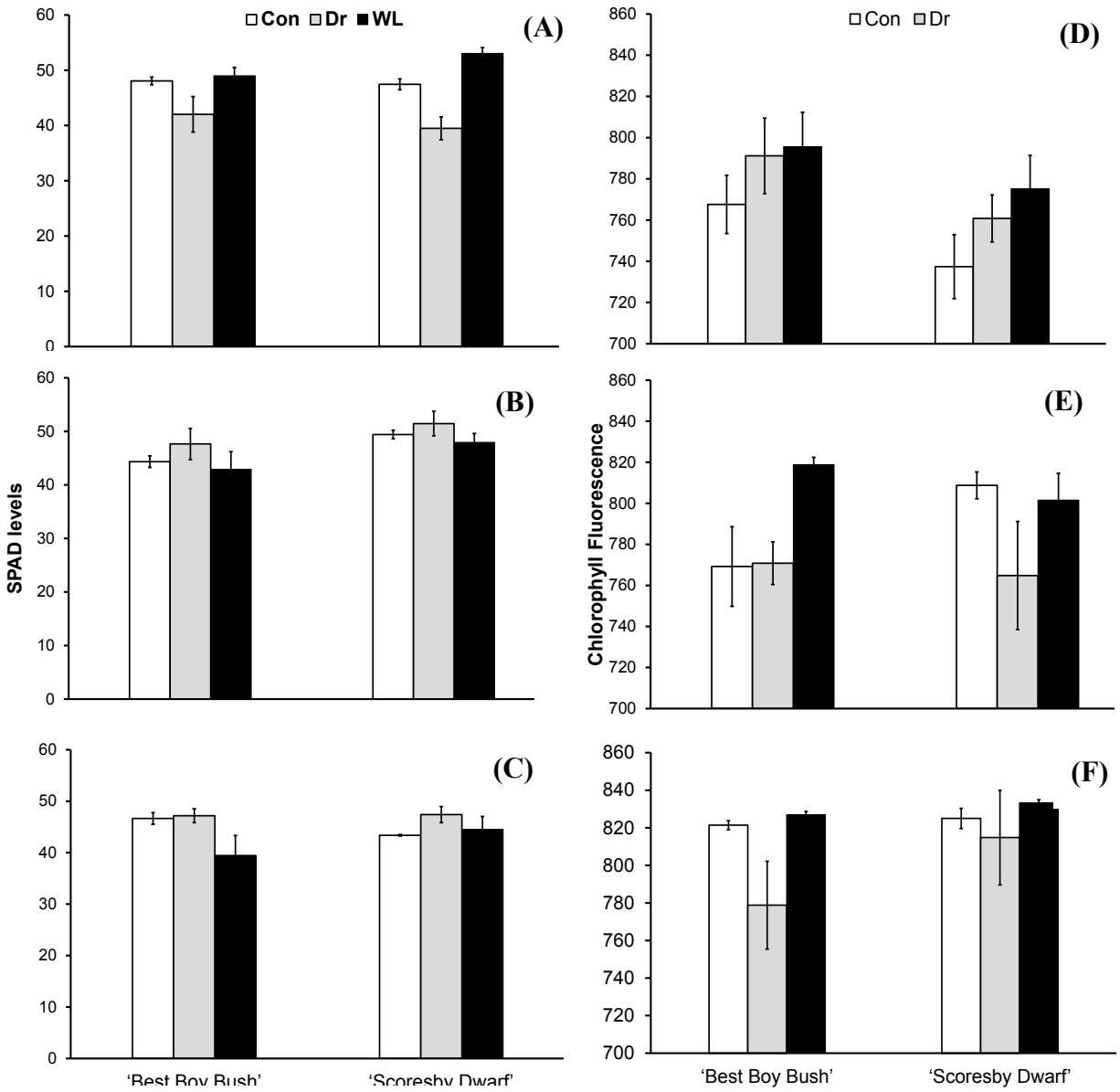
Flower number (A) and fruit number (B) of two tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' after eight days (Day8) of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

Appendix D

Supplementary information for Chapter 4

D.1 Glasshouse experiment

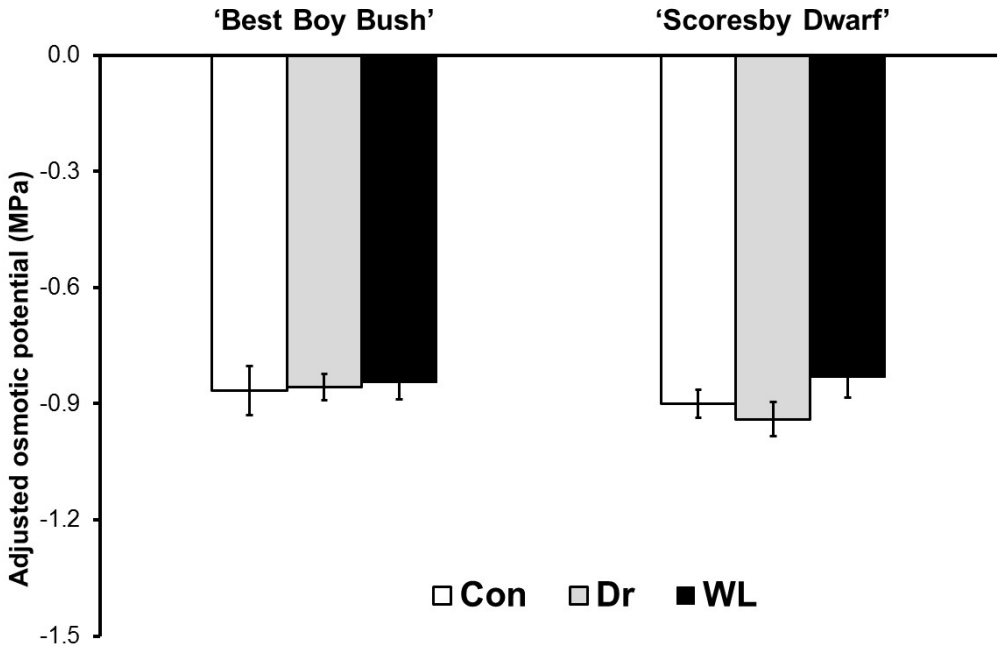
D.1.1 SPAD levels and fluorescence



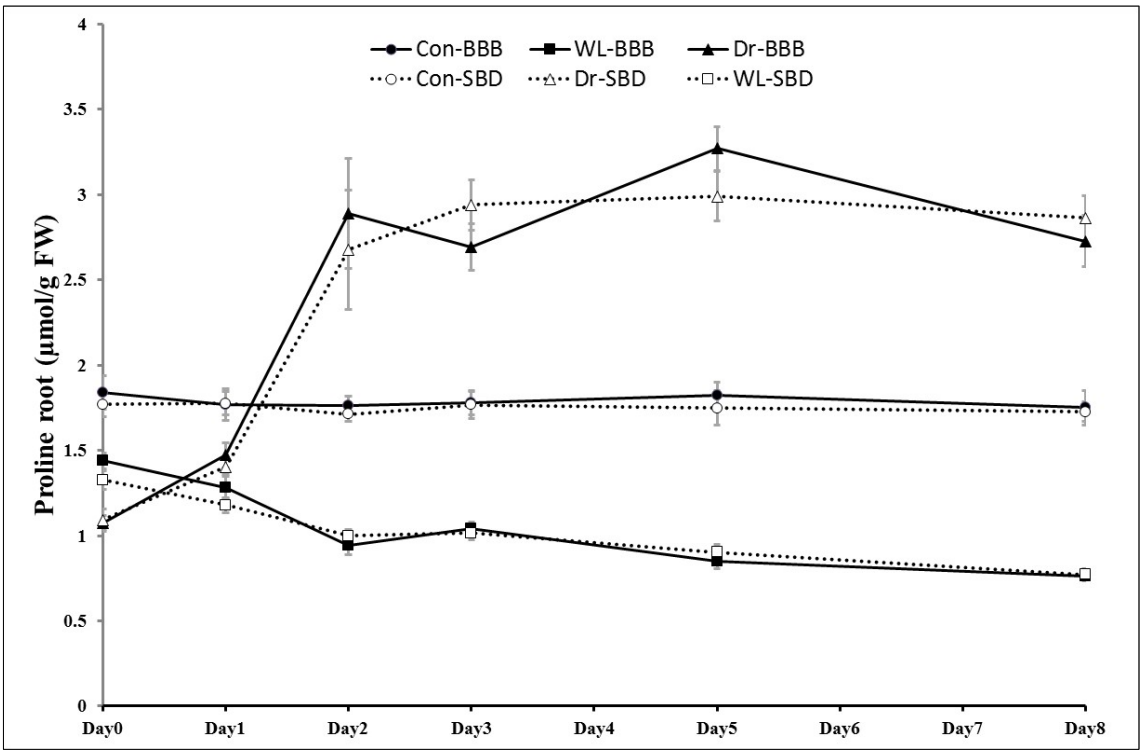
Leaf chlorophyll content at the vegetative (A), flowering (B), fruiting (C) stages and Chlorophyll fluorescence at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-water control conditions (Con), drought (Dr) and waterlogging (WL); values are mean (n=5) ± standard error.

D.2 Field experiment

D.2.1.1 Water status, osmotic adjustment and proline levels

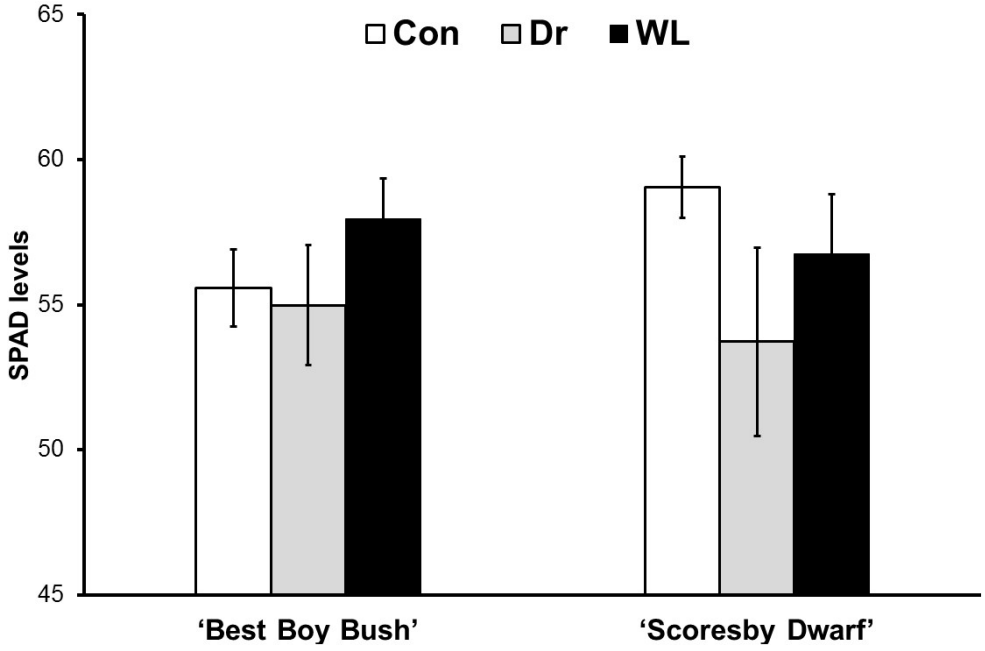


Adjusted osmotic potential of tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' after eight days (Day 8) of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL) conditions; values are means (n=10) ± standard error.



Proline content in root tissue of two tomato cultivars, 'Scoresby Dwarf' and 'Best Boy Bush', before (Day 0) to eight days (Day 8) after exposure to control conditions (Con), drought (Dr), and waterlogging (WL) conditions; values are means (n=10) ± standard error.

D.2.2 Leaf gas exchange



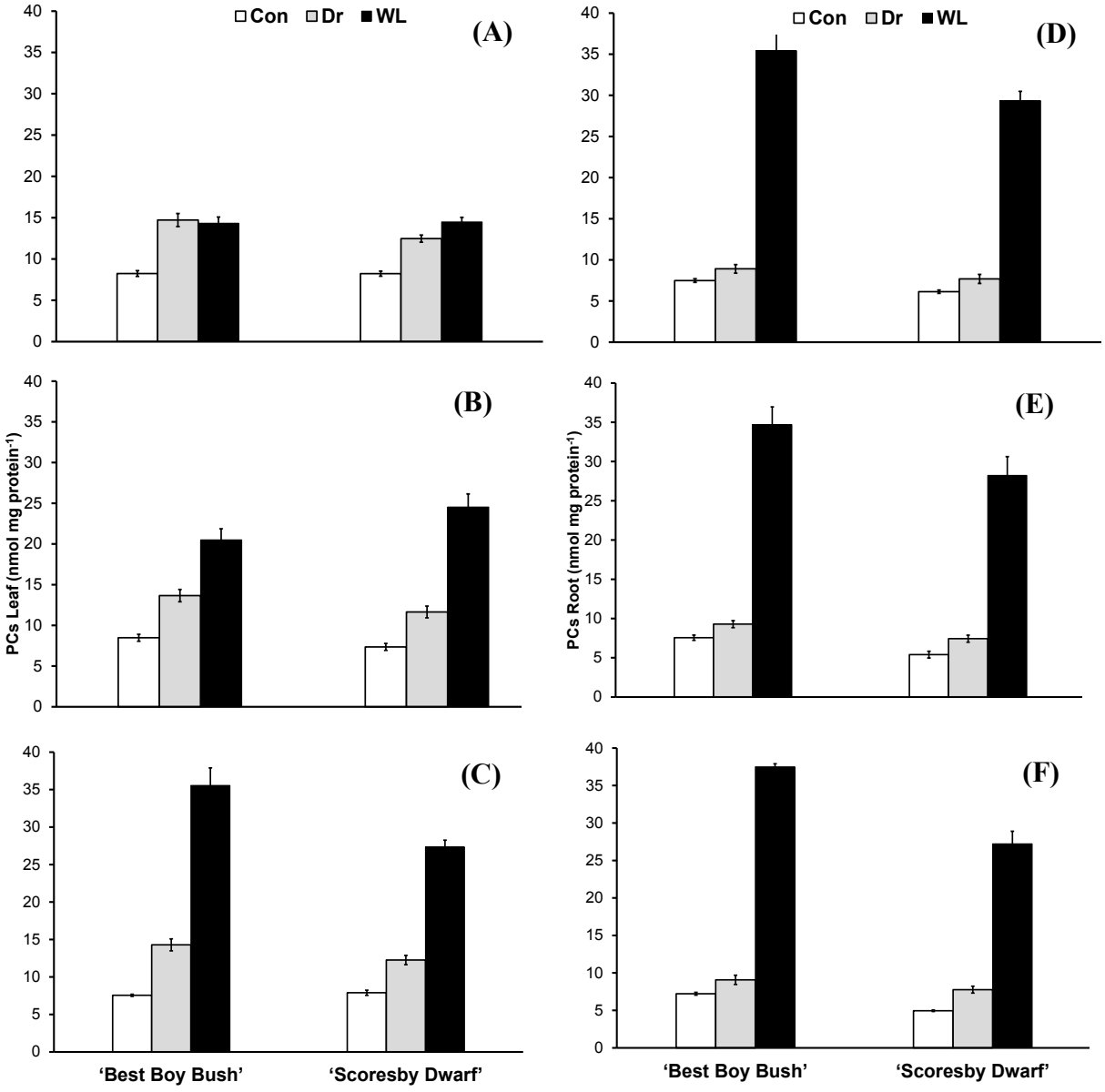
SPAD levels of two tomato cultivars 'Scoresby Dwarf' and 'Best Boy Bush' after eight days (Day8) of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

Appendix E

Supplementary information for Chapter 5

E.1 Stress markers: hydrogen peroxide production and oxidative damage

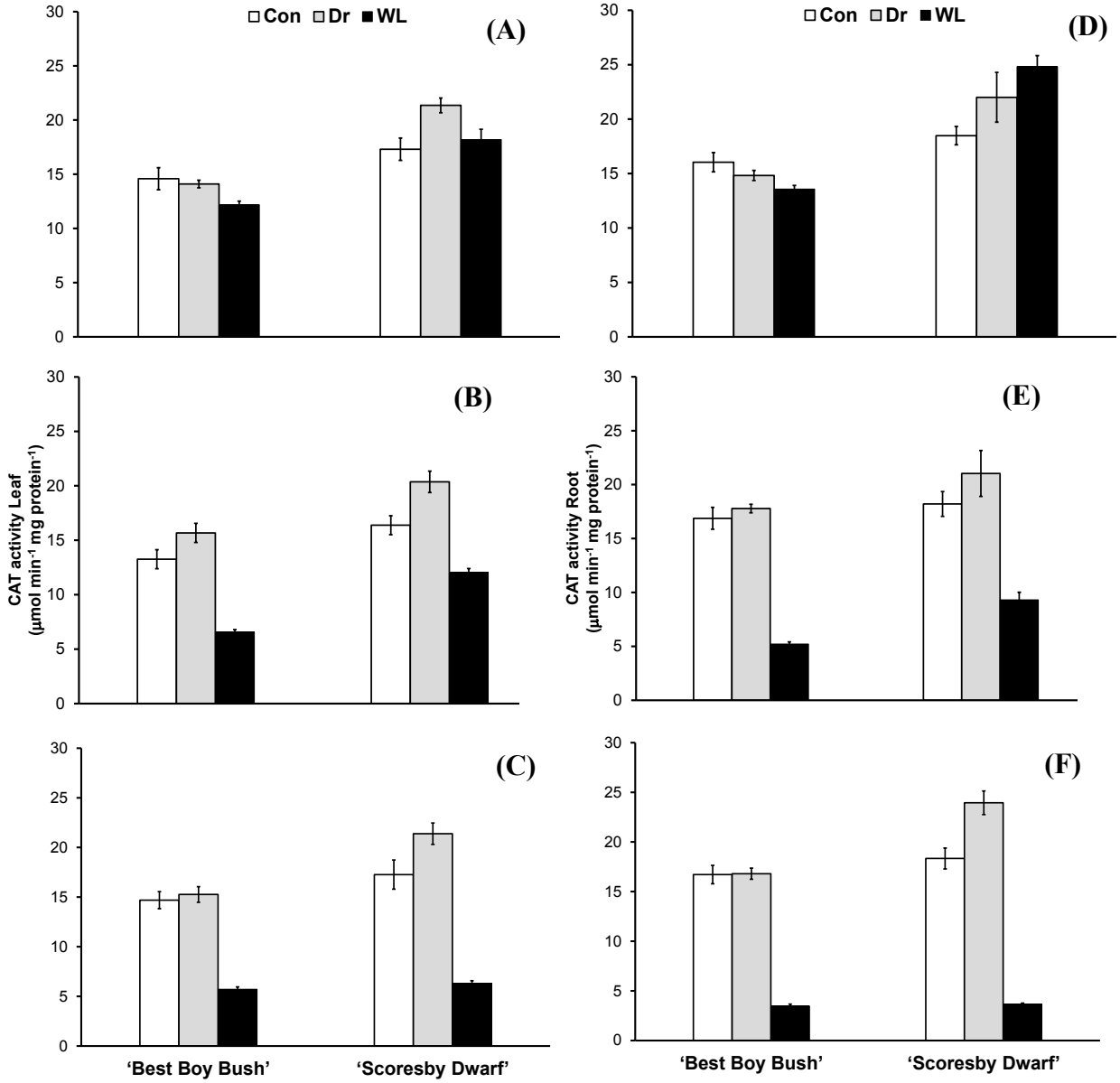
E.1.1 Protein carbonyls



Protein carbonyls (PCs) in leaf tissues at the vegetative (A), flowering (B), fruiting (C) stages and Protein carbonyls in root tissues at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato grown under well-watered control conditions (Con), drought (Dr), waterlogging (WL); values are means (n=5) ± standard error.

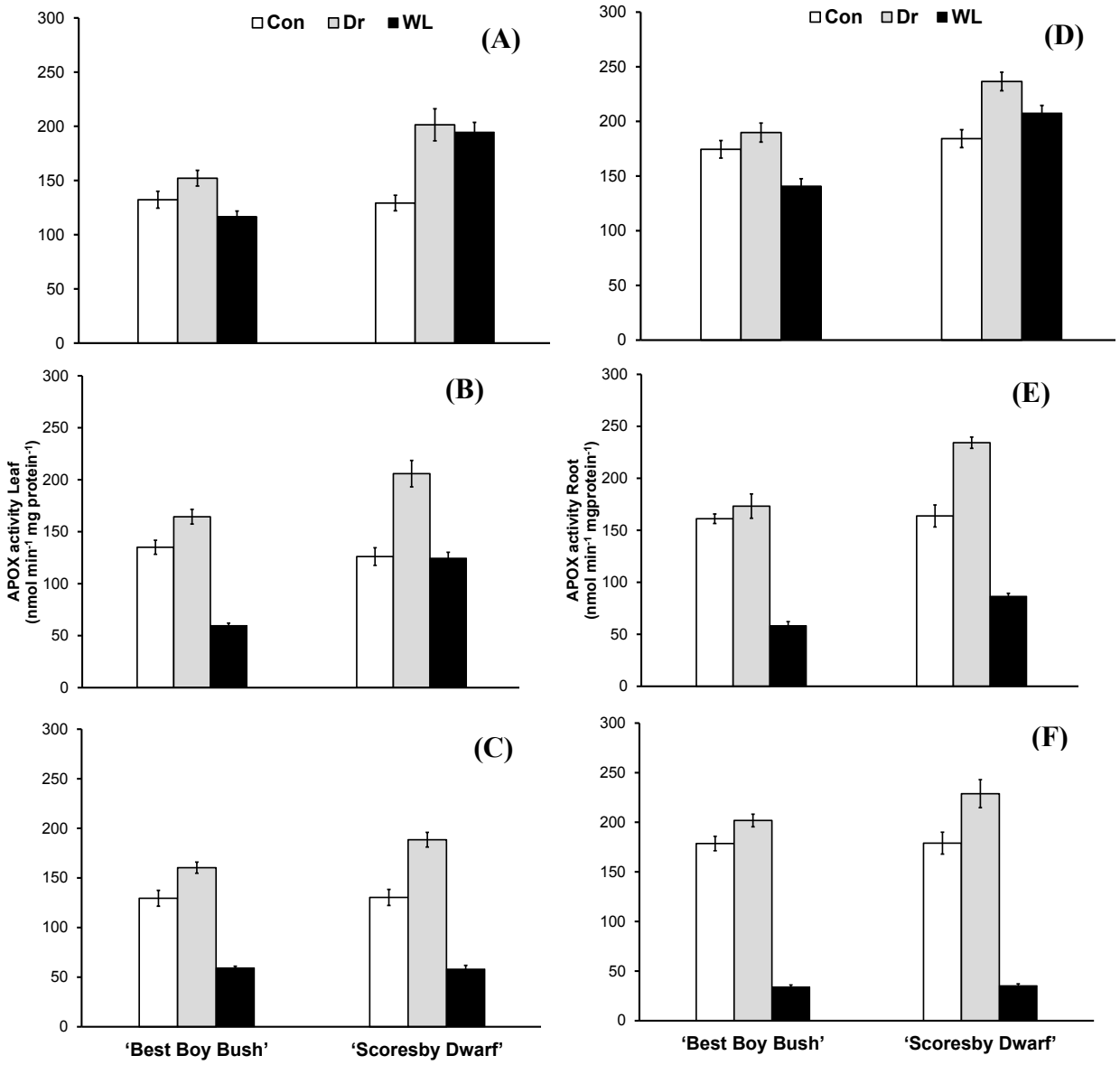
E.2 Antioxidant enzymes

E.2.1 Catalase



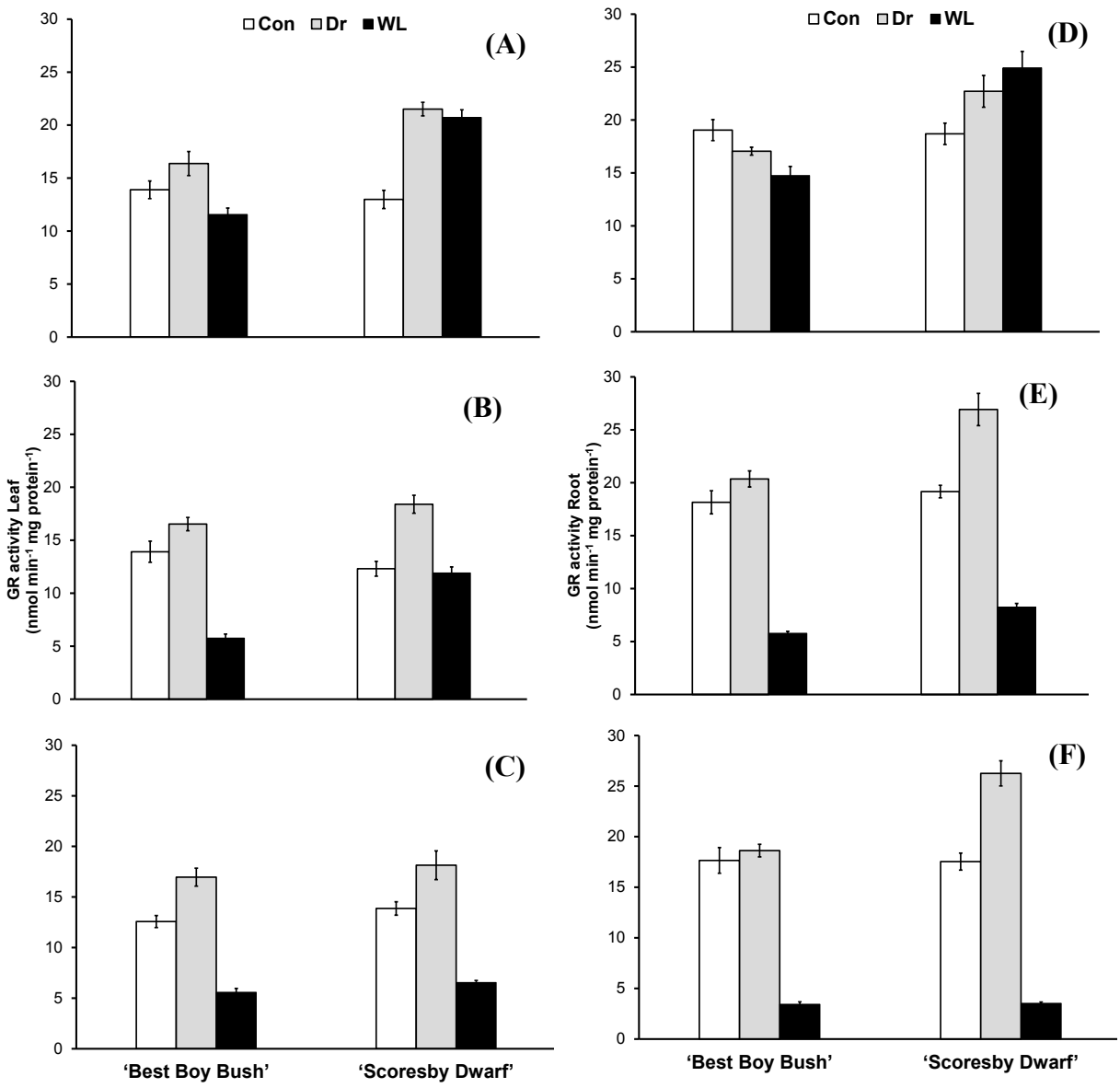
Catalase (CAT) in leaf tissues at the vegetative (A), flowering (B), fruiting (C) stages and CAT in root tissues at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

E.2.2 Ascorbate peroxidase



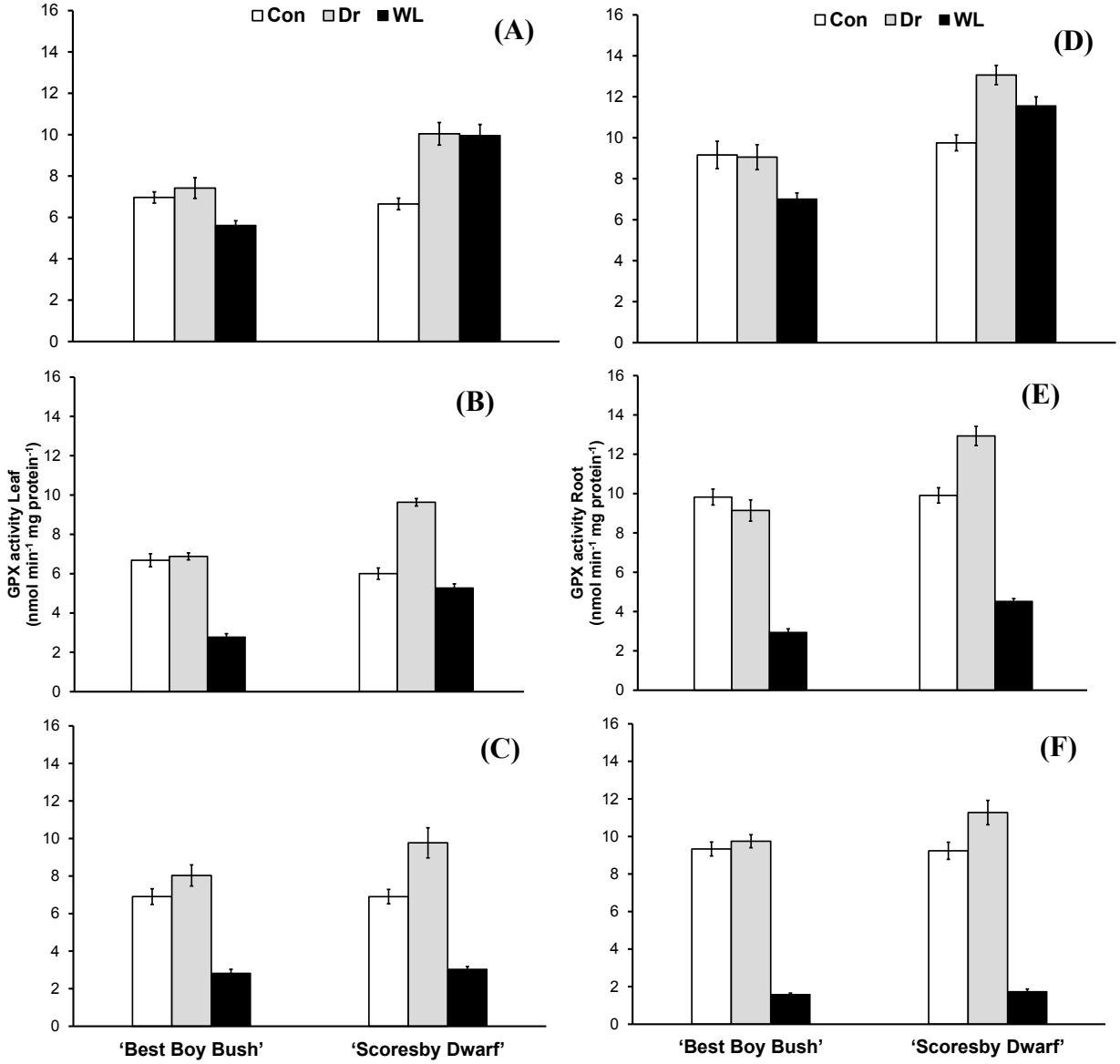
Ascorbate peroxidase (APOX) in leaf tissues at the vegetative (A), flowering (B), fruiting (C) stages and APOX in root tissues at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

E.2.3 Glutathione reductase



Glutathione reductase (GR) in leaf tissues at the vegetative (A), flowering (B), fruiting (C) stages and GR in root tissues at the vegetative (D), flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

E.2.4 Glutathione peroxidase



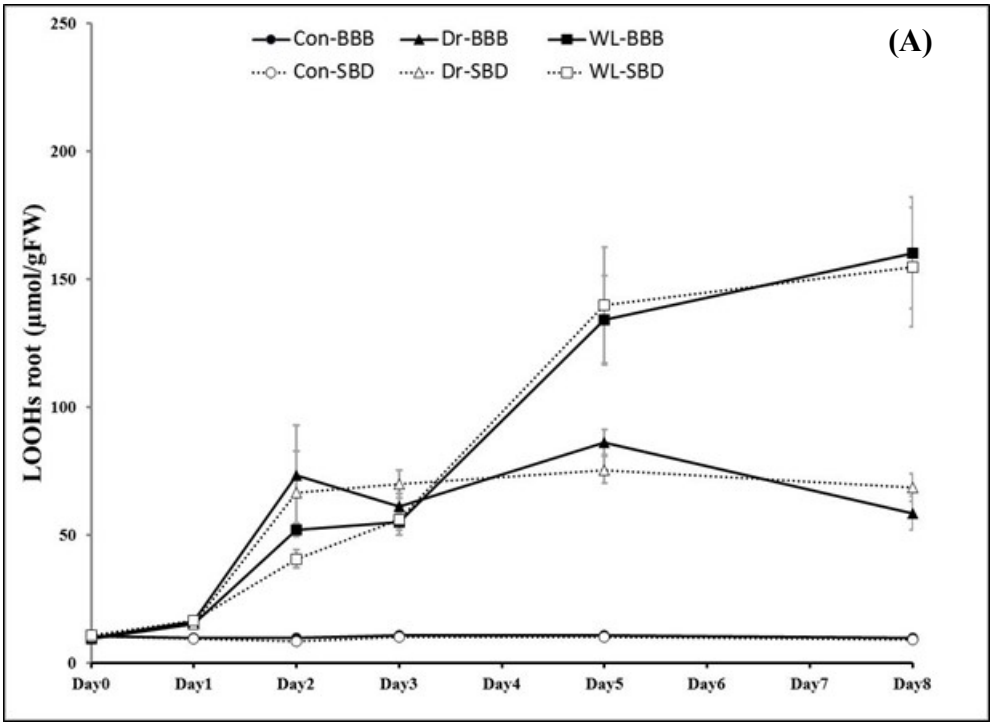
Glutathione peroxidase (GPOX) in leaf tissues at the vegetative (A) flowering (B) and fruiting (C) stages and GPOX in root tissues at the vegetative (D) flowering (E) and fruiting (F) stages of two tomato cultivars grown under well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

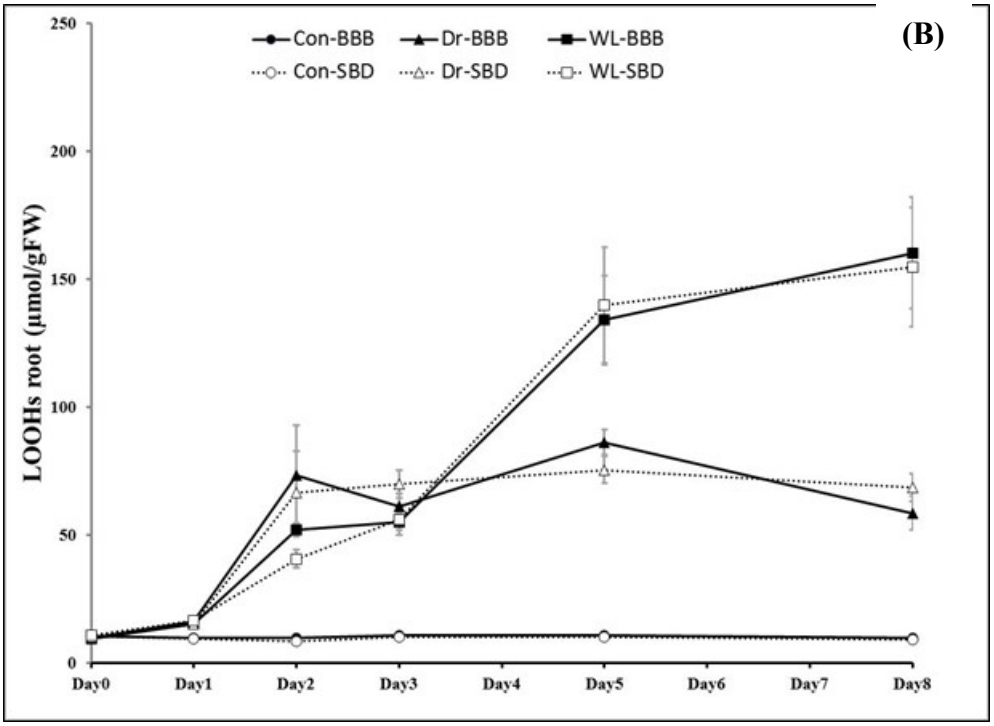
Appendix F

Supplementary information for chapter 6

F.1 Stress markers: hydrogen peroxide production and oxidative damage

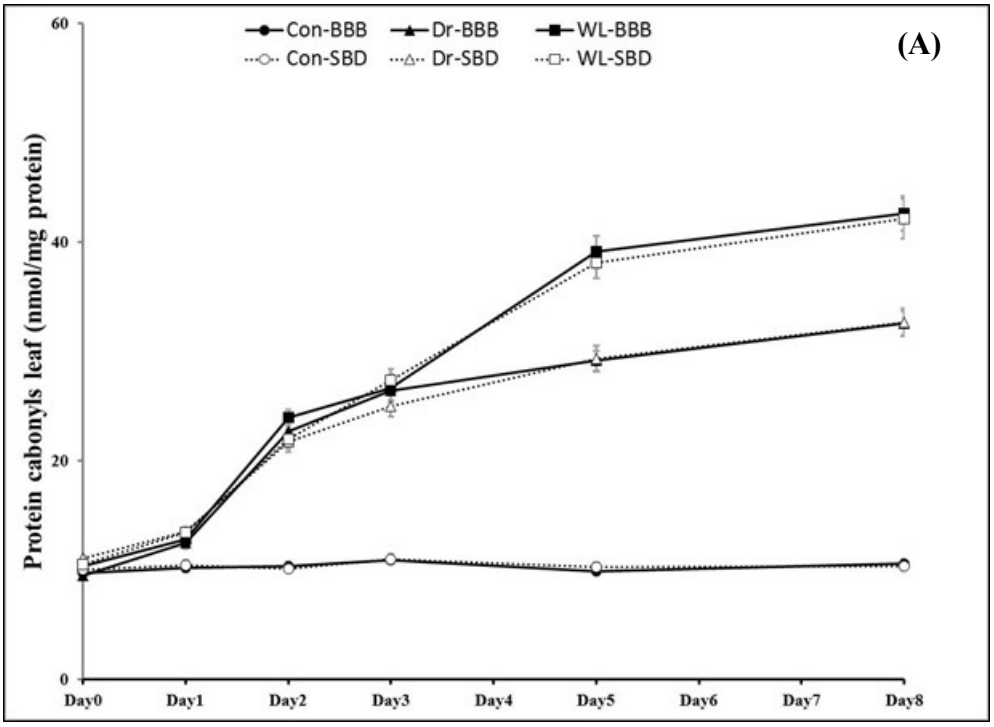
F.1.1 Lipid hydroperoxides

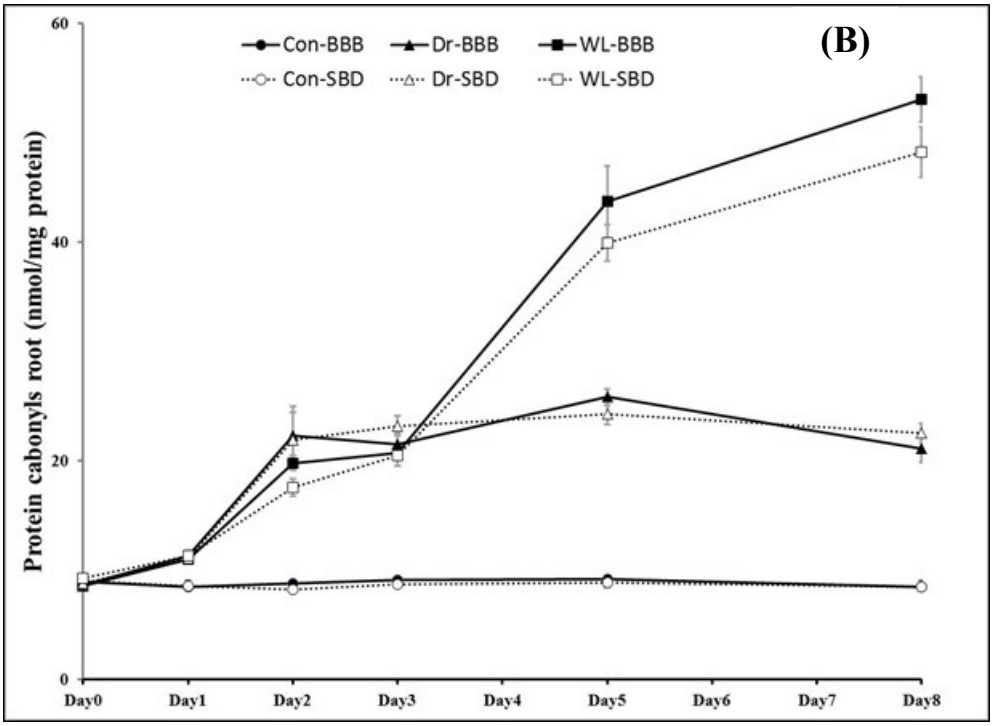




Lipid hydroperoxides (LOOHs) in leaf tissues (A) and root tissues (B) of two tomato cultivars 'Best Boy Bush' and 'Scoresby Dwarf' before during eight days period of exposure to control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

F.1.2 Protein carbonyls

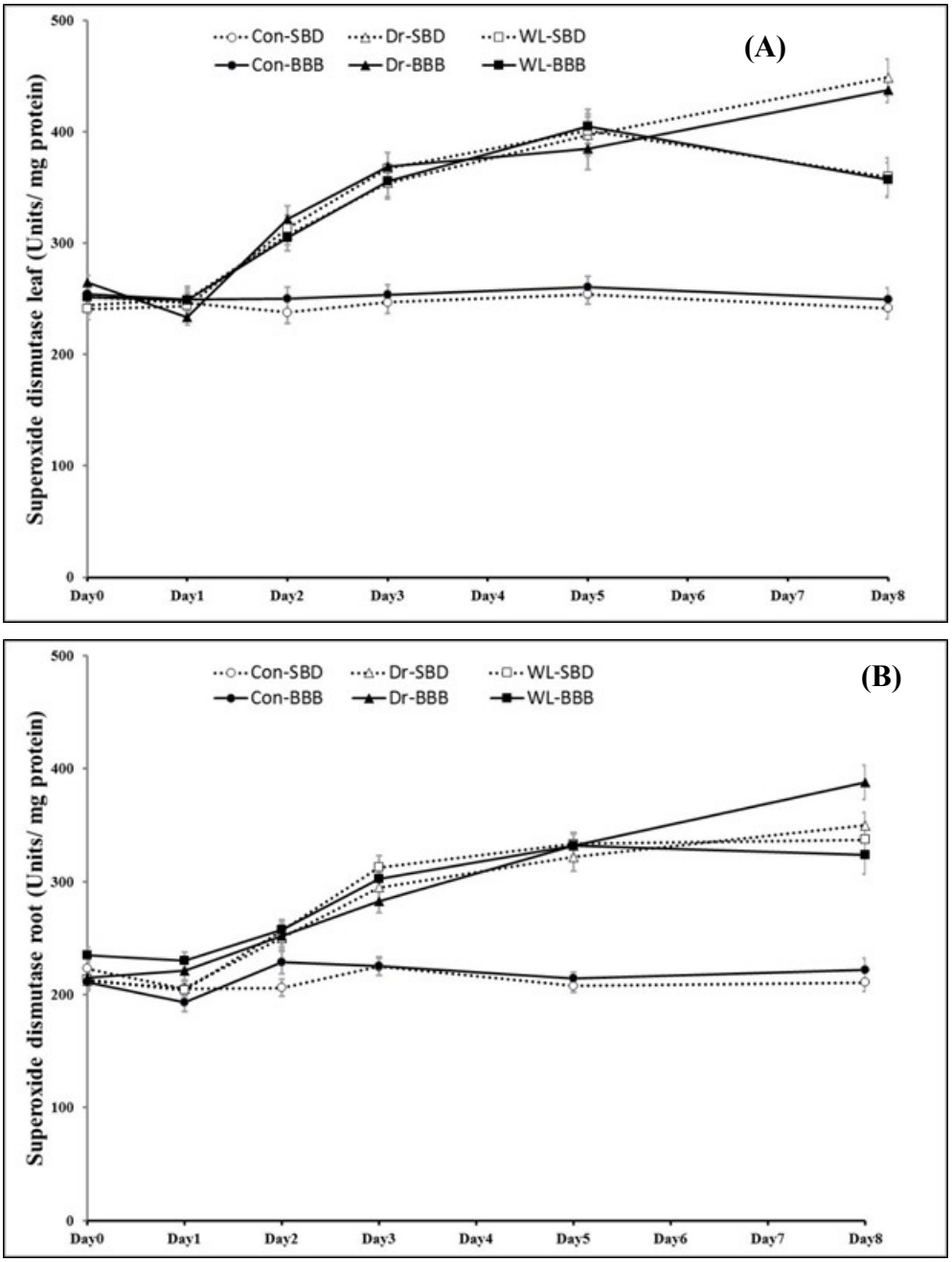




Protein carbonyls (PCs) in leaf tissues (A) and root tissues (B) of two tomato cultivars 'Best Boy Bush' and 'Scoresby Dwarf' during eight days period of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

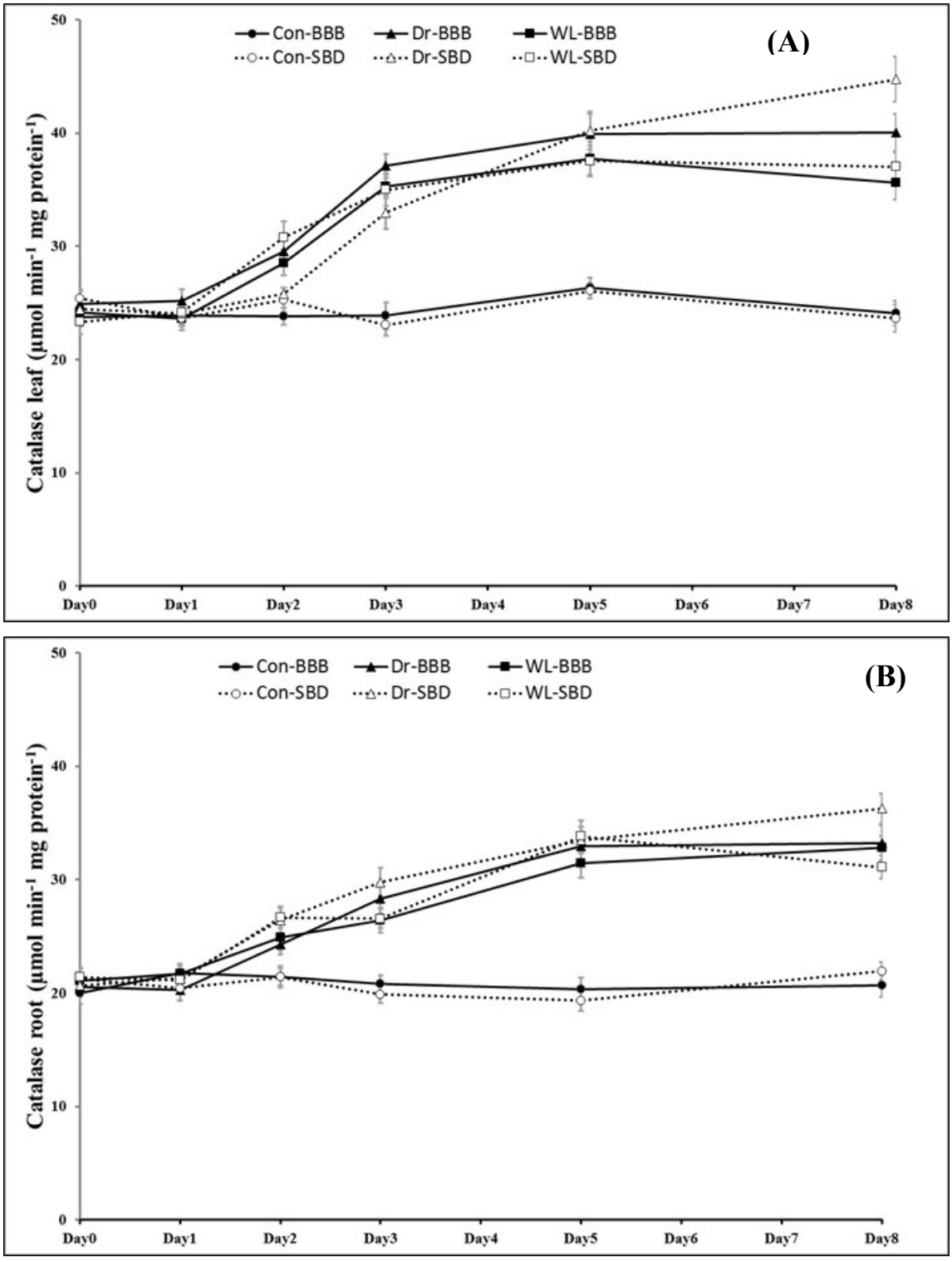
F.2 Antioxidant enzymes

F.2.1 Superoxide dismutase



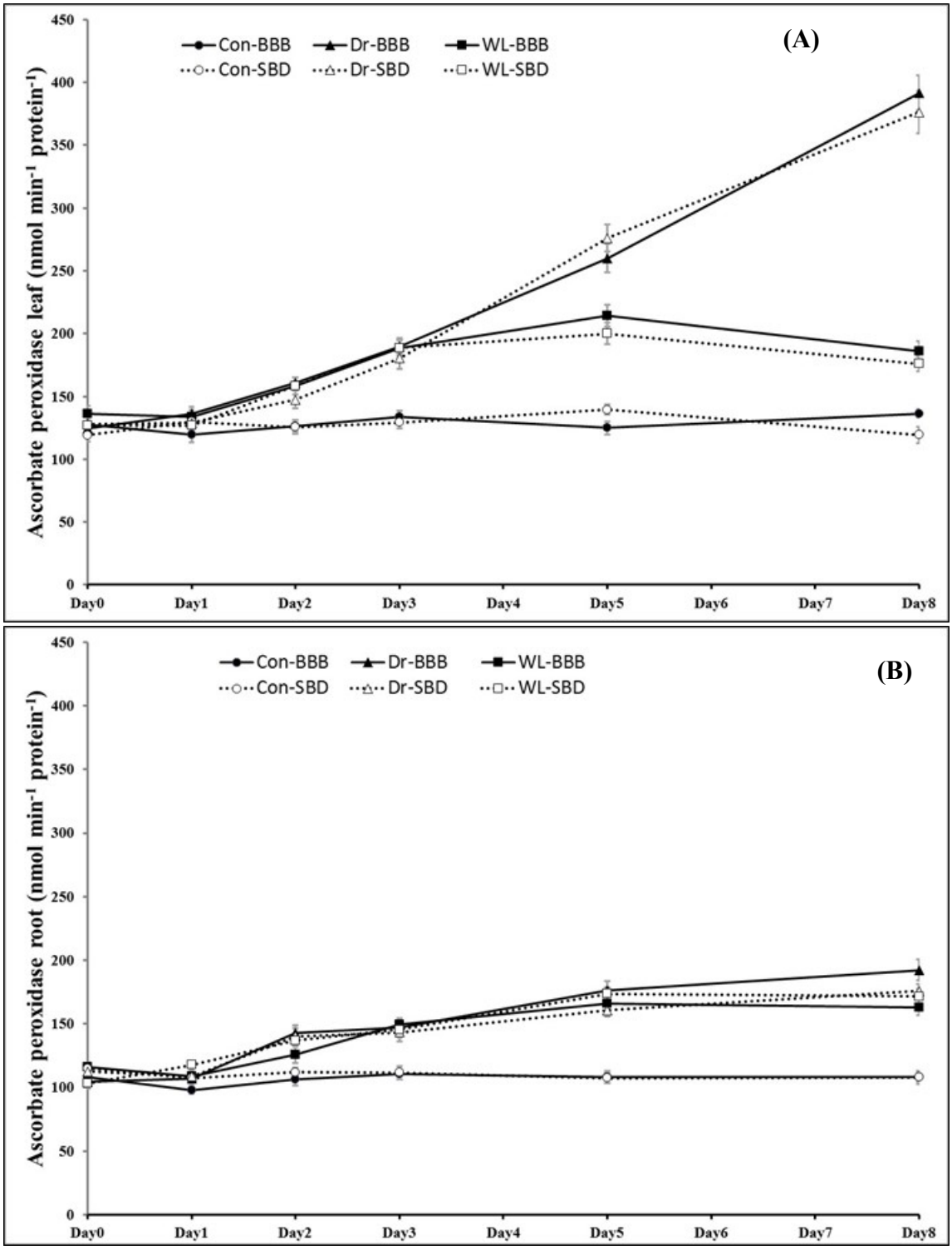
Superoxide dismutase (SOD) in leaf tissues (A) and root tissues (B) of two tomato cultivars 'Best Boy Bush' and 'Scoresby Dwarf' during eight days period of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

F.2.2 Catalase



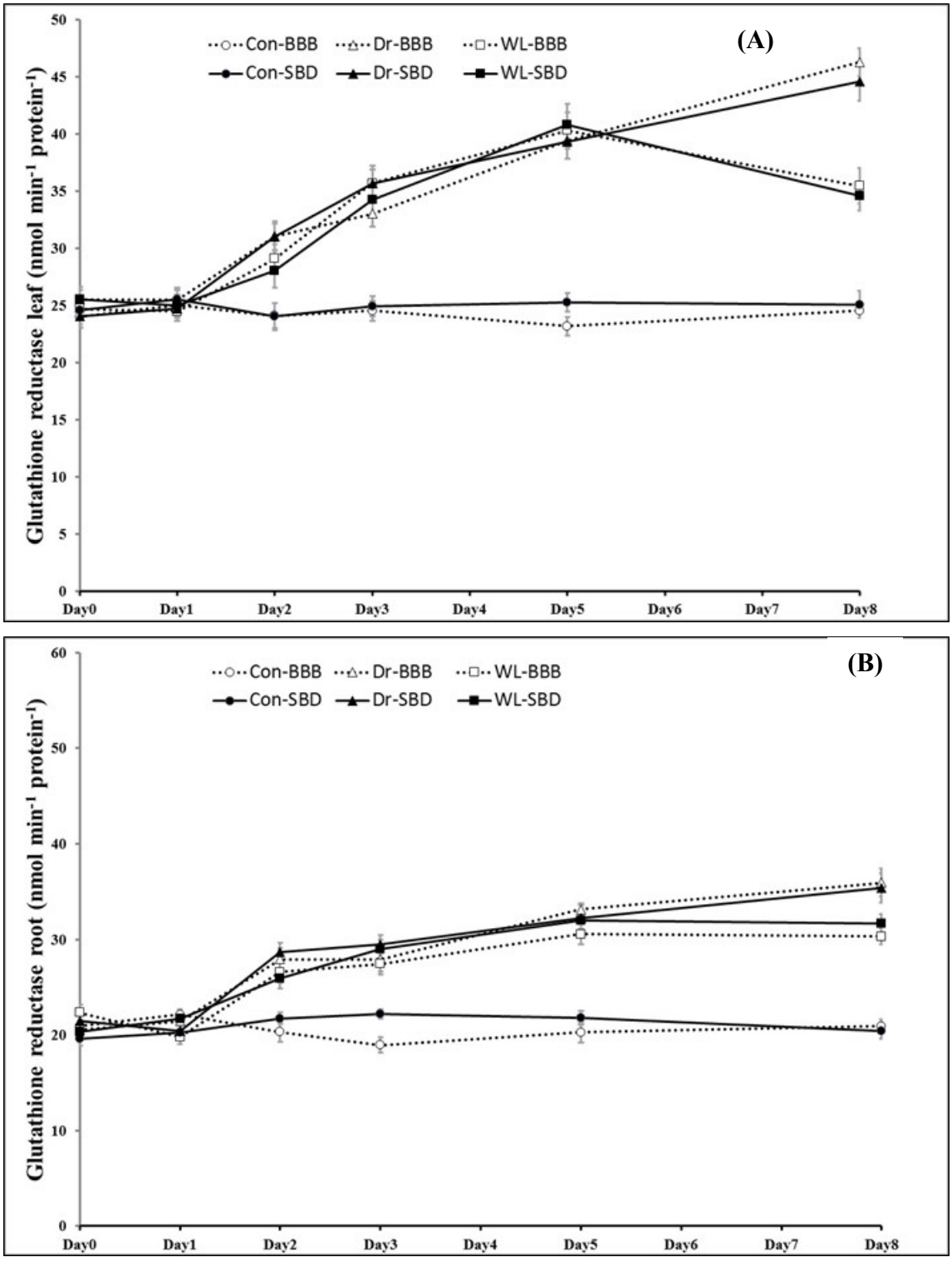
Catalase (CAT) activity in leaf tissues (A) and root tissues (B) of two tomato cultivars, 'Best Boy Bush' and 'Scoresby Dwarf' during eight days period of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

F.2.3 Ascorbate peroxidase



Ascorbate peroxidase (APOX) in leaf tissues (A) and root tissues (B) of two tomato culitvars 'Best Boy Bush' and 'Scoresby Dwarf' during eight days period of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

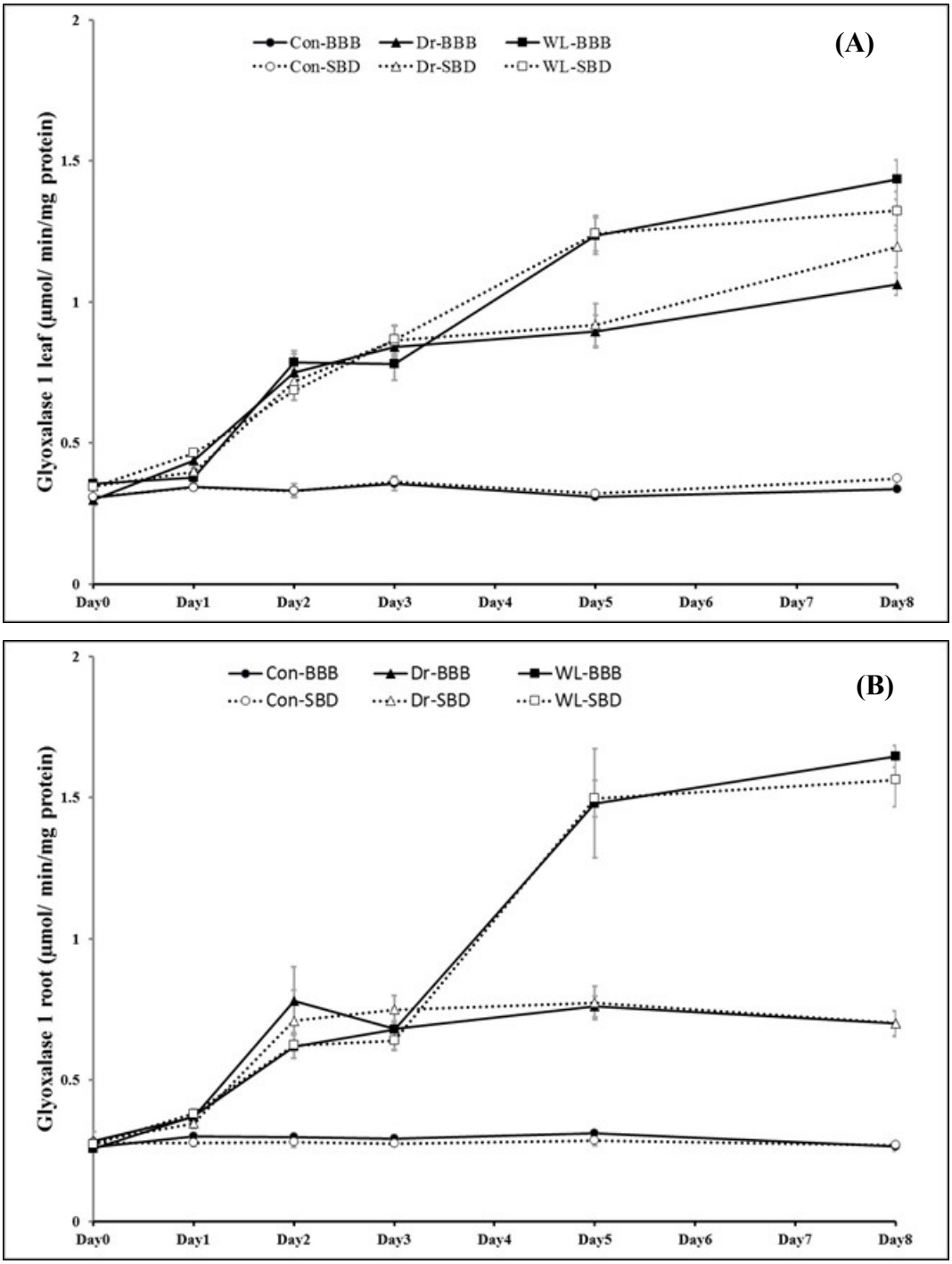
F.2.4 Glutathione reductase



Glutathione reductase (GR) in leaf tissues (A) and root tissues (B) of two tomato cultivars 'Best Boy Bush' and 'Scoresby Dwarf' during eight days period of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=10) ± standard error.

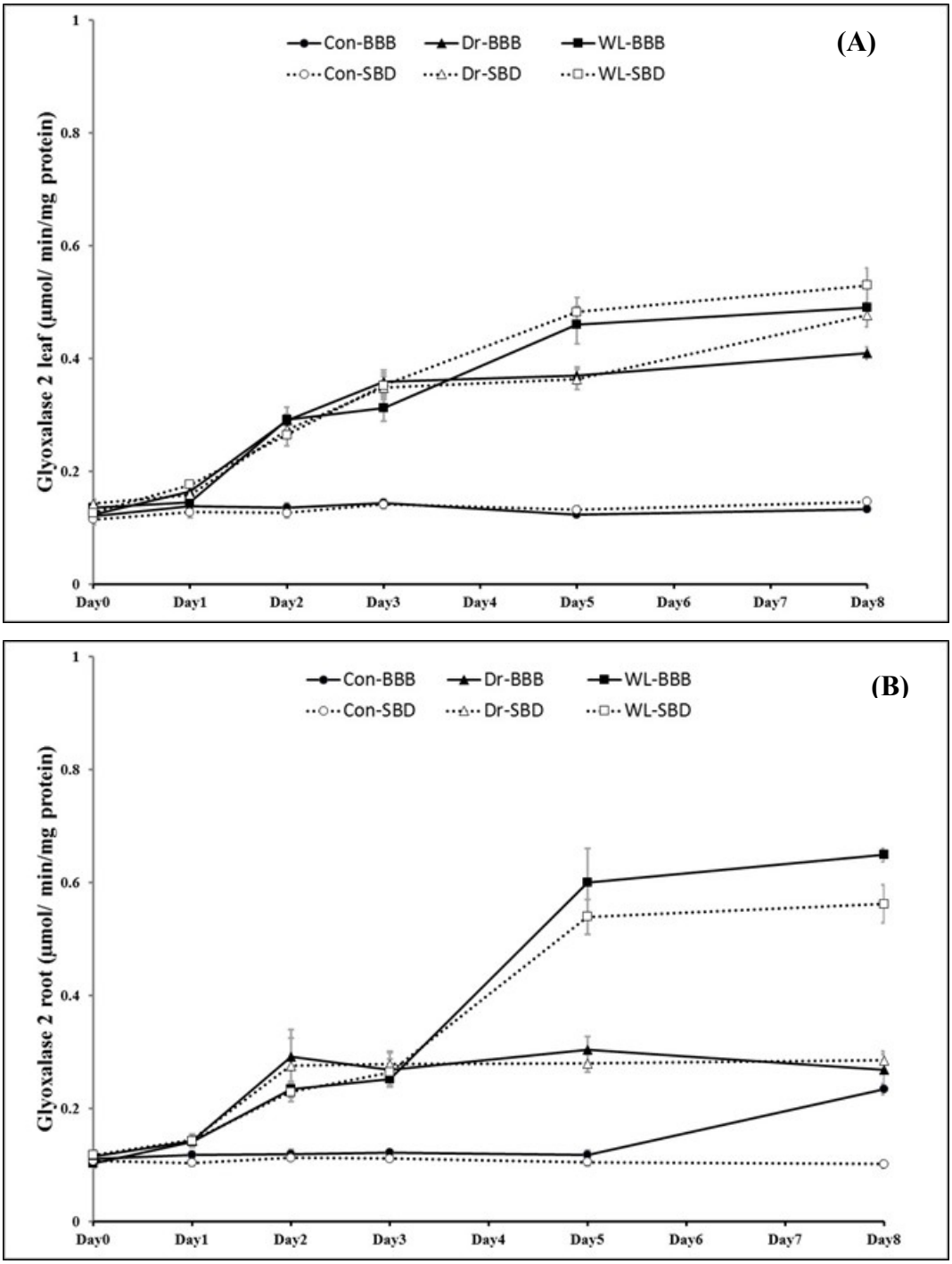
F.3 Methylglyoxal and glyoxalases

F.3.1 Glyoxalase I



Glyoxalase I in leaves (A) and in roots (B) of two tomato cultivars: 'Best Boy Bush' and 'Scoresby Dwarf' during eight days period of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

F.3.2 Glyoxalase II



Glyoxalase II in leaves (A) and in roots (B) of two tomato cultivars: 'Best Boy Bush' and 'Scoresby Dwarf' during eight days period of exposure to well-watered control conditions (Con), drought (Dr) and waterlogging (WL); values are means (n=5) ± standard error.

Appendix G

Raw data of the heat stress experiment

In the attached CD

Appendix H

Temperature records

In the attached CD

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