CRANFIELD UNIVERSITY

IKENNA CHRISTIAN OHANENYE

PhD THESIS

UNDERSTANDING THE REGULATION OF ONION DORMANCY AS INFLUENCED BY DEFICIT IRRIGATION AND POSTHARVEST STORAGE REGIMES

SCHOOL OF WATER, ENERGY AND ENVIRONMENT
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DOCTOR OF PHILOSOPHY (PhD)
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Supervisor: PROF. LEON A. TERRY
Associate Supervisor: PROF. ANDREW J. THOMPSON AND DR. M. CARMEN ALAMAR
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(NB. This section can be removed if the award of the degree is based solely on examination of the thesis)

ABSTRACT

Onion (*Allium cepa* L.) is a biennial crop that produces a bulb in the first season only, but the demand for onion bulbs is all-year-round and to meet demand, they are stored for up to nine months. Consequently, this extended bulb storage causes premature sprouting, which impacts negatively on onion storage quality. Ethylene and 1-methylcyclopropene (1-MCP) were revealed to inhibit sprout growth in stored onion bulbs, whether applied independently or in combination. Also, higher abscisic acid (ABA) and fructans (especially, those at a higher degree of polymerisation) are reported to positively correlate with delayed sprouting. Moreover, the accumulation of ABA and fructans have previously been reported in other plants grown under water-stress. This study investigated (1) the the impact of deficit irrigation (DI) on sprouting; (2) the effects of DI on ABA and fructans contents, and redistribution, in relation to onion bulb dormancy and quality in store; and (3) the molecular mechanisms of fructans and sucrose metabolism in stored onion bulbs during onion dormancy break and sprouting.

Across three seasons, onion cultivars 'Red Baron' sets, 'Sherpa' and double haploid (CUDH2107) seeds were grown and subjected to full irrigation (FI) (100% replacement of crop evapotranspiration) or deficit irrigation (DI) (50% of FI treatment) from bulb initiation to harvest. Bulbs were harvested at full maturity and stored at 1°C for five months and treated with or without 1-MCP (1 µL L^{-1}) for 24 h before storage under continuous ethylene supplementation (10 µL L^{-1}) or air. DI had no effect on dormancy-break, sprout emergence, total fructans and sugar content. Pre-harvest and end of curing ABA contents in the baseplate of DI bulbs was significantly higher compared to FI bulbs; however, no significant difference in sprouting between DI and FI bulbs was found. It was hypothesised that ABA accumulated under water-stress may not enhance onion

dormancy. Ethylene and 1-MCP independently reduce the rate of ABA decline in stored onion bulbs. However, while ethylene was found to delay sprouting for both DI and FI bulbs, 1-MCP did not. This therefore questions the role of ABA in onion dormancy; nonetheless, bulbs treated with 1-MCP and stored under ethylene produced the shortest sprouts. Sucrose and fructans contents declined in sprouted bulbs while the expressions of the genes *SPS* (sucrose phosphate synthase), *1-SST* (sucrose:sucrose 1-fructosyltranferase) and *6G-FFT* (fructan:fructan 6G-fructosyltranferase) involved in sucrose and fructans biosynthesis were significantly upregulated. This suggested posttranslational regulations, or a higher catabolic rate compared to biosynthesis. The concentration of DP3-8 fructans were higher in top and bottom sections compared to the baseplate. Prior to sprouting, fructans of DPs 7-8 were no longer present in the top and bottom wedges, while they accumulated in the baseplate, irrespective of pre- or postharvest treatments. This redistribution of fructans within the bulb indicated a transition in dormancy state and could be used as a predictive marker for sprouting in stored onion bulbs. The application of this finding could significantly reduce onion bulb losses due to unpredictable sprouting during storage.

Keywords:

Allium cepa L., dormancy, sprouting, fructans, non-structural carbohydrates, abscisic acid, abscisic acid glucose ester, 7′-hydroxy-abscisic acid, phaseic acid, dihydrophaseic acid.

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%	percent
<	less than
=	equals
>	greater than
°C	degree Celsius
µg	microgram
µL	microlitre
µM	micromolar
1-MCP	1-methylcyclopropene
1-SST	sucrose:sucrose 1-fructosyltranferase
6G-FFT	fructan:fructan 6G-fructosyltranferase
7′-OH-ABA	7′-hydroxyabscisic acid
Å	Ångström
ABA	abscisic acid
ABA-GE	abscisic acid glucose ester
ANOVA	Analysis of variance
BBSRC	Biotechnology and Biological Sciences Research Council
BLAST	Basic Local Alignment Search Tool
ca	approximately
CA	Controlled atmosphere
cDNA	complementary deoxyribonucleic acid

cm	centimetre
CO_2	Carbon dioxide
CUDH2107	Onion double haploid lines
cv.	cultivar
CYP707A2	abscisic acid 8'-hydroxylase 2
d.f.	Degree of freedom
DBA	dormancy-break for bulbs stored in air
DBE	dormancy-break for bulbs stored under ethylene
de novo	anew
DEFRA	Department for Environment, Food & Rural Affairs
DI	deficit irrigation
DIA	deficit irrigated bulbs stored in air
DIE	deficit irrigated bulbs stored under ethylene
DIMA	deficit irrigated bulbs treated with 1-MCP and stored in air
DIME	deficit irrigated bulbs treated with 1-MCP and stored under ethylene
DMC	dry matter content
DP	degree of polymerisation
DPA	dihydro-phaseic acid
DW	dry weight
EFSA	European Food Safety Authority
ELSD	Evaporative Light-Scattering Detector
et al	And others

ET_c	crop evapotranspiration
EU	European Union
FAOSTAT	Food and Agriculture Organization of the United Nations Statistics
FEH	Fructan exohydrolase
FI	full irrigation
FIA	fully irrigated bulbs stored in air
FIE	fully irrigated bulbs stored under ethylene
FIMA	fully irrigated bulbs treated with 1-MCP and stored in air
FIME	fully irrigated bulbs treated with 1-MCP and stored under ethylene
FW	fresh weight
g	gram
HaPI	Horticulture and Potato Initiative
HPLC	High Performance Liquid Chromatography
in vitro	outside a living organism
IPCS	International Programme on Chemical Safety
kg	kilogram
L	litre
LC-MS	Liquid Chromatography-Mass Spectroscopy
LSD	Least significant difference
MH	Maleic hydrazide
mL	millilitre
mm	millimetre

NCBI	National Center for Biotechnology Information
NCED9	9-cis-epoxycarotenoid dioxygenase
ng	nanogram
NSC	non-structural carbohydrates
PA	phaseic acid
PBI-365	8´-methylene ABA methyl ester phosphate
PCR	Polymerase chain reaction
PP2C	Phosphatase protein type-2C
pmol	picomoles
postH	postharvest
preH	pre-harvest
PTFE	Polytetrafluoroethylene
PYR	pyrabactin resistance
PYL	pyrabactin resistance-like
qPCR	Quantitative polymerase chain reaction
RB	‘Red Baron’
RCAR	regulatory component of ABA receptor
RIN	Ribonucleic acid integrity number
RNA	Ribonucleic acid
rpm	Revolutions per minute
RR	Real-time respiration rate
RT-qPCR	Real-time quantitative polymerase chain reaction

SH	‘Sherpa’
SnRK2	sucrose non-fermenting 1-related protein kinase 2
SpA	sprout emergence for bulbs stored in air
SpE	sprout emergence for bulbs stored under ethylene
SPS	Sucrose phosphate synthase
UK	United Kingdom
USA	United States of America
v/v	volume by volume
viz.	namely
ZEP	zeaxanthin epoxidase
β	beta

1 Chapter One: Introduction

1.1 Project background

Onion (*Allium cepa* L.) is a member of the *Allium* genus with shallot, chives (*Allium schoenoprasum*) and garlic (*Allium sativum*) as other members of the genus. The *Alliums* are flowering monocotyledonous plants, found mainly in the temperate climates of the Northern Hemisphere (Brewster, 2008). They can grow from about 5-150 cm and most produce bulbs, which vary in sizes according to specie, while some produce thickened leaves (e.g. *A. fistulosum*). Over 500 species of *Alliums* have been identified and only a handful of these are used for human food consumption. Domestication of onion dates to over 5000 years and thus, onion is no longer growing as a wild species.

Onion is the most cultivated member of the *Alliums* and the most cultivated vegetable worldwide (FAOSTAT, 2017). An onion bulb is composed of a compressed stem known as the baseplate and juicy edible scales (modified leaves). The baseplate is usually underground, and the roots are attached below the baseplate, while the scales are attached to the top of the baseplate where they are arranged in a rosette form. At the innermost part of the rosette is the bud, which envelopes the meristematic tissues.

1.1.1 The onion morphology and life cycle

Onion can be propagated from seed, sets (small bulbs) or plantlets. After germination, the onion plant goes through a vegetative growth before producing bulbs (Figure 1-1). In the vegetative growth stage, the onion plant produces hollow leave structures comprised of leaf blades and scales attached to the top of a flattened base or compressed stem known as the baseplate. Morphologically, the leaves are arranged in a rosette form with the older leaves on the outside and the roots are found below the baseplate. Emerging younger leaves usually grow bigger in size when compared to the ones preceding them. However, at bulb initiation, this trend reverses

and the emerging younger leaves stops growing bigger than the ones before them, instead they become smaller (Coolong, 2007). Bulb production is initiated well ahead of the arrival of adverse environmental conditions. Thus, bulb initiation in onions is a predictive, rather than a consequential response to adverse conditions. At the successful completion of bulb initiation, the onion bulb progress to bulb enlargement and maturity and full maturity is characterised by the lodging of onion leaves, often referred to as "fall-down". Moreover, it was reported that at bulb maturity, the onion bulb produces two or three foliage leaf initials at the base (Coolong, 2007). The exact point at which these leaf initials are produced is not known, however, they elongate and becomes the sprouts for the next growing season.

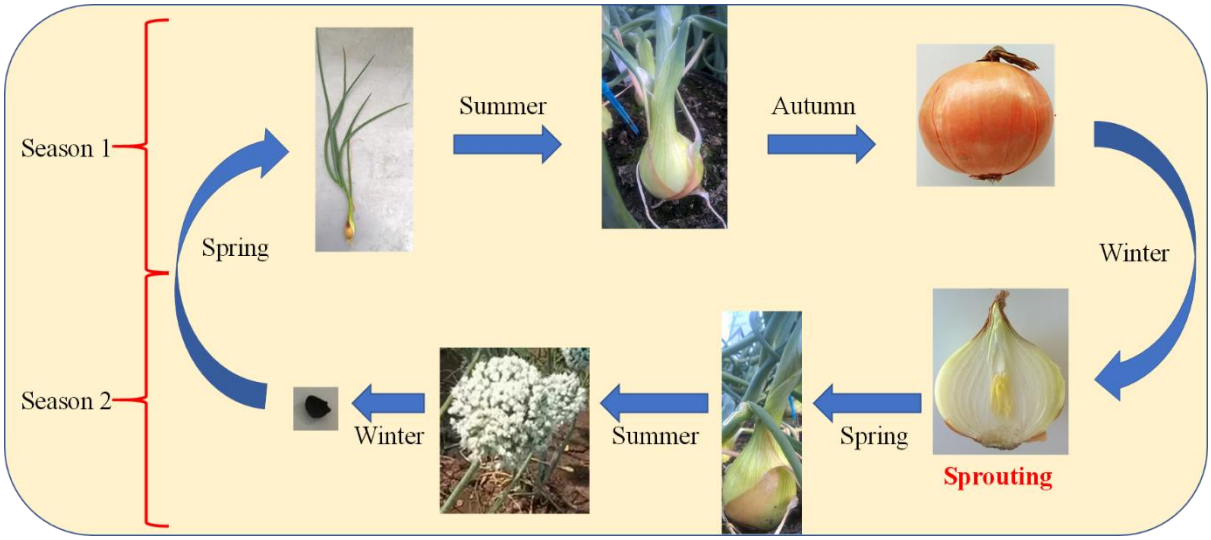

Figure 1-1. Depiction of onion biennial life cycle from seed through bulb formation, bulb dormancy and bulb regrowth

1.1.2 Onion ploidy and the production of the double haploid lines

Onion as with other *Alliums* belong to the order Asparagales, which are known to have the largest genome size of all eukaryotes (Kuhl *et al.*, 2004). Compared to other major crops such tomato and maize, the onion genome is 16 and 6 times larger, respectively (King *et al.*, 1998). Onion is a diploid (2n = 16) and its genome is 107 times larger compared to *Arabidopsis* (King *et al.*, 1998; Ohri *et al.*, 1998; Brewster, 2008; McCallum *et al.*, 2012; Shukla *et al.*, 2016).

Attempts to circumvent the hurdle of this large genome size led to the release of 10 000 onion expressed sequence tags (Kuhl *et al.*, 2004), which resulted in the mapping of some these genes (Martin *et al.*, 2005). Furthermore, McCallum *et al*., (2006) produced a map of the genes proposed to affect fructans content. Despite these, the heterozygosity of onion genome still poses a challenge in the analyses of sequence data and the sharing of germplasm. The development of onion double haploid (DH) lines 'CUDH2107' at Cornell University (USA) by Khosa *et al*., (2016) seems promising to the future of onion genetics studies, as the DH lines have a single copy for each gene. Thus, it becomes easier to use more common molecular techniques (e.g. gene knockout) to study and characterise genes. Briefly, the development of onion DH lines involved crossing of open pollenated onion (*Allium cepa* L.) with *Allium roylei*, a wild relative of onion that is resistant to *Botrytis* leaf blight used as the selection marker. Afterwards, the F_1 and F_2 and backcross generations (BC_1F_1 x BC_2F_2) were used to generate individual flower buds that were sterilised by treating the flowers with dichloroisocyanuric acid disodium salt (at 16.6 g L^{-1}) for 10 mins with a few drops of Tween 20. The sterilised flowers were then used in cell culture to regenerate plantlets used for ploidy selection and eventual identification of the DH lines. More detailed method of onion DH line development has been fully described elsewhere (Alan *et al*., 2003, 2004). Notwithstanding, these plausible efforts towards improving onion genetic studies, investigations into the molecular regulations of the biosynthesis of the metabolites (e.g. fructans) whose content have been linked to dormancy is stored onion bulbs is conspicuously lacking.

1.1.3 Economic importance of onion

Onion is the most cultivated vegetable, and only second to tomato on the list of the most cultivated fruit and vegetables worldwide (FAOSTAT, 2017). Over 111 million tonnes of onion bulbs were produced globally in 2016 (FAOSTAT, 2017). In the same year, the UK produced over 394 kilo tonnes (kT) (DEFRA, 2016), which corresponded to 30% and 1.5% increases on the global and UK productions, respectively, when compared to 2014. This is consistent with previous reports that the UK onion demand increases year-on-year (Terry *et al.*, 2011). Despite its home-grown onion production, the UK is not self-sufficient yet as it produces only half of its demand. Consequently, the UK further imported 392.7 kT in 2015 (DEFRA, 2016) to supplement the home production. Presently, the UK onion industry is valued at £245.8 million per year, with the value of home produced and imported onion bulbs valued at £106.1 million and £139.7 million, respectively (DEFRA, 2016). Nevertheless, up to 10%

of the UK total onion bulbs is lost during postharvest to sprouting and diseases (Terry *et al.*, 2011).

1.1.4 Sprout mitigation practices

1.1.4.1 Curing

Onion practitioners have adopted certain practices (e.g. curing, treatments and manipulation of the storage conditions) aimed at delaying sprout growth in onion bulbs meant for storage purposes. Curing is the process of enhancing water-loss from the outermost scales of onions to create the dried outer layers and colours associated with the onion bulb skin. At the end of curing, the bulb neck is sealed to reduce water-loss and pathogenic infections (Downes *et al*., 2009; Schroeder and du Toit, 2010; Eshel *et al.*, 2014; Mishra *et al.*, 2014). In addition, curing enhances the appeal of onion bulbs by improving the skin colour, typical of cultivars (Cools, 2010). Commercial curing involves the stacking of onion bulbs into crates in a tunnel-like enclosure and forcing heated humid air (at 28 – 30℃ and 65 – 75% humidity) circulation through the crates. In the UK, onion bulbs are cured for 3 - 6 weeks after which the temperature is slowly reduced to a desired cold storage temperature. There are no clear markers to identify when curing is complete, the decision is upon the practitioner, however, curing is thought to be complete when the onion skin is dry and papery. Furthermore, Nega *et al*. (2015) showed that curing delayed sprout growth in stored onion bulbs. That said, curing alone is not enough to delay sprouting for the length of onion bulb storage. Thus, there is need for the combination of curing with other layers of treatments to delay sprout growth in stored onion bulbs.

1.1.4.2 Maleic hydrazide

Sprout growth impacts negatively on the two major selling points of onions -taste and flavour. Current practices and the industrial standard for sprout mitigation involve the use of maleic hydrazide (MH), a synthetic chemical applied as a foliar spray on onion plants. MH is then assimilated into the leaves and translocated to the bulbs, where they elicit the widely reported sprout suppression in stored onion bulbs (Ilić *et al.*, 2011). Despite its effective sprout suppression attributes, MH residues are known to linger in onion bulbs (Ilić *et al.*, 2011). In addition to the potential link of MH to human health conditions (Ribas *et al.*, 1996), the European Union is continuously re-evaluating the use of MH in fresh produce (European Commission, 2002). These taken together have raised concerns and uncertainties around the continued use of MH in the future and thus, have created the need to find alternatives to MH.

1.1.4.3 Ethylene and 1-methylcyclopropene

The quest for alternatives to MH have revealed that the application of ethylene and its antagonist 1-methylcyclopropene (1-MCP) delay sprout growth in stored onion bulbs. Despite being low producers of ethylene, the exogenous application of ethylene to onion bulbs has been widely reported to delay and suppress regrowth during storage (Bufler, 2009). In the fruit industry, ethylene hastens ripening, a desired outcome for consumers. However, unpredictable and uncontrolled ripening is a source of major losses to the fruit industry. To slowdown the ripening process of climacterics, the fruits are treats with 1-methylcyclopropene (1-MCP) to inhibit the ethylene-related ripening. Accordingly, 1-MCP is perceived as an antagonist of ethylene activities. However, onion bulbs treated with 1-MCP and ethylene, whether independently or in combination were reported to experience a delay in sprout growth (Chope *et al*., 2006; Downes *et al*., 2010; Cools *et al*., 2011). Thus, 1-MCP and ethylene are touted as potential replacements for MH.

1.1.5 Deficit irrigation and its implication on onion

In addition to pre- and postharvest substance applications and treatments, water-stress during pre-harvest such as deficit irrigation have been reported to affect the sprouting in stored onion bulbs (Biswas *et al.*, 2010; Leskovar *et al.*, 2012; Vickers, Grove and Monaghan, 2015). Onion is a shallow rooted plant whose roots are found mainly in the top 40 cm soil depth with none found beyond 76 cm (Weaver and Bruner, 1927) and with roots this close to the soil surface, onions is susceptible to drought stress. There are various methods for plant water-stress measurement, for example, pressure chamber ("pressure bomb"), gas exchange, chlorophyll fluorescence and chlorophyll content. Water-stress is known to alter a plants water potential as reflected in the sap pressure, which is defined as the pressure needed to push back the sap flow from a cut plant's surface back into the plant (Waring and Cleary, 1967). The less this pressure needed, the more water-stressed the plant is and this can be measured using a pressure chamber (Scholander *et al.*, 1965). Water-stress also causes a reduction in photosynthesis as reflected in reduced transpiration and CO_2 assimilation (Baker, 2008). Nevertheless, some adjustment is required to ensure the accurate amount of CO_2 (measured using infrared gas analysers) is recorded. This is due to the overlap in the CO_2 and water absorption spectra (Long, Farage and Garcia, 1996). Chlorophyll fluorescence measured with chlorophyll fluorometers shows the photosynthesis efficiency of plants and water-stressed plants are known to have a low photosynthesis efficiency, both in light and in the dark states. Chlorophyll content is measured

with a chlorophyll content and the reading could be correlated to nitrogen deficiency (Majid *et al.*, 2015). A low chlorophyll content indicates a reduction in photosynthesis and transpiration and thus, evapotranspiration could be used to differentiate between water-stressed and non-water-stressed plants. Due to its root morphology, the onion plant requires constant but light water application to achieve maximum yield. Recently, the reliance on irrigation as a supplement to rainfall or the major source of water for crop production is on the rise. Thus, there is need for some irrigation water management strategies. Deficit irrigation (DI), the application of irrigation water below a plant's water need, has become the preferred strategy. The rationale behind DI practice is such that water saved from DI could be used to irrigate other high-quality crops that are not usually irrigated (FAOWater, 2015). Thus, any yield penalties that may have been accrued from DI practice will be compensated for by the extra yield from the irrigated high-quality crops. Notwithstanding that the storage quality of onion bulbs is key to its continuous supply, majority of DI studies on onion have focused on the bulb yield penalties with little or no interest on the potential impacts on the storage quality.

For instance, studies that evaluated the impact of DI on onion plants subjected to the replenishment of evapotranspiration (ET_c) at 100 (full irrigation – FI) or 50% (DI) (Bekele and Tilahun, 2007; Ayas and Demirtaş, 2009; Igbadun *et al*., 2012; Leskovar *et al*., 2012) reported that DI significantly reduced bulb yield compared to FI. Leskovar *et al*. (2012) reported that DI at 50% reduced onion yield by 19 – 27% over a two-year experiment, which is similar to the 15.5 – 23% reduction reported by Igbadun *et al*. (2012). These amongst other depths of onion DI treatments such as reported elsewhere (Al-Jamal *et al.*, 2000; Nagaz *et al*., 2012; Wakchaure *et al.*, 2018) have all reported some levels of yield deficits. This could be the reason for the slow adoption of DI practices in onion production. The success of DI treatments, however, is dependent on a sound knowledge of a crop's responses to DI at different growth and developmental stages. This knowledge is paramount in the determination of what level of DI to apply, at what stage and for how long to minimise yield losses while saving water. Four major stages of growth and development have been identified in onion namely: initial stage (up to the first 20 days after germination), crop development (40 – 55 days after germination), mid-season (50 – 110 days after germination) and late season (the last 40 - 45 days) (FAOWater, 2015). Successful application of DI is also dependent on the understanding of the crop coefficient (K_c) and yield responses to water-stresses associated with a crop's growth and developmental stages. The K_c reported for onion are 0.4 – 0.7 (K_c for initial and crop development stages), 0.85 – 1.05 (mid-season) and 0.6 – 0.85 (late season), corresponding to

the following yield responses 0.45, 0.8 and 0.3, respectively (Pérez Ortolá and Knox, 2014; FAOWater, 2015). K_c and yield response define the levels of sensitivity to water deficit, for example, applying DI at higher K_c (e.g. mid-season) will have the most impact on the plant as reflected on the yield response of 0.8. Besides transplanting of onion seedlings (which could lead to young plants losses), bulb initiation is the most water sensitive stage, thus, applying DI at this time is known to have the most impact on yield (Al-Jamal *et al*., 2000; Martín de Santa Olalla *et al*., 2004; Pérez Ortolá and Knox, 2014; FAOWater, 2015). In contrast, the vegetative growth stage is the most water-stress resistant stage. These taken together suggests that the application of DI at bulb initiation will have the most impact on the onion bulb. Moreover, studies on the effects of DI on onion bulb storage quality is scarce and the few available studies are contradictory. Rattin *et al*. (2011) and Vickers *et al*. (2015) reported that DI caused earlier sprouting in stored onion bulb, while Biswas *et al*. (2010) and Leskovar *et al*. (2012) reported the opposite. Notwitstanding, none of these studies applied any postharvest treatments such as ethylene or 1-MCP, which have been revealed to delay sprouting in stored onion bulbs.

1.1.6 Hormonal regulation of dormancy

Although the current sprout mitigation practices have focused on physiological tackling of sprout growth, it is thought that understanding the mechanisms behind the regulation of onion bulb dormancy may be key to a more robust sprout mitigation practice. Dormancy in onion is a state when the growth of all the meristematic tissues is arrested (Terry *et al*., 2015). Onion bulbs at full maturity goes into a quiescence state known as dormancy and sprouting only occurs at the expiration of the dormancy period known as dormancy-break. At dormancy-break, the meristematic tissues are released for regrowth to complete the onion biennial cycle. Therefore, a more efficient sprout control may depend on the understanding of the mechanisms underlying dormancy regulation and dormancy-break, the processes preceding sprouting. An area that is not currently receiving the attention it deserves.

The regulation of dormancy in plants involve these phytohormones auxin, cytokinin, gibberellin (GA), ABA and ethylene. Auxin mediates phototropism, geotropism, ion fluxes and sex expression (Wang and Irving, 2011). In relation to dormancy, auxin is known to regulate cell division, cell expansion and the hyperpolarisation of the plasma membrane necessary for triggering the actions attributed to auxin (Arteca, 1996; Kepinski and Leyser, 2002; Kulaeva and Prokoptseva, 2004; Wang and Irving, 2011). It has also been suggested that auxin may stimulate the biosynthesis of GA while suppressing ABA biosynthesis and ethylene (Koshioka

et al., 1994; Nitsch *et al.*, 2009). Cytokinin is usually found at high concentrations in the meristematic tissues, young leaves, roots, seeds and developing fruits where it modulates cell division, organ differentiation, senescence and controlling stomatal movement (Arteca, 1996; Kulaeva and Prokoptseva, 2004). GA is known to promote flowering, cause plant elongation, and releases seed dormancy (Bewley, 1997; Brady and McCourt, 2003; Finkelstein *et al.*, 2008; Wang and Irving, 2011). GA is synthesised from geranylgeranyl diphosphate and its perception involves intracellular Ca^{2+}/calmodium increase, corresponding to a decrease in pH with a concomitant elevation of cGMP as the second messenger (Richards *et al.*, 2001). This GA-related Ca^{2+}/calmodium signalling is important in GA-induced dormancy release in seeds as it causes the synthesis and secretion of hydrolases, a process known to be inhibited by ABA (Sun and Gubler, 2004). ABA is known to be a mediator of plant stress and regulates the guard cells opening and closing of the stomata in accordance with drought responses. ABA was initially thought to cause abscission, due to its activation of cellulase and polygalacturonase (Cracker and Abeles, 1969). Cellulase and polygalacturonase catalyse the breakdown of cellulose and pectin, the two most common compounds responsible for the intactness of plant organs (Zhang and Zhang, 2009). However, later studies have assigned the regulation of abscission to ethylene and auxin (Taylor and Whitelaw, 2001), whereas ABA mediates senescence (Suttle, 2004). Senescence precedes abscission, therefore, the high content of ABA in abscised leaves and fruits quantified by Cracker and Abeles, (1969), may have been from ABA residues left over from senescence. That said, dormant plant tissues and organs are known to contain a high quantity of ABA, and regrowth occurs with the decline of ABA content. ABA inhibits seed germination by blocking the synthesis and secretion of hydrolases (Sun and Gubler, 2004). The hydrolases are necessary for the remobilisation of the energy reserve to make available the energy needed for regrowth to occur. ABA-induced dormancy and seed germination is dependent on the ABA/GA ratio, where seeds remain dormant so long as the balance favours ABA, and dormancy is released once it tilts towards GA (Khan, 1967). Hormones are known to cross-talk with one another. For instance, there is an overlap in auxin and GA functions in cell elongation, and auxin plays a role in GA biosynthesis. GA regulates cell elongation, which occurs after cytokinin regulated cell division (Khan, 1971) and the exogenous application of ethylene represses the expression of GA metabolic genes (Dugardeyn, Vandenbussche and Straeten, 2008). These taken together shows that phytohormones interconnect in a cascade of networks with one another in a synergistic, additive or antagonistic fashion to achieve the responses attributed to them. Moreover, auxin, cytokinin, GA and ethylene collectively are categorised as growth promoters and dormancy inhibitors, whereas ABA is the only dormancy-

promoting phytohormone (Finkelstein and Lynch, 2000; Finkelstein *et al*., 2002; Nambara *et al*., 2010; Garciarrubio *et al*., 2015; Kang *et al*., 2015). Thus, justifies the interest on ABA in the regulation of dormancy in onion bulbs as with other plants. Notwithstanding, the ABA-induced dormancy is dependent on the balance between its biosynthesis and catabolism.

1.1.7 ABA biosynthesis and signalling network

ABA biosynthesis starts in the plastid from carotene and ends with ABA production in the cytosol (Figure 1-2). The last five steps of ABA biosynthesis are catalysed by *ABA1*, *ABA4*, *NCED*, *ABA2*, and *ABA3*, respectively (Seo and Koshiba, 2002; Seiler *et al*., 2011; Finkelstein, 2013). However, the step catalysed by *NCED* is thought to be the committed step in ABA biosynthesis and this occurs in the plastid after which the succeeding steps occur in the cytosol (Thompson *et al*., 2000; Okamoto *et al*., 2006). In addition to the leaves, ABA is also synthesised in the roots and the presence of ABA in a soil solution has been reported, which can be taken up by the root (Hartung *et al*., 2002). Oftentimes, the site of a hormone synthesis differs from the site of its activities, as such there is a need for transportation from the source (site of synthesis) to the sink (site of activity). Thus, creating three major possible condition of hormonal signalling and response conditions: (1) when the signalling cells are far from the target cells, it is called a long distance response; (2) when the cells are adjacent to each other it is called a paracrine signalling and (3) autocrine is when the signlalling and target cells are the same cells (Norman and Litwack, 2014). Hormonal transduction is key to the accurate and timely responses to stimuli. In the ABA signalling pathway, the major players are *PYR/PYL/RCAR* receptors which are localised in the cytoplasm, where they exist as active dimers (Wasilewska *et al.*, 2008). However, upon the binding of ABA, the dimers dissociate and inhibits the activities of *PP2C*, which in turn causes *SnRK2/OST1* protein kinases to activate various cascade of downstream phosphorylation and activation steps. In turn, these enhances the synthesis of proteins and molecules responsible for remediation of stress-related damages through transcription factors and ion channels (Rodriguez, 1998; Wasilewska *et al*., 2008; Hauser *et al*., 2018). Nevertheless, there are more players involved in the ABA signalling pathway such as the receptors *FCA* and *CHLH* shown to bind to ABA *in vitro*, including *IP3* and phosphatic acid referred to as second messengers, which were reported to activate the Ca^{2+} channels within the guard cell's endoplasmic reticulum (Wasilewska *et al.*, 2008). For ABA transport, the *DTX/MATE-type* transporter *DTX50* and the *NRT1/PTR* family (*NPF*), which are dominantly localised in the plasma membrane have been characterised as the transporters

responsible for ABA export/import from source to sink (Hauser *et al.*, 2018). These *NPF* transporters are part of the bigger ATP-binding cassette (*ABCG*) transporter family, while some members of the *ABCG* have been found to be importers, the *DTX50* are the exporters (Hauser *et al.*, 2018). ABA synthesised in the leaves are transported through the phloem while the root synthesised ABA are transported through the xylem. As would be expected, each of these would experience hindrances and these are provided by the plasma membranes present in the exo- and endodermis. But due to its solubility, ABA is circulated in the water-based xylem sap and could move from root-to-shoot or vice versa. ABA movement within the plant is thought to be in its inactive glycosylated ester form, which can be rapidly activated be the excision of the glucose moiety by β-glucosidases in the apoplast and endoplasmic reticulum (Wasilewska *et al.*, 2008).

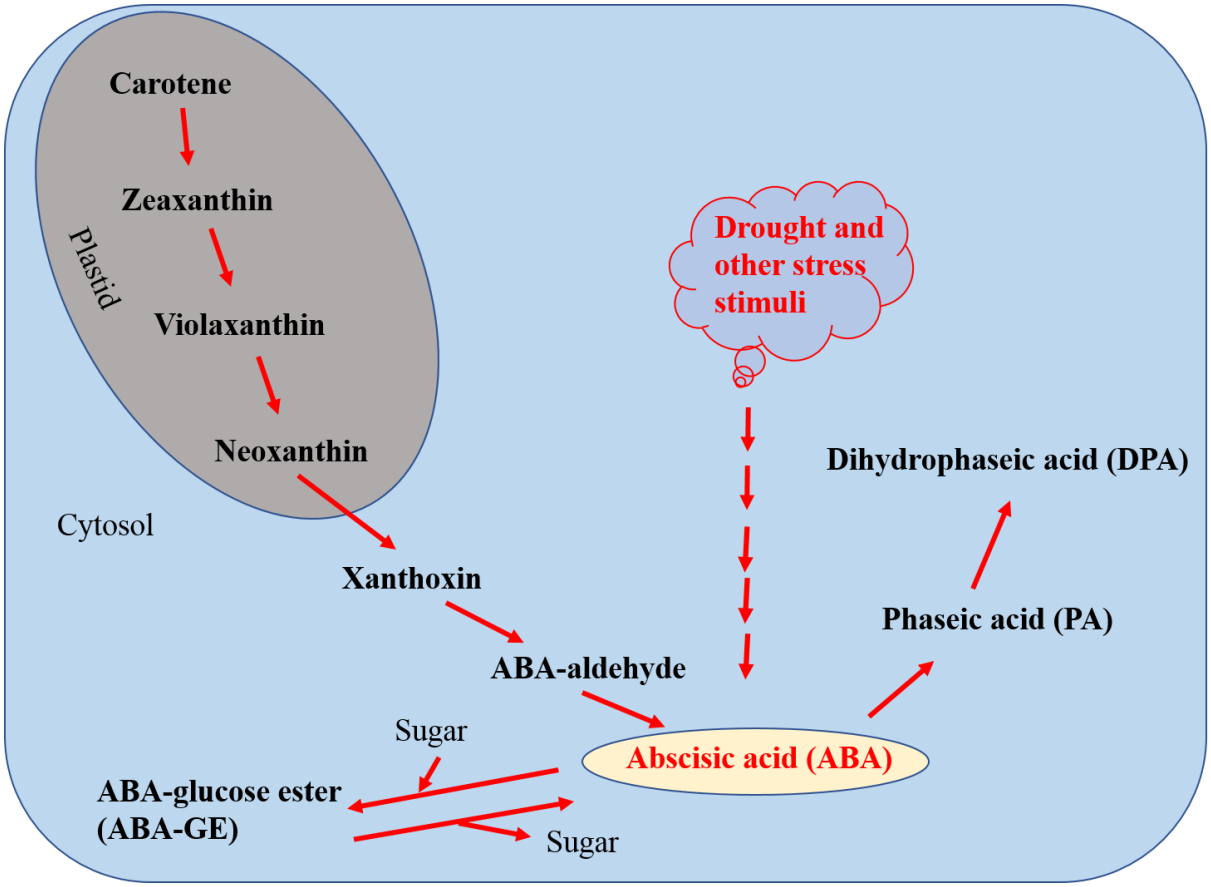

Figure 1-2. Schematic representation of the major steps of abscisic acid biosynthesis pathway

1.1.7.1 ABA catabolism and its metabolites

Dormancy-break in plant tissues and organs is characterised by the decline in ABA content, and ABA decline is either due to hydrolysis or catalysed by ABA 8'-hydroxylases (*CYP707A*)

or glycosylation (conjugation with glucose) catalysed by ABA glucosyltransferase. Glycosylation of ABA produces ABA-glucose ester (ABA-GE), the preferred form of ABA transport around the plant (Hartung, Sauter and Hose, 2002; Wasilewska *et al.*, 2008). Whereas, hydroxylation of ABA involves oxidation at the 8'-carbon position catalysed by 8'-OH-abscisic acid hydroxylase (*C707A2*) to form 8'-hydroxyabscisic acid (8'-OH-ABA) as the major route for catabolism (Rodriguez, 2016). Having said that, hydrolysis of ABA at carbon positions 7'- and 9' have been reported. The 8'-OH-ABA produced is quickly converted to phaseic acid (PA) and then to dihydroxyabscisic acid (DPA). The production of DPA from ABA through hydroxylation is thought to be an irreversible inactivation of ABA, while glycosylation to ABA-GE is a reversible deactivation of ABA, since the cleavage of the sugar moiety catalysed by β-D-glucosidases regenerates ABA. Moreover, ABA-GE is preferred to ABA as a means of circulation as it is easily absorbed into the xylem due to its higher hydrophilicity compared to ABA, and unlike ABA, ABA-GE experiences minimal losses or enrichments during transport (Hartung *et al*., 2002).

1.1.7.2 Implication of ABA in the regulation of onion bulb dormancy

Several studies on ABA regulation of dormancy were conducted either in seeds or fruit trees, while studies on other plants are scarce. In onion, ABA content was reported to decline continuously from a higher concentration prior to storage to a minimum during storage before sprouting occurs (Matsubara and Kimura, 1991; Chope *et al*., 2006). Chope *et al*., (2006) further found a higher concentration of ABA in long- and medium-storing onion bulbs when compared to the poor-storing onion bulbs. Thus, they postulated that an increase in the endogenous ABA content may delay sprouting, which inspired their attempts to increase the endogenous ABA content of onion bulb prior to storage. In one experiment, they applied an ABA analogue (8′-methylene ABA methyl ester; PBI-365) as a foliar spray and in the other they dipped onion bulbs in ABA solution before storage. Neither of these increased the ABA content or delayed sprout growth in onion bulb during storage. Moreover, despite the widely reported roles of ABA on the regulation of dormancy, its primary role is in the mediation of plant responses to water deficit (Socias *et al*., 1997; Jiang and Zhang, 2002; Manzi *et al*., 2015; Kuromori *et al*., 2018) under which ABA is known to accumulate. Interestingly, no studies have investigated whether water stress could be used to manipulate onion bulb ABA content.

1.1.8 Fructans biosynthesis and roles in onion dormancy

In addition to hormonal regulation of dormancy, non-hormonal regulations such as that of fructans are known. Fructans are polymers of fructose and together with fructose, glucose and sucrose are the only non-structural carbohydrates of onion. Similar to ABA, onion bulb fructans content was reported to be highest at harvest but declined continuously during storage until a minimum when sprout growth occurred (Suzuki and Cutcliffe, 1989; Jaime *et al*., 2001; Chope *et al*., 2012). Fructans vary in accordance with their degree of polymerisation (DP) and their biosynthesis starts with the transfer of fructosyl from one sucrose to another to synthesise 1-kestose, a fructan with a DP of 3 (Figure 1-3). The further elongation of 1-kestose to produce fructans with various DPs involves the transfer of fructosyl from sucrose or fructan to another fructan. The first step of the fructan biosynthesis is catalysed by sucrose:sucrose 1-fructosyltransferase (*1-SST*) or sucrose:fructan 6-fructosyltransferase (*6-SFT*) while the elongation is catalysed by fructan:fructan 6G-fructosyltransferase (*6G-FFT*), fructan:fructan 1-fructosyltransferase (*1-FFT*) or sucrose:fructan 6-fructosyltransferase (*6-SFT*) (Van den Ende *et al*., 1996; Hisano *et al*., 2008; Lasseur *et al*., 2011). In addition to DP, fructans also vary according to the fructosyl linkage types present in their structures. There are three major classes of fructans found in plants *viz.* inulin, levan and gramian; and these differ in the type of fructosyl linkages present in their structure. At the initial steps of fructans biosynthesis, the fructosyl moiety from a donor (sucrose or other fructans) could be attached to either one or the two primary hydroxyl groups at positions 1 or 6 to form β-2,1-linkages, β-2,6-linkages, or both β-2,1- and β-2,6-linkages to produce the inulin, levan or gramian classes of fructans, respectively (Ende, 2013). The class of fructans found in onion are the inulin type, and while fructans with DP of up to 100 are thought to occur in various plants, the highest number reported for onion so far is fructans with DP15 (Ernst *et al.*, 1998). Thus, due to the shorter fructans molecules found in onion, its fructans are often referred to as fructo-oligosaccharides. The first step of onion fructan biosynthesis is catalysed by sucrose:sucrose 1-fructosyltransferase (*1-SST*) while elongation is catalysed by fructan:fructan 6G-fructosyltransferase (*6G-FFT*) (Shiomi, 1989; Vijn *et al*., 1997,1998; Havey *et al*., 2004), which are localised in the vacuole. Thus, fructans biosynthesis and catabolism are known to occur in the vacuole. It is presently unclear how fructans are transported out of the vacuole and around the plants. That said, one possibility is that fructans are broken down and transported as sugars and could be re-synthesised if there is need for storage, since both the anabolic and catabolic enzymes are present in the vacuole. However, this changed when fructans, their

biosynthetic and catabolic enzymes were found in the apoplast of cold-stressed plants, thus, Valluru *et al.* (2008) suggested the possibility of a vesicle-mediated transport system for fructans in plants. Overall, the comprehensive understanding of fructans transport within the plant will depend on future studies.

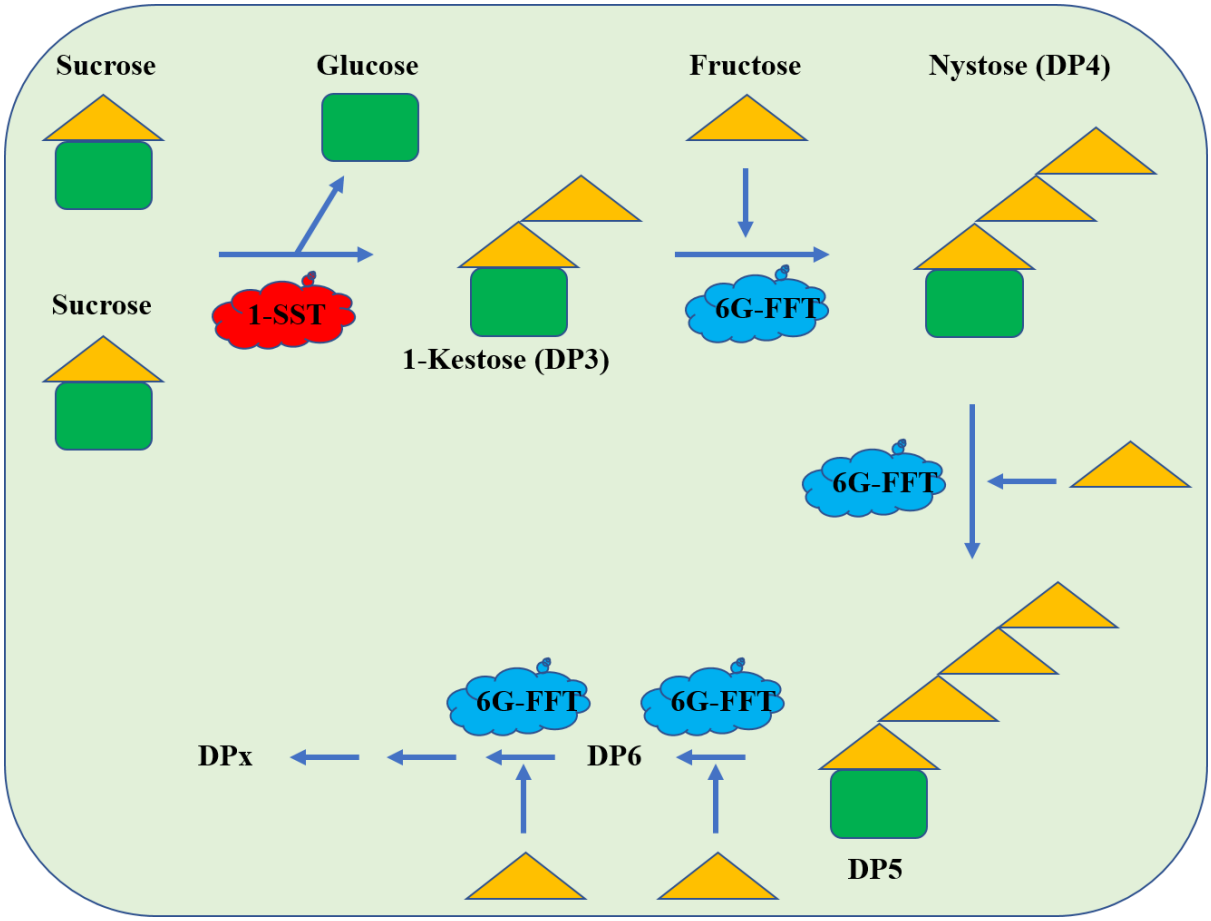

Figure 1-3. Depiction of onion biosynthesis pathway showing the steps catalysed by *1-SST* (sucrose:sucrose 1-fructosyltranferase) and *6G-FFT* (fructan:fructan 6G-fructosyltransferase); DP is degree of polymerisation while DPx represents fructans with higher DPs.

1.1.8.1 Fructans accumulation in response to environmental factors

In addition to the primary function of fructans as an energy reserve, they also offer some protection against environmental stress such drought, salt-stress and frost (Vijn and Smeekens, 1999; Kerepesi and Galiba, 2000; Ritsema and Smeekens, 2003; Livingston *et al.*, 2009). Under water stress, excess sugars are mopped up through fructans biosynthesis and at the return of normalcy fructans are hydrolysed to into sugars to balance the osmotic pressure. Under frost

conditions, a fructans-membrane interaction have been reported where fructans attach to the lipid headgroups in the cell membranes, thus, eliciting some anti-freezing effects (Vereyken *et al.*, 2003). Overall, fructans content are known to increase in plant tissues grown under water-stress such as DI (Vijn and Smeekens, 1999; Kerepesi and Galiba, 2000; Livingston *et al.*, 2009; Asega *et al.*, 2011; Cimini *et al.*, 2015), even in genetically engineered rice (A. Kawakami, Sato and Yoshida, 2008), including transgenics such as tobacco (Pilon-Smits *et al.*, 1995; Li *et al.*, 2007;) and maize (Ober and Setter, 1992). Despite these and the implication of fructans in the regulation of onion dormancy, it is not known whether DI could be used to enhance fructans accumulation in relation with onion bulb storage qualities.

1.1.8.2 Fructans catabolism and the underlying mechanisms

Fructans accumulation starts when the rate of energy production from photosynthesis becomes higher than its consumption (Pollock, 1984). The onion bulb during its vegetative growth serves as a sink organ, especially for fructans that are synthesised and stored in the vacuole. However, the onion bulb becomes the source organ upon which the energy to survive its dormancy period must be drawn. Thus, the onion bulb transitions from being a sink to a source organ, although it is still unclear at what point this transition occurs. That said, the transition from sink to source organ is characterised by a decline in fructans content signifying a pronounced hydrolysis of fructans. An occurrence associated with dormancy-break and active sprout growth. Fructans hydrolysis is catalysed by fructan exohydrolase (*FEH*) where the terminal fructose is hydrolysed creating fructans of shorter DP and fructose. Thus, the hydrolysis of fructans yields fructose. Fructan 1-exohydrolase (*1-FEH*) is reported to evolve from cell wall invertase, a glycoprotein also known as beta-fructofuranosidase, which catalyses the cleavage of the terminal non-reducing betafructofuranoside residues (Kulshrestha *et al.*, 2013). These taken together would suggest that the reported decline in the fructans content of onion bulb during storage (Suzuki and Cutcliffe, 1989; Jaime *et al.*, 2001; Chope *et al.*, 2012) is due to *FEH* hydrolyses. Having said that, unlike 1-SST and 6G-FFT that have already been cloned and characterised in onions (Vijn *et al.*, 1997, 1998), the quest for onion *1-FEH* continues. Moreover, despite the cloning and characterisation of *1-SST* and *6G-FFT* and their potential roles at maintaining onion bulb dormancy, their activities in relation to fructans metabolism in stored onion bulbs has not been reported.

Overall, ethylene and 1-MCP delay sprout growth in stored onion bulbs and higher accumulation of ABA and fructans contents have been correlated with extending onion bulb

dormancy. ABA and fructans are known to accumulate in plants grown under DI, no studies have investigated this in onion. DI treatment at 50% replenishment of ET_c is known to cause significant yield deficit and the application of water-stress at bulb initiation is known to have the biggest impact on onion bulbs. Thus, starting water-stress at bulb initiation may have the most impact on ABA and fructans accumulation. That said, no studies have investigated this in onions. The roles of ABA and fructans on the regulation of dormancy hinge on their metabolism and probably distribution, however, the influence of DI on these in relation to dormancy in stored onion bulbs is not known. The two genes (*1-SST* and *6G-FFT*) involved in onion fructans biosynthesis have been cloned and characterised, but their activities in onion bulbs during storage is not known, probably due to the knowledge gap on onion genetics. This knowledge gap is mainly due to the large size of onion genome and its ploidy. However, the development of the onion DH lines was a breakthrough and promising for the future of onion genetics. Whether these onion DH lines could enhance the understanding of the molecular regulation of onion bulb dormancy during storage has not been reported. This project was sponsored by the Biotechnology and Biological Sciences Research Council (BBSRC: BB/K02065X/1) to investigate the regulation of onion dormancy using deficit irrigation.

1.2 Aim and objectives

1.2.1 Aim

The aim of this PhD project was to understand the mechanisms of onion bulb dormancy as influenced by deficit irrigation and postharvest storage regimes.

1.2.2 Objectives

1. To investigate the sprout responses in stored onion bulbs as influenced by pre-harvest deficit irrigation (DI), and postharvest treatments such as continuous ethylene supplementation and 1-MCP, in relation to dormancy and sprouting in stored onion bulbs.
2. To determine the combined effects of DI, continuous ethylene supplementation and 1-MCP, and the interactions thereof, on the accumulation, distribution and metabolism of ABA, in relation to onion dormancy.
3. To evaluate how DI, continuous ethylene supplementation and 1-MCP treatments regulate the accumulation and distribution of fructans and the molecular responses of the genes involved in fructans biosynthesis in relation to dormancy in stored onion.

1.3 Thesis structure

The thesis adopted a "paper-format" and is divided into six chapters. Chapter 1 introduces the background of the project such as the choices of treatments and timing of pre- and postharvest treatments, and the metabolites analysed. This chapter also included the aim and objectives and concludes with the thesis structure (Figure 1).

Chapter 2 details the effects of deficit irrigation (DI) on onion bulb dormancy and sprouting during storage. The effects of ethylene, 1-MCP and combined treatments of ethylene and 1-MCP on onion bulbs grown under DI have been shown for the first time.

Chapter 3 showed a higher accumulation of ABA in the baseplate (containing the meristematic tissues) of DI bulbs compared to the fully irrigated (FI) bulbs, and how these changed during curing and throughout the storage period. The differences in the longitudinal distribution and how these responded to bulb progress towards sprouting was revealed. The metabolites of ABA were often thought to possess no biological activities, the discussion of the results in this chapter questions these assumptions. This chapter concluded with the evaluation of the current cross-talk between ABA and sugars, by suggesting that the metabolites of ABA may be involved in this cross-talk.

The results described in Chapter 4 showed that DI did not cause a differential accumulation in the total fructans content. Fructans with higher degree of polymerisation (DP) (DP7 and DP8) were found in the top and bottom but not in the baseplate sections of the onion bulbs prior to storage. However, before sprouting these higher DP fructans had disappeared from the top and bottom sections and accumulated in the baseplate. It was unclear if this redistribution was due to translocation or localised synthesis; therefore, the gene expressions of the gene involved in sucrose and fructans biosynthesis were conducted. The expression analyses suggested that these higher DP fructans were mainly translocated to the baseplate. That said, this redistribution was interpreted to have identified dormancy-break. Dormancy-break and sprouting were often used interchangeably, however, the discussion in Chapter 4 used this redistribution of fructans prior to sprouting to clearly differentiate between dormancy-break and sprouting in stored onion bulbs. Chapter 4 concluded that this redistribution of fructans could be used as a marker to predict dormancy-break and sprouting.

A list of where the results from this study have been presented or published is shown in 5.9Appendix L

The highlights, recommendations for the industrial applications, and how this project aligns with the goal of improving storage qualities of onion bulbs and potentials for future works are integrated into a general discussion in chapter 5.

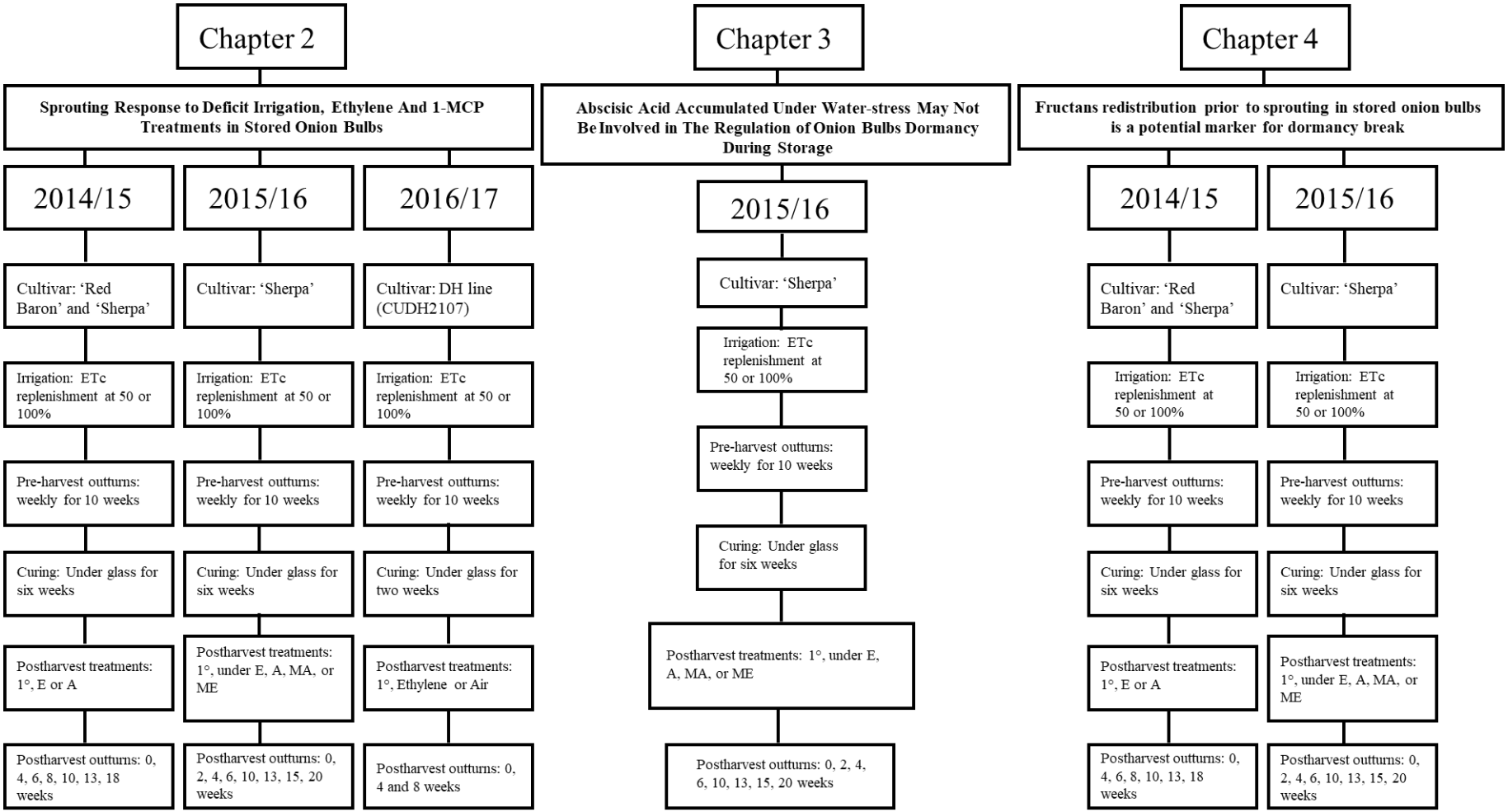

Figure 1-4. Depiction of the plant materials, pre- and postharvest treatments and methods used for each experimental chapter. Where ETc = crop evapotranspiration, E = storage under ethylene, A = storage in air, MA = 1-MCP-treated and stored in air, and ME = 1-MCP-treated and stored under ethylene.

1.4 Declaration

All experiments and analyses reported here was conducted by the author.

2 Chapter Two: Sprouting Response to Deficit Irrigation, Ethylene and 1-MCP Treatments in Stored Onion Bulbs

2.1 Abstract

A three-year study on onion cultivars 'Red Baron', 'Sherpa' and double haploid (CUDH2107) seeds was conducted to investigate (1) the impact of deficit irrigation (DI), (2) ethylene supplementation and (3) 1-methylcyclopropene (1-MCP) on dormancy and sprouting in stored onion bulbs. Ethylene and 1-MCP have previously been shown to inhibit sprout growth in stored onion bulbs, whilst studies on the effect of DI on sprouting in stored onion bulb are sparse. However, no studies have studied the combined effect of DI and postharvest treatments. Onion plants were grown in the glasshouse and were subjected to full irrigation (FI - ET_c replenished at 100%) or DI (ET_c replenished at 50%) from bulb initiation until two weeks before harvest. Bulbs were harvested at full maturity (i.e. at 100% fall-down when all plants have lodged) and cured under glass for six weeks. Thereafter, the bulbs were treated with or without 1-MCP at 1 µL L^{-1} 24h prior to storage and stored at 1°C under continuous ethylene supplementation at 10 µL L^{-1} or air. DI significantly reduced the amount of water-loss during curing and bulb weights for all years. DI and 1-MCP as independent factors neither influenced sprout emergence nor suppressed sprout growth. However, ethylene delayed sprout growth in both DI and FI bulbs and in combination with 1-MCP suppressed sprout growth in both DI and FI bulbs. These results suggested that DI can be adopted in regions where water availability is a constraint to onion production without compromising onion bulb storage qualities, nevertheless, with some yield deficit.

2.2 Introduction

Onion is the second most economically important crop of all fruits and vegetables produced worldwide. With an annual production of over 88 million tonness globally (FAOSTAT, 2017). The UK produces over 394 thousand tonnes of onion yearly, which is about half of its market demand with the rest imported to meet demand (DEFRA, 2016). An onion bulb is composed of 85 % moisture (Mitra *et al.*, 2011); but the plant is shallow rooted with 80 % of its roots within the top 40 cm of soil depth and none beyond 76 cm (Weaver and Bruner, 1927). Therefore, onion plants require frequent but light irrigation

throughout the growing season to maximise yield. Water is one of the major limiting factors in agricultural crop production and it was estimated that to produce 1 g of organic matter, plants will need to transpire approximately 500 g of water (Gonzalez and Reigosa, 2001). With this and the high moisture content of onion bulbs, they can be classed as a high-water requirement plant. Agricultural crop production accounts for over 70 % of the total freshwater extractions by humans (Davies and Bennett, 2015) – an unsustainable rate of water extraction that demands a change in the strategy of water management in crop production. One of the evolving strategies in agricultural water management is deficit irrigation (DI), an irrigation strategy where water applied is below the crop evapotranspiration (ET_c) at certain growth and developmental stages. Previous DI studies on onion often reported certain levels of yield deficit (Kadayifci *et al.*, 2005; Ayas and Demirtaş, 2009; Nagaz *et al*., 2012), however, the storage quality of onion bulbs is equally important, since they are stored for up to ten months.

Onion is a biennial crop, which produces a bulb in the first season and seeds in the second season. The bulb producing cycle is exploited for human food production. Thus, onions are cultivated mainly as an annual crop. Although a biennial crop, the demand for onion bulbs is all-year-round and extended storage to ensure continuous supply is required. However, onion bulbs are known to produce sprouts during storage. Sprouting, although a natural phenomenon to the onion bulb in the completion of its biennial life cycle, is accompanied by various changes which adversely affect the quality and marketable attributes of onions (e.g. visual appearance - size and finish, firmness, flavour and taste). Sprouting is a major contributor to bulb losses during storage (Terry *et al.*, 2011). Reports on the role of DI on postharvest quality of onion bulbs are contradictory. Rattin *et al*. (2011) and Vickers *et al*. (2015) reported that DI caused early sprouting and greater postharvest losses; Leskovar *et al*. (2012) reported an increase in postharvest storage life and quality; while Martín de Santa Olalla *et al*. (2004) and Enciso *et al*. (2009) found no such differences. Notably, none of these previous studies combined DI with any postharvest sprout delay treatments in accordance with the industrial standard.

Presently, the standard sprout mitigation practice adopted by the onion industry currently involves the use of maleic hydrazide (MH – 1, 2-dihydro-3, 6-pyridazinedione). MH is a synthetic chemical applied as a foliar spray when the leaves are still green, two or three

weeks prior to lodging of the leaves and harvest (Wittwer *et al.*, 1950; Isernberg, 1956; Gajewski and Majewski, 2008). The application of MH whilst the leaves are green is to ensure successful absorption and translocation to the bulb, prior to senescence after which translocation will be impossible. That said, earlier application is discouraged as it could be broken down before storage. Therefore, MH is applied 2 – 3 weeks prior to lodging and harvest (Wittwer *et al.*, 1950; Isernberg, 1956; Reynolds *et al.*, 2002; Gajewski and Majewski, 2008). Although there is a paucity in knowledge on the mechanism of MH sprout suppression activities, a few suggestions have been made. These include enhancement of sister chromatid and chromosomal aberrations (Rank and Nielsen, 1997; Del Campo and Coletto, 1998), inhibiting lipid synthesis (Müller and Mondy, 1977) and arrest of the breakdown of the spindle (Rees *et al*., 2012), which is important in cell division associated with sprouting. Despite being an effective sprout suppressant, MH residues have been reported in onion bulbs (Ilic *et al.*, 2011) and food samples, even after cooking (IPCS Inchem, 1976). There are also concerns over some potential human health implications (Swietlinska and Zuk, 1978; Ribas *et al.*, 1996). Moreover, the EU has an ongoing re-evaluation of MH aimed at reducing its use in fresh produce (European Commission, 2002; EFSA, 2011). Therefore, even though MH is an effective sprout suppressant in stored onion bulbs (Ilić *et al.*, 2011), the continued future use of MH is uncertain.

Studies to identify alternative sprout suppressants have revealed that exogenous application of ethylene at 10 µL L^{-1} suppressed sprout growth - whether applied for 24h prior to storage (Downes *et al*., 2010), or continuously during storage (Chope and Terry, 2008; Bufler, 2009; Cools *et al*., 2011 Chope *et al*., 2012). Ethylene, structurally is the simplest known plant growth hormone and the first gaseous signalling molecule found in any organism (Sisler and Yang, 1984; Ruduś, Sasiak and Kępc-zyński, 2013). Despite its structural simplicity and gaseous state, ethylene is involved in the regulation of complex plant growth and developmental activities such as seed germination, root growth and development, shoot growth, senescence of flowers and leaves, and fruit ripening (Abeles, Morgan and Saltveit, 1992; Chang, 2016). The most harnessed of these ethylene activities is the regulation of ripening in climacteric fruits, which is pivotal to the fruit supply chain. Ripening, although a desired outcome for fruit consumers an uncontrolled and unpredictable ripening is of concern to the fruit industry (Kende, 1993). Therefore, to

slow down the ethylene-induced ripening, fruits are treated with 1-methylcyclopropene (1-MCP), as such, 1-MCP is a known antagonist of ethylene activities. However, onion bulbs treated with 1-MCP at 1 µL L^{-1} applied for 24h prior to storage was also reported to suppress sprout growth, whether stored in air (Chope *et al*., 2006b) or in combination with continuous ethylene supplementation at 10 µL L^{-1}, which according to Cools *et al*. (2011) enhanced sprout suppression. Therefore, there are potential uses for ethylene and 1-MCP in onion storage in the future.

The aim of this study was to investigate how DI, continuous ethylene supplementation and 1-MCP treatments influenced sprouting in cold stored onion bulbs.

2.3 Materials and methods

2.3.1 Plant material

In this study, two different onion cultivars ('Red Baron' and 'Sherpa') and an onion double haploid line [CUDH2107] were used. In 2015, 'Red Baron' sets and 'Sherpa' seeds were sourced from Elsom seeds, Lincolnshire, UK, and Steve Howe Seeds, Lincolnshire, UK, respectively; while in 2016, 'Sherpa' seeds were sourced from Limagrain, Lincolnshire, UK. The onion double haploid lines used for the 2017 experiment was supplied by John McCallum of Cornell University, U.S.A. For all years, onion seeds were planted into trays with John Innes Compost No 1 (J. Arthur Bower's, The Garden Superstore, West Sussex, UK) and seedlings were transplanted into pots six weeks after germination, while 'Red Baron' sets were planted directly into pots. Final growth media was John Innes Compost No 3 (J. Arthur Bower's, Westland Horticulture Ltd, Huntingdon, UK). Equal weights of the growth media (7.3 kg) were measured into 96 pots of 8 L capacity per cultivar for 2015, 264 pots for 2016 and 96 pots for 2017 experiments. Plants were transplanted at the rate of three plants per pot (used as pseudo-reps) for experiments. Plants were split into three completely randomised blocks (replicates) formed across the benches in the glasshouse. The first and last rows of plants in blocks 1 and 3, respectively, served as guard plants (excluded from the analysis) for all years (Figure 2-1). Plants were fertilised with 800 mL of Hoagland's solution as two single 400 mL applications per treatment initially and four weeks after transplanting. Bulbs were harvested manually at full maturity when all plant foliage had lodged (100%

fall-down) and cured under glass for six weeks (August - September) for 2015 and 2016, while 2017 samples were cured for two weeks owing to sprout incidence found at harvest.

Bulbs from the onion double haploid lines (CUDH2107) used in the 2017 experiment had already sprouted at harvest. Although unclear why, it seems storage qualities was compromised in the circumvention of the hurdles of onion diploidy. Therefore, the CUDH2107 bulbs were excluded from the biochemical and molecular analyses in this study and were only used to investigate the effects of ethylene on already sprouted bulbs.

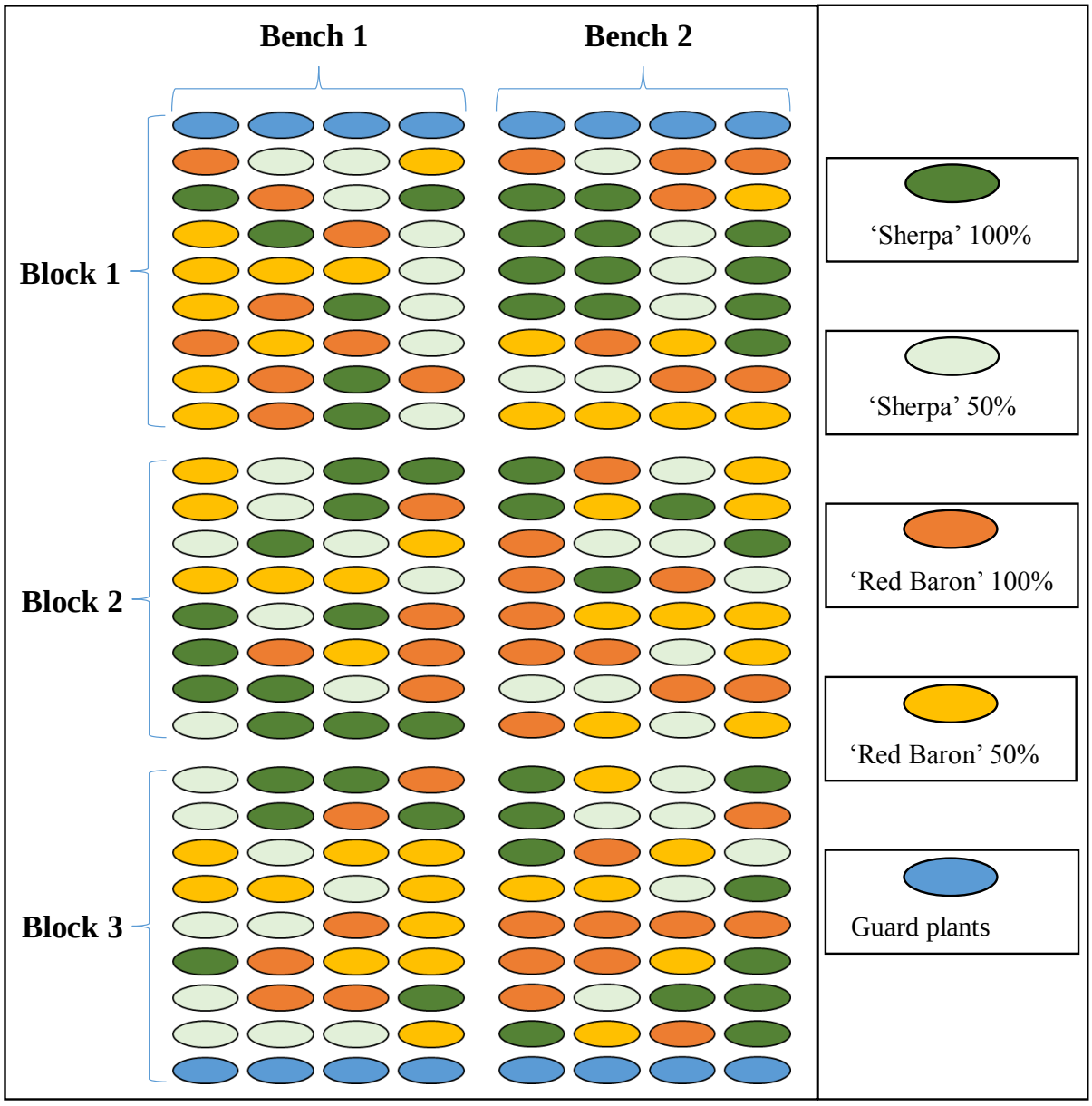

Figure 2-1. Schematic representation of the completely randomised block and pot arrangements in the glasshouse

2.3.2 Experimental design

Plants were subjected to full irrigation (FI) or deficit irrigation (DI) from bulb initiation; where FI and DI defined the replenishment of crop evapotranspiration (ET_c) at 100 and 50%, respectively. Irrigation was uniformly applied using an automated irrigation system (Hozelock model: AC4, Hozelock UK, Garden Irrigation & Automatic Watering Systems, Herts, UK). Polyvinyl pipes (20 mm diameter) were fitted with pressure-compensating emitters of 1.2 L min^{-1} flow rate, which were connected to polyvinyl tubes (5 mm diameter) to the pots (emitter per pot). Soil moisture content was monitored weekly both gravimetrically (based on pot weight) and volumetrically (using Delta-T soil moisture probes type: HH2 and ML2x, Cambridge, UK); three pots per treatment per block were assessed. The sensors of the soil moisture probe can only access the top 12 cm of soil depth, therefore, the volumetric soil moisture measurement in this study was conducted on the top 12 cm of the soil depth. All pre-harvest irrigation was stopped two weeks before harvest.

All three bulbs per pot were harvested manually, tagged, weighed together, and placed into a mesh bag. Afterwards, the bulbs were spread out in a single layer over the benches in the glasshouse for curing (18 – 35°C and 40 – 90% relative humidity). Bulbs were weighed weekly throughout the six-week curing period. After curing, bulbs were transferred to 100 L storage boxes and stored at 1°C for 18, 20 and 12 weeks in 2015, 2016 and 2017, respectively. A total of 12 storage boxes were used (3 per treatment used as replicates), and each box contains approximately 60 bulbs. For 2015 and 2017, bulbs were subjected to two treatments: continuous ethylene supplementation at 10 µL L^{-1} or continuous air, as described elsewhere (Chope *et al.*, 2006a; Amoah *et al.*, 2016). Ethylene gas source was from a 50 L cylinder of ethylene concentration of 5000 µL L^{-1} certified standard in nitrogen (BOC, Surrey, UK). This concentration was reduced to the targeted 10 µL L^{-1} by mixing ethylene with air through a manifold (HNL, Engineering Ltd, Stockton-on-Tees, UK) at a flow rate of 0.04 L min^{-1} and 9.46 L min^{-1}, respectively; air was supplied at a flow rate of 9.5 mL h^{-1}. For 2016, bulbs were treated with or without 1-MCP treated at 1 µL L^{-1} for 24 hours before storage; and stored in air or under continuous ethylene supplementation at 10 µL L^{-1} as described elsewhere (Cools *et al.*, 2011). At each sampling point, three bulbs were collected in triplicates, per treatment and

throughout the storage duration [3 bulbs x 3 replicates x treatments]. All experiments (pre- and postharvest Figure 2-2) were arranged in a completely randomised design.

In 2015, 2016 and 2017, six, seven and three postharvest sampling points were conducted, respectively. In 2016, two pre-storage sampling points (pre-harvest and mid-curing) were added to make a total of nine sampling points. The pre-harvest samples for 2016 were collected at the termination of irrigation at 100% fall-down (two weeks before harvest), while the mid-curing samples were collected three weeks into curing. Time 0 (week 0) samples for all years were collected at the end of curing, prior to bulbs being transferred to the cold room for storage. Afterwards, sampling was conducted bi-weekly until sprout length was 40 % in proportion to bulb height (Chope *et al*., 2006). At each sampling point, bulb weight, respiration rate and sprout assessment (sprout length as a percentage of total bulb height) were recorded.

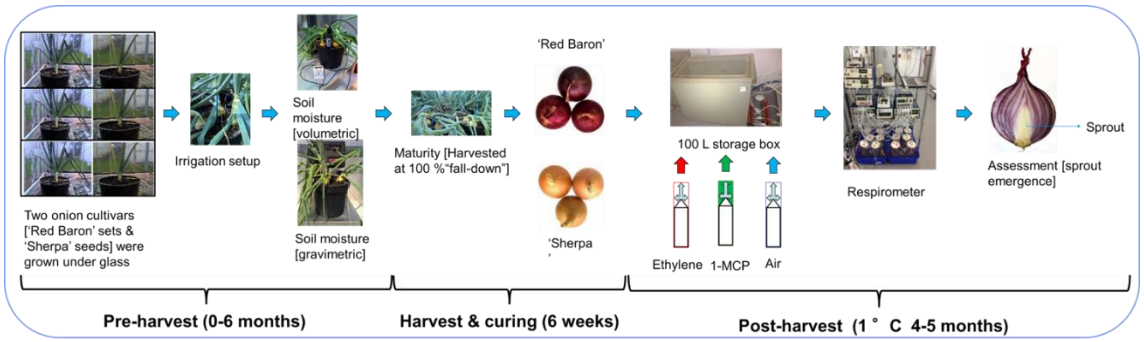

Figure 2-2. A schematic representation of the pre- and postharvest experimental design

2.3.3 Estimation of crop evapotranspiration (ET_c) and soil moisture measurements for the potted onion plants

Three pots per block, per treatment, per cultivar were randomly selected, weighed, and reweighed after 24 hours. The ET_c was then calculated as the difference between the recorded initial and final weights (in grams). Measurements were conducted weekly, from bulb initiation to harvest (June – August) for both years. Where there was a need, irrigation was readjusted according to the treatment.

2.3.4 Curing and storage bulb weight-loss

Onion bulb curing weight-loss was measured weekly during curing, while storage weight-loss were measured at each sampling point during storage. Thus, weight-loss during

curing was calculated as the difference between bulb weight at harvest and weight at sampling. While postharvest, weight-loss was determined as the difference between bulb weight at the start of storage (time 0) and at each sampling point. Curing and storage weight losses are presented as a percentage in proportion relative to the bulb weight.

2.3.5 Bulb weight

After curing for six weeks, the bulbs were defoliated and weighed; the weight of the cured bulbs were used for bulb yield measurements for all years.

2.3.6 Sprout assessment

Sprout incidence were assessed biweekly as previously described elsewhere (Chope *et al.*, 2006). Onion bulbs were cut in half (vertically from top to bottom) and internal sprout length was measured as a percentage of the total bulb height (Figure 2-3). Sprout vigour (i.e. the sprout length in relation to time after sprouting) was used to further understand the actual effects of each postharvest treatment on sprout growth, in relation to the pre-harvest treatments.

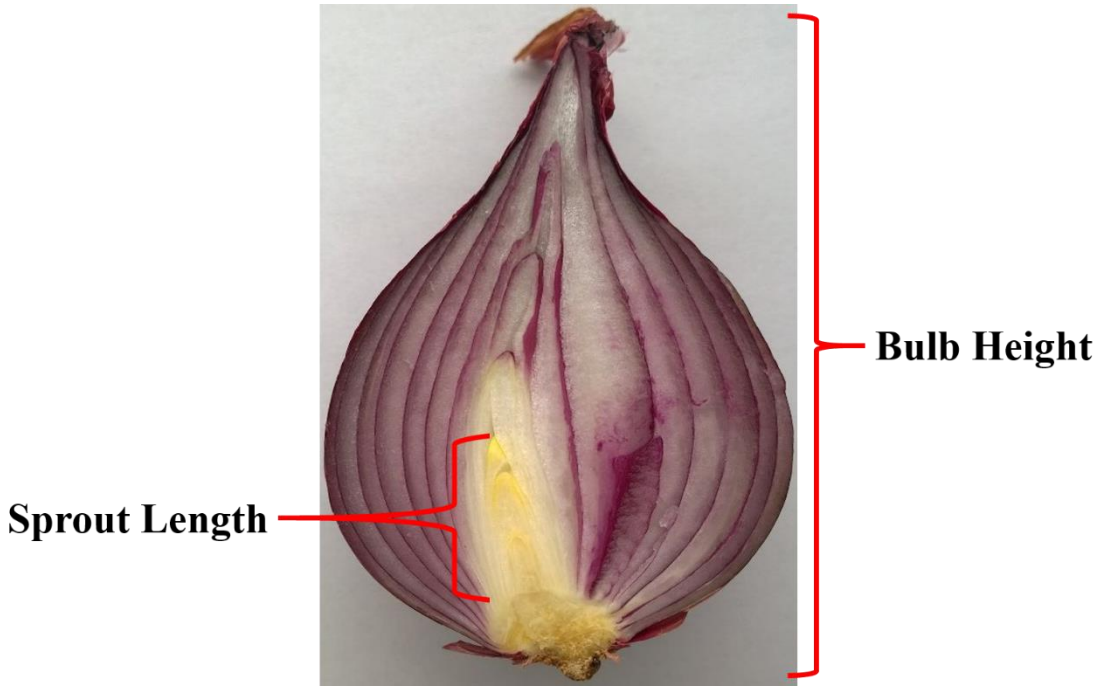

Figure 2-3. Depiction of sprout assessment

2.3.7 Real-time respiration rate (RR)

Real-time respiration rate measurements were taken at each sampling point using the Sable Respirometry System (Model 1.3.8 Pro, Sable Systems International, NV, USA), as described previously by Collings *et al*. (2013). Each replicate (3 replicates x 3 onion bulbs per replicate) were taken out from the storage boxes and placed on the laboratory bench for a minimum of one hour to be acclimatised to room temperature. Each replicate was then placed in a 3 L sealed gas jar with gas inlet for air supply and outlet for respiration rate measurement as CO_2 production. The measured CO_2 produced (in mL h^{-1}) was adjusted to get the final respiration rate values as CO_2 produced in milligrams per kilogram of bulb weight per hour (mg CO_2 kg^{-1} h^{-1}) as previously described (Collings *et al*., 2018).

2.3.8 Dry matter content

Onion bulbs were sliced into two from top to bottom, weighed and freeze-dried at -55°C for 7 days using a freeze-dryer (Scanvac, Lynge, Denmark). The freeze-dried samples were reweighed. The dry matter content was reported as a proportion of the dry weight against the fresh weight.

2.3.9 Statistical analyses and plots

All statistical analyses were conducted using Genstat for Windows 10th Edition (VSN International Ltd, Herts., UK). Analysis of variance (ANOVA) was performed to identify factors that significantly affected variance in the physiological and biochemical data collected. ANOVA was performed on the data specifying a nested treatment structure of a common baseline (observation before postharvest treatments). Least significant difference (LSD) values were calculated from each analysis, for comparison of appropriate treatment means, using general analysis of variance (shown in 5.9I.1.2). A significance threshold of $p<0.05$ was adopted, for all analyses. SigmaPlot for Windows SPW13 (Systat Software, Inc., London, UK) was used for all plots.

2.4 Results

2.4.1 Crop evapotranspiration, soil moisture content, curing and storage weight-loss

In 2015, rainfall leading to a significant drop in temperature affected the evapotranspiration (ET_c) between three and four weeks after start of treatment (Figure 2-4 A). However, the mean crop evapotranspiration, especially in 2016 [Figure 2-4 A and B], soil moisture content (Figure 2-4 C and D) and curing weight-loss (Figure 2-5 A and B) were all higher in the FI plants when compared to the DI plants. The same trends were found in 2017 (Figure C-1). A comparison between cultivars (for 2015) showed that curing weight-loss were 15.0 and 8.6% for 'Red Baron' FI and DI bulbs, respectively, compared to 9.8 and 6.2% for 'Sherpa' FI and DI bulbs, respectively. While a comparison between years showed that curing weight-loss for 'Sherpa' in 2015 were 9.8 and 6.2% for FI and DI bulbs, in 2016, they were 6.8 and 3.6% for FI and DI bulbs, respectively. Overall, curing water-loss was twice as high in FI compared to DI, irrespective of cultivar or year. Bulb storage weight-loss was continuous throughout the storage period, however, there were no significant differences between pre- or postharvest treatments (Figure 2-5 C and D).

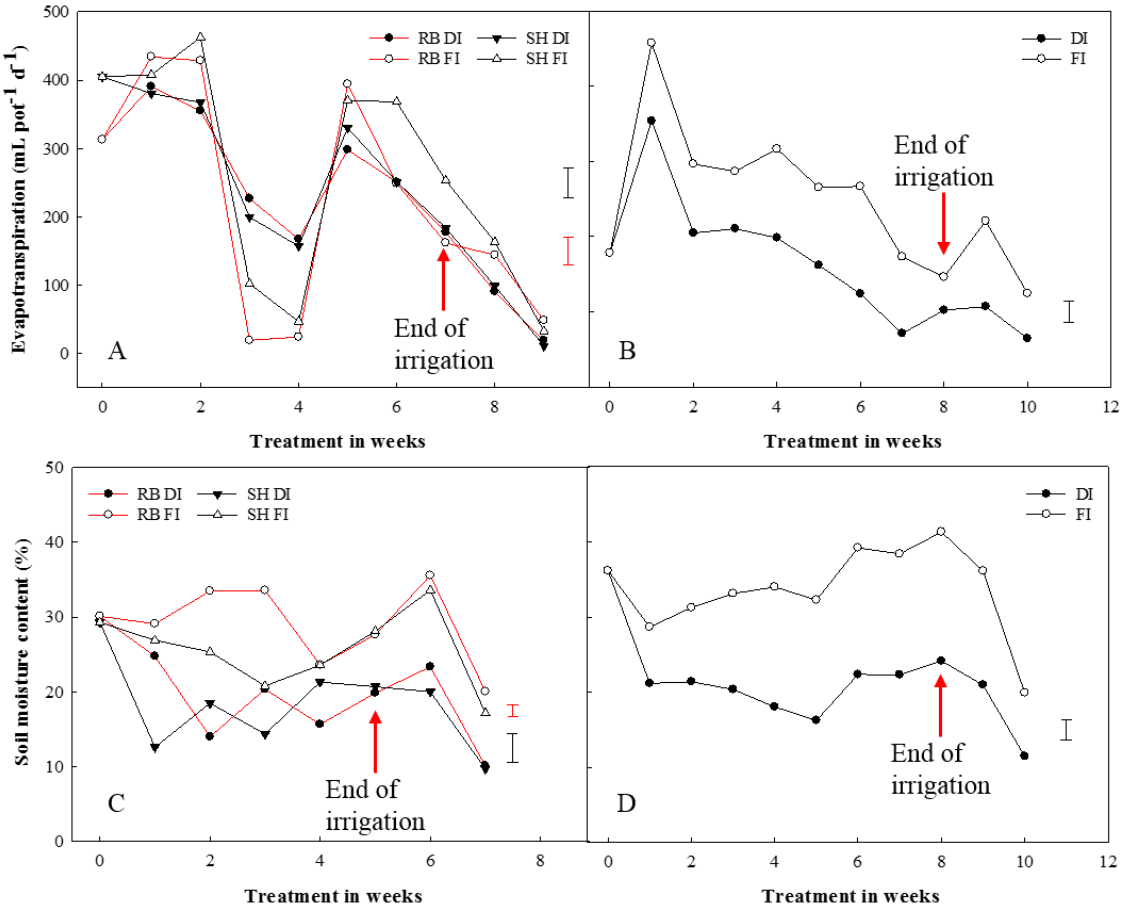

Figure 2-4. Crop evapotranspiration and soil moisture content. Onion plants of cultivars 'Red Baron' (RB) and 'Sherpa' (SH) were grown in 2015 (A and C) and 2016 (B and D) under full irrigation (FI) or deficit irrigation (DI), where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. LSD bar at 95% confidence shown for interactions between treatments and time in weeks.

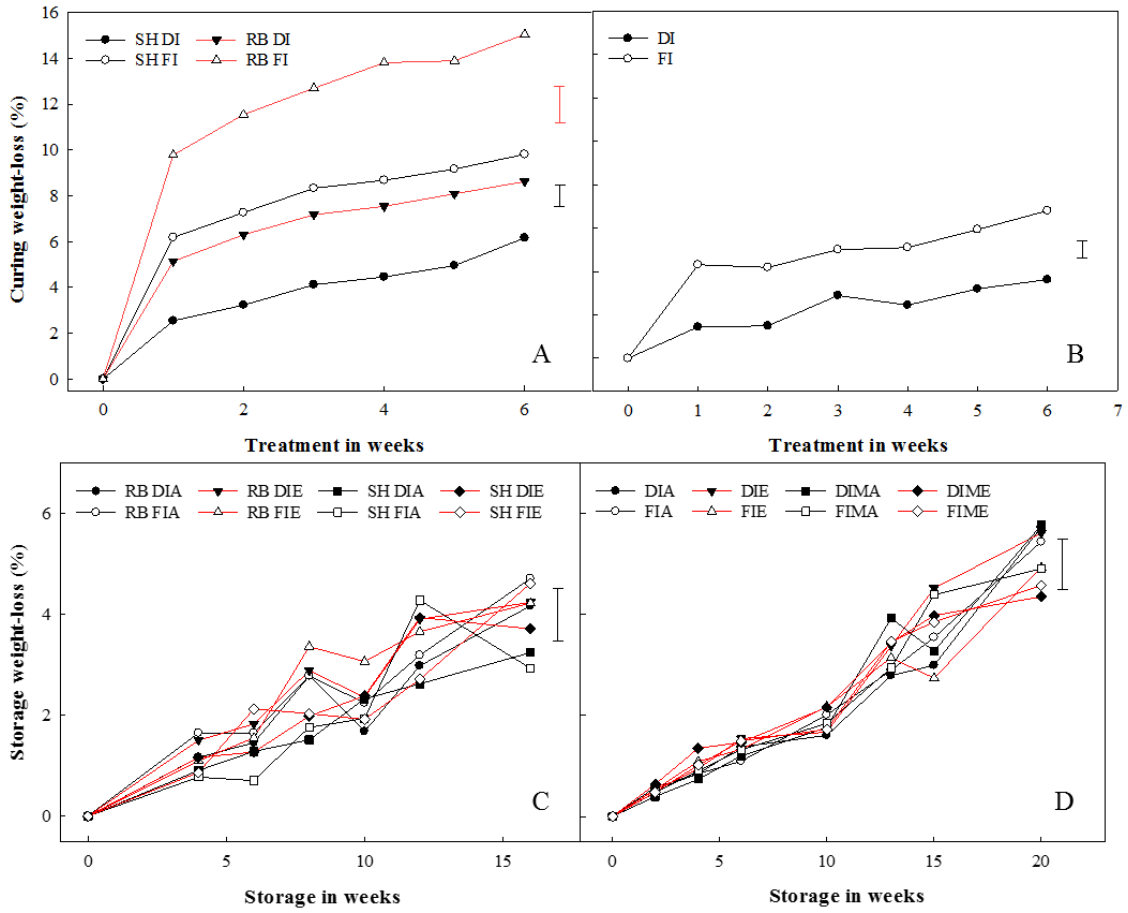

Figure 2-5. Postharvest curing water-loss [A and B] and storage weight losses [C and D] in stored onion bulbs. Onion bulbs of cultivar 'Red Baron' [RB] and 'Sherpa' [SH] were grown in 2015 (A and C) and 2016 (B and D) under full irrigation [FI] or deficit irrigation [DI]; where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. Post-curing, bulbs were either treated with 1-MCP at 1 µL L^{-1} for 24 h or untreated before storage; and then, stored at 1°C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIA and FIA are DI and FI bulbs in air, DIE and FIE are DI and FI bulbs stored under ethylene, DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. LSD bar at 95% confidence shown for interactions between treatments and curing/storage in weeks.

2.4.2 Bulb weight

Average bulb weights (12.7 and 11.3 kg for 'Red Baron FI and DI; 28.1 and 19.8 kg for 'Sherpa' FI and DI year 1; 40.6 and 35.7 kg for 'Sherpa' FI and DI year 2 and 23.8 and

20.5 kg for onion CUDH2107 year 3, respectively) were significantly higher in the FI compared DI bulbs for all years; such that in 2015, bulb weights were 12.2 and 41.8% higher for 'Red Baron' and 'Sherpa' FI bulbs when compared to the DI bulbs. For 2016 and 2017, the average bulb weights were 14 and 16.2% higher for FI compared to DI bulbs, respectively.

2.4.3 Sprout length

There were no significant differences in sprout emergence between DI and FI bulbs, irrespective of cultivar or year (Figure 2-6). However, sprout emergence and sprout length varied according to the postharvest regimes. Ethylene delayed sprout emergence by two and four weeks (2015 and 2016, respectively) when compared to bulbs stored in air. In 2017, although the bulbs had already sprouted, ethylene caused a plateau in sprout length in the first three weeks of storage (Figure A-1). Mean sprout length for 1-MCP treated bulbs (2016) stored in air was significantly longer (*ca*. 42%) in DI bulbs when compared to FI bulbs (*ca*. 30%). Overall, 1-MCP-treated bulbs stored under ethylene produced the shortest sprouts followed by untreated bulbs stored under ethylene supplementation, untreated bulbs stored in air and 1-MCP treated bulbs stored in air.

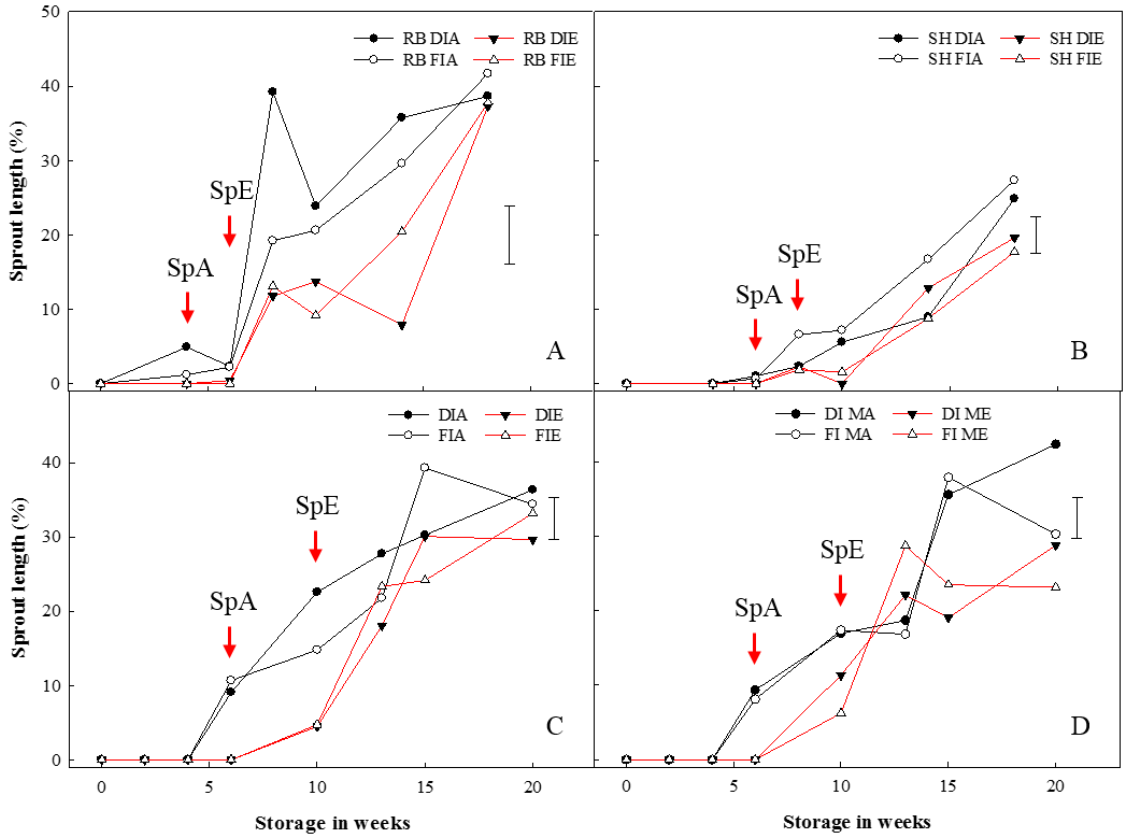

Figure 2-6. Sprout length in stored onion bulbs. Sprout length was measured in proportion to bulb height represented as a percentage for onion bulbs of cultivar 'Red Baron' [RB] and 'Sherpa' [SH] grown in 2015 (A and B) and 2016 (C and D) under full irrigation [FI] or deficit irrigation [DI]; where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. After curing, bulbs were either treated with 1-MCP at 1 µL L^{-1} for 24 h or untreated before storage; and then, stored at 1°C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIA and FIA are DI and FI bulbs in air, DIE and FIE are DI and FI bulbs stored under ethylene, DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95% confidence shown for interactions between treatments and storage in weeks.

The comparison and analyses of the sprout vigour in relation to the postharvest treatments showed that onion bulbs treated with 1-MCP and stored under ethylene had a 1.5-fold reduction in sprout vigour (i.e. suppressed sprout growth) from seven weeks after sprout

emergence (Figure 2-7 A and B); while ethylene as an independent factor showed no such suppression.

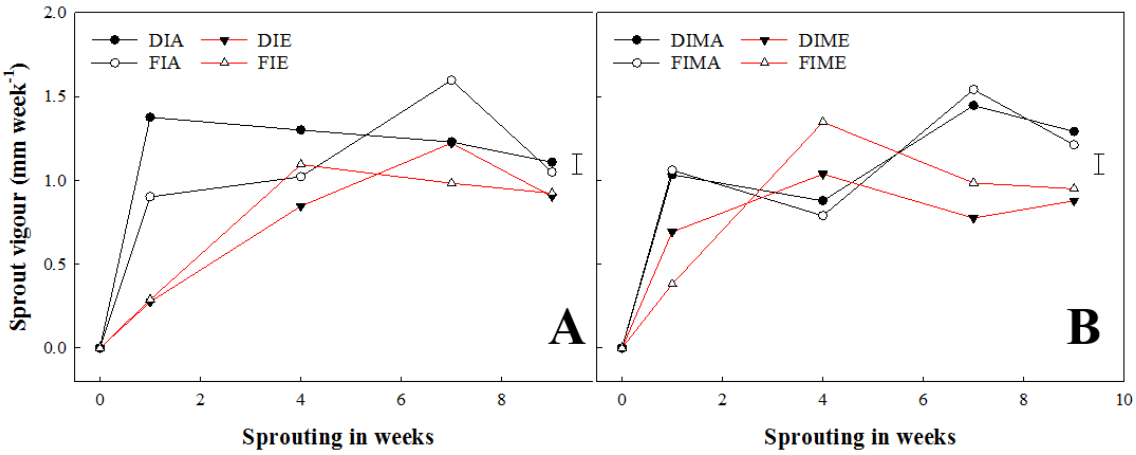

Figure 2-7. Sprout vigour in stored onion bulbs. Sprout vigour measured as sprout growth in milimeter per week for onion bulbs of cultivar 'Sherpa' [SH] grown in 2016 under full irrigation [FI] or deficit irrigation [DI]; where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks. After curing, bulbs were treated with or without 1-MCP (at 1 µL L^{-1}) for 24 h and stored at 1°C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIA and FIA are DI and FI bulbs in air, DIE and FIE are DI and FI bulbs stored under ethylene, DIMA and FIMA are DI and FI treated with 1-MCP and stored in air and DIME and FIME are DI and FI treated with 1-MCP and stored under ethylene. LSD bar at 95% confidence shown.

2.4.4 Respiration rate

In 2015, respiration rates (RR) measured after curing prior to storage were 9.58 and 6.73 mg kg^{-1} h^{-1} of CO_2 for 'Red Baron', and 9.64 and 7.03 mg kg^{-1} h^{-1} of CO_2 for 'Sherpa'; for DI and FI bulbs, respectively (Figure 2-8 A and B). At the end of curing, RR was not significantly different between pre-harvest treatments within cultivars (Figure 2-8 C and D). After four weeks of storage, mean RR increased by at least 50 and 25% for 'Red Baron' and 'Sherpa' bulbs, respectively. Overall mean RR during storage was significantly higher (25%) in cv. 'Red Baron' when compared to 'Sherpa', for the 2015 experiment.

To understand the impact of curing on RR, during the 2016 experiment, RR was measured for onion bulbs two weeks prior to harvest (pre-harvest), at harvest, at mid-curing, and after curing (Figure 2-8 C and D). RR declined continuously from 22.8 mg kg^{-1} h^{-1} of CO_2 at the pre-harvest stage to 4.6 mg kg^{-1} h^{-1} of CO_2 at the end of curing (i.e. pre-harvest > harvest > mid-curing > post-curing). Although RR declined 6-fold from pre-harvest to the end of curing, there were no significant differences in RR between DI and FI bulbs (Figure 2-8). During cold storage, RR increased steadily until sprout emergence at week 6 (7.9 – 18.0 mg kg^{-1} h^{-1} of CO_2), when the RR for 1-MCP-treated bulbs were twice as high when compared to the untreated bulbs stored under ethylene or air. The RR at sprout emergence were: 1-MCP treated bulbs under ethylene > 1-MCP treated bulbs stored in air > bulbs stored under ethylene > bulbs stored in air. Thereafter, RR declined until week 13, after which the RR for bulbs stored under ethylene became significantly higher in DI treated bulbs compared to FI bulbs.

For 2017, where a double haploid line was tested, respiration rate measured at pre-harvest was 18.4 and 15.4 mg kg^{-1} h^{-1} of CO_2 for DI and FI bulbs, respectively. At harvest, there was a spike in RR (30.8 and 33.1 mg kg^{-1} h^{-1} of CO_2 for DI and FI bulbs, respectively), probably due to the sprout incidence recorded at harvest. However, at the end of two weeks curing, RR had steeply declined to 4.5 and 6.3 mg kg^{-1} h^{-1} of CO_2 (Figure B-1). During storage, RR ranged between 2.5 – 6.4 mg kg^{-1} h^{-1} of CO_2, there were no significant differences between pre- or postharvest treatments. Overall, RR was not influenced by pre- or postharvest treatments.

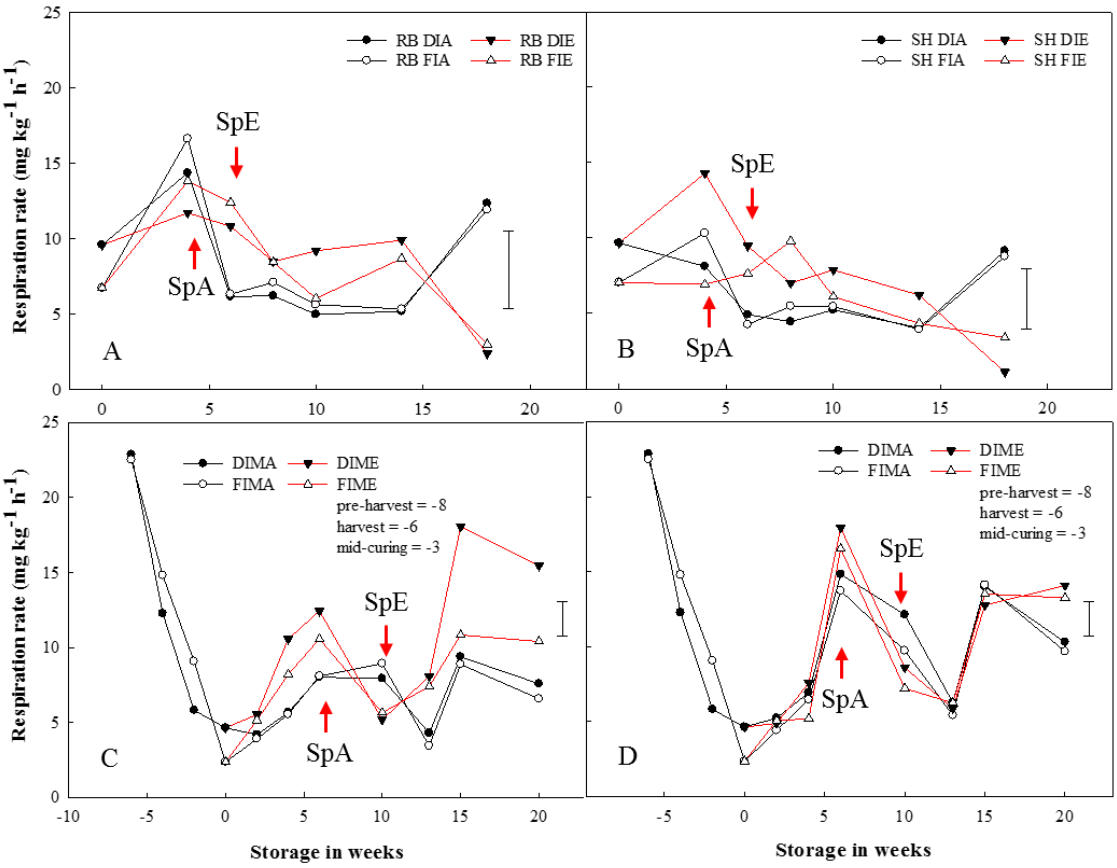

Figure 2-8. Real-time respiration rates as CO_2 production (mg kg^{-1} h^{-1}) of stored onion bulbs. Onion bulbs of cultivar 'Red Baron' (RB) and 'Sherpa' (SH) were grown in 2015 (A and B) and 'Sherpa' in 2016 (C and D) under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. Post-curing, bulbs were treated with or without 1-MCP at 1 µL L^{-1} for 24 h before storage; and then, stored at 1 °C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIA and FIA are DI and FI bulbs in air, DIE and FIE are DI and FI bulbs stored under ethylene, DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95% confidence shown for interactions between treatments and storage in weeks.

2.4.5 Dry matter content

Average dry matter content (DMC) for DI and FI onion bulbs (Table 2-1) across all postharvest treatments and time points were 127.3 and 129.1 g kg^{-1} FW, respectively.

There were no significant differences in DMC between DI and FI at pre-harvest, harvest, and throughout the curing period. DMC fluctuated during storage, and after 20 weeks it was 17% higher in the FI compared to DI bulbs stored in air, while there was no significant difference between DI and FI bulbs stored under ethylene.

Table 2-1. Percentage changes in dry matter content of onion bulbs grown under full or deficit irrigation, treated with or without 1-methylcyclopropene (1-MCP) at 1 µL L^{-1} for 24 h and stored under continuous ethylene supplementation (10 µL L^{-1}) or air

Weeks	DIA	FIA	DIE	FIE	DIMA	FIMA	DIME	FIME
0	13.22	13.03	13.22	13.03	13.22	13.03	13.22	13.03
4	12.00	12.22	12.78	12.91	12.65	13.51	13.25	12.68
6	12.62	13.21	12.19	13.55	13.00	12.84	12.52	12.47
10	12.61	13.45	13.02	13.07	12.83	13.08	12.68	13.71
13	12.12	12.19	12.85	13.13	11.82	12.64	11.80	12.35
15	12.44	13.07	12.61	12.16	12.60	11.84	12.42	12.27
20	12.24	14.01	13.69	13.97	13.09	13.63	13.79	13.58
LSD	0.511							

Key: DI and FI are deficit and fully irrigated bulbs, respectively. DIA and FIA are DI and FI stored in air, DIE and FIE are DI and FI stored under ethylene, DIMA and FIMA are DI and FI treated with 1-MCP and stored in air, and DIME and FIME are DI and FI treated with 1-MCP and stored under ethylene.

2.5 Discussion

The data herein showed that the highest respiration rate (RR) occured pre-harvest, after which it steeply declined continuously through harvest, mid-curing with the lowest RR recorded at post-curing. These results suggested that whilst endo-dormancy may have occurred earlier in the meristematic tissues, this decline in respiration rate may have shown the progression of the onion bulbs towards eco-dormancy. Notably, irrigation was stopped after the pre-harvest samples were collected. Despite the steep decline in RR, there were no significant differences between DI and FI bulbs suggesting that the entrance into eco-dormancy was not influenced by the pre-harvest treatments. Moreover, RR increased after four- and six-weeks storage in 2015 and 2016, respectively. This coincided with sprout emergence for bulbs stored in air, in concordance with previous findings (Benkeblia *et al*., 2002; Benkeblia, 2003; Yasin and Bufler, 2007). Although the bulbs stored under ethylene sprouted much later, the increase in RR occurred across all treatments. This indicated that dormancy-break may have occurred when RR increased while the delay in sprout emergence was caused by the postharvest ethylene supplementation. Dormancy in onion bulb was defined as when the growth of all meristematic tissues is arrested (Terry *et al*., 2015) and is characterised by a reduction in metabolic and RR. Three classes of dormancy exist *viz.* endo-dormancy, para-dormancy and eco-dormancy; where endo-dormancy is time-dependent irrespective of the environmental condition; para-dormancy is dependent on the transfer of biochemical compounds; while eco-dormancy is dependent on environmental conditions (Campbell, 2006). Of these, endo- and eco-dormancies are typical of onion bulbs, and Chope, (2006) suggested that endo-dormancy was the true dormancy in onion bulbs. Endo-dormancy is thought to occur around bulb initiation (Terry *et al*., 2015). Therefore, breaking of dormancy (endo-dormancy-break) is when all meristematic tissues are released for regrowth while sprouting is a result of dormancy-break. Dormancy-break is characterised by an increase in RR. Overall, neither ethylene nor 1-MCP influenced RR prior to or at dormancy-break, suggesting that the ethylene and 1-MCP-related sprout suppression reported in this study and previously (Chope *et al*., 2006a; Bufler, 2009; Downes *et al*., 2010; Cools *et al*., 2011) may take effect after dormancy-break and not before. Nonetheless, there is a need for further investigations into the molecular basis of ethylene-related sprout suppression.

Sprout emergence was not affected by the pre-harvest treatments, irrespective of cultivar or year; which showed that DI had no negative impact on the storage qualities of onion bulbs under cold storage. This data contrasted with Vickers *et al*. (2015), who reported that DI increased sprouting in stored onion bulbs. Their DI treatments, however, involved withholding irrigation for up to 36 days, suggesting more drastic DI applications pre-harvest may impact on the quality of stored onion bulbs.

Although the pre-harvest treatments in this study had no effect on sprout emergence, the postharvest treatments had an influence on sprouting. Bulbs stored in air sprouted first and produced the longest sprouts throughout the storage periods, irrespective of cultivar or year, while bulbs stored under continuous ethylene supplementation experienced a delay in sprout emergence across all cultivars and years. This is supported by previous studies where ethylene was reported to delay sprouting in stored onion bulbs (Chope *et al*., 2006a; Bufler, 2009; Downes *et al*., 2010; Cools *et al*., 2011). Interestingly, there were no significant differences in ethylene sprout suppression between FI and DI bulbs. This showed that ethylene-related delay in sprouting in stored onion bulbs is independent of the pre-harvest irrigation treatments. As such, in regions where water is a major constraint to onion cultivation, ethylene supplementation can be used to extend storage and availability of onion bulbs. In addition, 1-MCP (a known ethylene inhibitor) was reported to suppress sprout growth when applied alone (Chope *et al*., 2006b) or in combination with ethylene (Downes *et al*., 2010; Cools *et al*., 2011). 1-MCP, as an independent factor, was not found to suppress sprout growth in stored onion bulbs; which contrasted the finding by Chope *et al*. (2007). Nevertheless, they only found significant sprout suppression for 1-MCP-treated onion bulbs stored at 12°C; a factor that was not investigated here. 1-MCP-treated bulbs stored under continuous ethylene supplementation produced the shortest sprouts throughout storage for both DI and FI bulbs. Although Cools *et al*. (2011), had previously reported a similar synergistic sprout suppression for bulbs treated with 1-MCP and stored under ethylene, the data in this study further showed that a similar additive effect occurs in both DI and FI bulbs.

DI onion plants had a reduced evapotranspiration when compared to FI plants. Begum *et al*. (1993) previously reported that differences in evapotranspiration between DI and FI plants resulted from differential transpiration rates. In addition, plants with higher

transpiration are known to produce more yield when compared to those with low transpiration. This would explain the higher percentage of bulb weight measured for FI compared to DI bulbs across all years. Interestingly, curing weight-loss was higher in FI plants compared to DI plants, irrespective of cultivar or year. Although the aim of curing is to cause water-loss from the outer skin of onion bulbs to improve storage quality (Cools, 2010), Pak *et al*. (1995) linked water-losses in onion bulbs to the bulb sugar content. As such, the internal translocation of sugars from the outer skin towards the inside would increase the water potential in the outer scales and water-loss. This would suggest that the DI bulbs may have accumulated more sugars compared to the FI bulbs. Thus, the differences in curing water-loss. DI had previously been reported to cause the accumulation of sugar in plants (Bordonaba and Terry, 2010). This may explain the differences in curing water-loss between DI and FI plants. However, it is still unclear why no such significant differences in weight-loss were found during storage. Therefore, other factors besides differential sugar accumulation, such as leaf water content, may play a role in curing weight-loss. Notably, the measurement of water-loss during curing was on the whole plant unit.

The data in this study clearly showed that ethylene delayed sprout emergence irrespective of pre-harvest treatments. However, there was no clear indication it reduced sprout vigour when compared to bulbs stored in air. In contrast, the combined treatment of onion bulbs with 1-MCP and ethylene delayed and suppressed sprout growth, including a reduction in sprout vigour. Previous studies often attributed sprout delay and sprout suppression to ethylene. Indeed, both terms are commonly used interchangeably. Understandably, since ethylene delayed sprout emergence, comparing sprout length at a single timepoint will often show differences between bulbs stored under ethylene and air. This could be due to the differences in sprout age, since sprout emergence occurred first in bulbs stored in air. Moreover, 1-MCP when combined with ethylene delayed and suppressed sprout growth. Interestingly, in already sprouted bulbs (see Figure A-1), ethylene reduced sprout elongation. However, a similar reduction was not found when ethylene was applied to bulbs prior to sprout emergence. Despite these interactions and responses, the mechanisms behind ethylene and its antagonists (1-MCP) eliciting synergistic sprout suppression remains unclear.

2.6 Conclusions

Deficit irrigation can be implemented in onion crops with no significant negative impact on bulb storage qualities. The adoption of deficit irrigation in combination with certain postharvest regimes has potential use in onion crop production, especially in regions where water is a major constraint to onion production. This may, however, be accompanied by some levels of yield deficit. A differential effect was found for both postharvest treatments; thus, whilst ethylene delayed sprout emergence, in combination with 1-MCP they further suppressed sprout growth in stored onion bulbs. Further biochemical and molecular studies are required to understand the mechanism behind the regulation of sprouting in stored onion bulbs.

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3 Chapter Three: Water-stress Induced Abscisic Acid Accumulation in the Baseplate of Onion Bulbs but Had No Influence on Dormancy During Cold Storage

3.1 Abstract

The effects of deficit irrigation (DI), ethylene and 1-methylcyclopropene on the endogenous content and longitudinal distribution of abscisic acid (ABA) in relation to dormancy and sprouting were examined in onion bulbs (*Allium cepa* L. cv. 'Sherpa'). 'Sherpa' seeds were grown in the glasshouse and subjected to full or deficit irrigation from bulb initiation to harvest. Bulbs were harvested at full maturity and stored at 1°C for five months. Bulbs were treated with or without 1-MCP (at 1 µL L^{-1}) for 24 h before being stored under continuous ethylene supplementation (10 µL L^{-1}) or air. There was a significantly higher accumulation of ABA in the baseplate (containing the meristematic tissue) of DI bulbs at pre-harvest and at the end of curing compared to the FI treatment. However, sprout emergence was not delayed. Ethylene as an independent factor reduced the decline in ABA content and delayed sprouting for both DI and FI bulbs; while 1-MCP did not delay sprout emergence. Positive correlations between ABA and its metabolites (abscisic acid glucose ester [ABA-GE], 7′-hydroxyabscisic acid [7′-OH-ABA], phaseic acid [PA] and dihydroxyphaseic acid [DPA]) was strongest with 7′-OH-ABA at the pre-storage stage and with DPA during storage. This switch from hydroxylation to glycosylation indicated that the pathway for ABA regulation may differ depending on onion bulbs dormancy state. The results indicate that: 1) the ABA-linked sprout delay is not enhanced by stress-induced ABA accumulation; 2) the ethylene-related sprout suppression may be dependent on the reduction of ABA catabolism; and 3) the mechanism of the ABA-related regulation of dormancy in onion bulbs remain largely unclear.

3.2 Introduction

Premature sprouting is a major contributor to postharvest losses and a threat to the year-round supply of quality onion bulbs. Onion bulbs are harvested dormant and the length of this dormancy state dictates the length of storage and is a paramount factor in the continuous supply of onion bulbs. Therefore, understanding the mechanisms and the regulation of onion bulb dormancy and sprouting are important in ensuring the continuous supply of onion bulbs, and to avoid wastage during sprouting in store. Currently, the majority of practices for extending the storage qualities of onion are focused on exogenous treatments such as maleic hydrazide (Wittwer *et al.*, 1950; Isernberg, 1956; Gajewski and Majewski, 2008; Ilić *et al.*, 2011) ethylene and 1-methylcyclopropene treatments (Chope *et al*., 2006b; Chope *et al*., 2007; Bufler, 2009; Downes *et al*., 2010). However, abscisic acid (ABA) content in onion bulbs, which accumulate during various growth stages (Matsubara and Kimura, 1991) have been postulated to play some roles in the regulation of onion bulb dormancy and sprouting (Chope *et al*., 2007; Chope and Terry, 2008).

The regulation of dormancy and regrowth involves complex interactions with both environmental (e.g. drought, salt and temperature) and endogenous components, such as the plant hormones. There are five classical known plant hormones, auxin, cytokinin, gibberellin, ABA and ethylene. Auxin regulates cell division and expansion (Arteca, 1996; Kepinski and Leyser, 2002; Kulaeva and Prokoptseva, 2004), cytokinin regulates cell division and organ differentiation (Arteca, 1996; Kulaeva and Prokoptseva, 2004), gibberellin promotes cell elongation, promotoes the synthesis of hydrolases and releases dormancy (Bewley, 1997; Brady and McCourt, 2003; Finkelstein *et al.*, 2008; Wang and Irving, 2011), ABA maintains dormancy and blocks the synthesis and secretion of hydrolases while ethylene promotes germination (Johnson and Ecker, 1991; Ecker, 1995; Wang *et al*., 2002; Wang and Irving, 2011). Therefore, since four of the five classic phytohormones promote dormancy break, ABA is an inhibitor of dormancy- break. Recently, other compounds eliciting hormonal effects (e.g. brassinosteroid, jasmonic acid, salicylic acid) have been identified, including strigolactones that has been found to regulate branching and enhances mycorrhisation (Wang and Irving, 2011). However, ABA is the only classic phytohormone hormone mainly involved in the maintenance of

dormancy. Dormant seeds are known to have a higher accumulation of ABA, however, a decline in ABA is accompanied by an increase in gibberellin at which point dormancy is released and germination occurs (Khan, 1967). ABA synthesis is known to occur in plants roots and leaves from where they are transported around the plant through the xylem and phloem, respectively (Hartung, Sauter and Hose, 2002). ABA is transported around the plant in its inactivated glycosylated form, which is activated by the actions of β-glucosidases (Hartung, Sauter and Hose, 2002; Wasilewska *et al.*, 2008). Free ABA and its glycosylated form are known to be present in soil solutions where they are taken up by the roots along with water into the plant to elicit the activities attributed to ABA such as the control of the opening and closing in relation to drought stress.

Although ABA is involved in seed germination (Nambara *et al.*, 2010; Kang *et al.*, 2015), can have positive and negative effects on vegetative growth (Finkelstein and Lynch, 2000; Finkelstein *et al*., 2002; Garciarrubio *et al*., 2015) and tends to promote senescence (Suttle *et al*., 2012), its major role is in the regulation of plant response to water deficit (Kuromori *et al*., 2018). The relationship between ABA, water content and dormancy was illustrated in seeds, where the drying associated with dormancy was reported to decrease the ABA content due to catabolism by ABA 8′-hydroxylases (Okamoto *et al.*, 2006). ABA accumulation in relation to dormancy was also renewed through imbibition by dormant seeds (Matsubara and Kimura, 1991; Grappin *et al.*, 2000; Finkelstein *et al.*, 2008). The genes *9-cis-epoxycarotenoid dioxygenase* (*NCED9*) and *zeaxanthin epoxidase* (*ZEP*) were positively correlated with dormancy, while dormancy is released through ABA catabolism catalysed by abscisic acid 8'-hydroxylase 2 (CYP707A2) (Cadman *et al.*, 2006; Millar *et al.*, 2006). Thus, ABA content and its metabolism has become central to the study of dormancy in many plant species.

In onion, endogenous ABA content was reported to be highest in bulbs at lodging and harvest, but to decrease to a minimum as bulbs progressed to sprouting (Matsubara and Kimura, 1991; Chope *et al*., 2006). As such, it was postulated that increasing endogenous ABA content in onion bulbs might delay sprouting (Chope *et al*., 2007). To address this, Chope *et al*. (2007) applied ABA analogues (8′-methylene ABA methyl ester; PBI-365) as foliar sprays on onion leaves in one experiment and in another, they dipped onion bulbs in ABA solution. Neither of them caused a significant increase in the endogenous ABA

content nor delayed sprouting. However, ABA accumulation is known to occur in water-stressed plants (Ober and Setter, 1992; Jiang, 2002; Balint and Reynolds, 2013; Manzi *et al.*, 2015; Puértolas *et al.*, 2015); from the whole plant level (Davies and Zhang, 1991) down to the cellular level (Shinozaki and Yamaguchi-Shinozaki, 1997). Whether pre-harvest water stress treatments such as deficit irrigation (DI) could be used to delay sprouting by increasing the endogenous accumulation of ABA in onion bulbs has not been reported. Ethylene and its known inhibitor 1-methylcyclopropene (1-MCP), whether applied independently or in combination, delayed sprouting and inhibited sprout growth (Chope *et al.*, 2006b; Chope and Terry, 2008; Cools *et al.*, 2011; Chope *et al.*, 2012). However, the interaction with ethylene or 1-MCP with endogenous ABA levels has also not been previously studied.

The aim of this study was to investigate the effects of DI on the accumulation and distribution of ABA, its metabolites and to test the hypothesis that ethylene and ABA interact in stored onion bulbs to control dormancy and the rate of sprout growth.

3.3 Materials and methods

3.3.1 Plant material

Onion cultivar 'Sherpa' seeds were sown into trays filled with John Innes Compost No 1 (J. Arthur Bower's, The Garden Superstore, West Sussex, UK) and six weeks after germination, seedlings were transplanted into pots filled with 7.3 kg of John Innes Compost No 3 (J. Arthur Bower's, Westland Horticulture Ltd, Huntingdon, UK) in 264 pots of 8 L capacity. Seedlings were transplanted at three seedlings per pot (pseudo-reps) and all potted plants were equally divided into three completely randomised blocks, with one replicate per block formed across two 1 X 10 m benches in the glasshouse. The first and last rows of plants on each block served as guard plants (excluded in the analysis). Plants were fertilised with 800 mL of Hoagland's solution as two single 400 mL applications per treatment at two and four weeks after transplanting. Bulbs were harvested manually at full maturity when the foliage of all plants had lodged (100 % fall-down) and cured with foliage intact in the glasshouse for six weeks (August - September).

3.3.2 Experimental design

Pre-harvest, plants were subjected to irrigation treatments *viz*. full irrigation (FI) or deficit irrigation (DI). FI plants had their crop evapotranspiration (ET_c) replenished at 100% while ET_c for DI plants were replenished at 50% (i.e. half of FI treatment). Automatic irrigation valve timers (AC4, Hozelock, Warwickshire, UK) were used to control the volume of irrigation applied. Benches in the glasshouse were fitted with two polyvinyl tubing arrays (20 mm diameter), one per irrigation treatment. These tubes were fitted with pressure-compensating emitters of 1.2 L min^{-1} flow rate. Polyvinyl tubes (5 mm diameter) were connected to the emitters and into the pots (one tube per emitter per pot). Irrigation to meet ET_c was readjusted was based on soil moisture content measured gravimetrically (pot weight) and volumetrically using soil moisture probes (HH2 and ML2x, Delta-T, Cambs., UK). This was done weekly using three pots per treatment per block. Differential irrigation treatment was started from bulb initiation stage and stopped two weeks before harvest.

All three bulbs per pot were collected at harvest, tagged, and weighed together. After which, the bulbs (with foliage intact) were spread out to cure on the benches in the glasshouse. Post-curing, the foliage was trimmed 2 cm above the neck and then bulbs were transferred to 100 L storage boxes. Half of the bulbs were treated with or without 1-MCP (1 µL L^{-1} for 24 hours before storage); and then stored at 1°C in air or in air continuously supplemented with ethylene at 10 µL L^{-1} (Cools *et al*., 2011) for 20 weeks. Ethylene gas was sourced from a 50 L cylinder (5000 µL L^{-1} certified standard in nitrogen; BOC, Surrey, UK). The gas mixture was as previously described (Amoah *et al*., 2017). At each sampling point, three bulbs were collected in triplicate, per treatment and throughout the storage duration (3 bulbs [pseudo-reps] x 3 replicates [blocks] x 4 treatments). All experiments (pre- and postharvest) were arranged in a completely randomised block design.

Four pre-storage sampling points (pre-harvest, harvest, mid-curing and end of curing [time 0]) and four storage sampling points (4, 6, 10 and 20 weeks of cold storage) were assessed. The pre-harvest samples were collected at full maturity, two weeks before harvest at the termination of irrigation. Further samples were collected at harvest, three weeks into curing (mid-curing), six weeks into curing (end of curing), four weeks after

storage, at sprout emergence for bulbs stored in air (six weeks into storage), at sprout emergence for bulbs stored under ethylene (10 weeks storage) and at the end of storage (20 weeks). Bulbs were sliced into two equal parts from top to bottom with a sharp stainless-steel knife. The individual baseplates were excised, after which the remaining section (including the skin) was divided equally into top and bottom (Figure D-1). All samples were quickly snap-frozen in liquid nitrogen and stored at -80 °C before lyophilisation at -55 °C in a freeze-drier (Scanvac, Lynge, Denmark) in the dark for 7 days. Freeze-dried samples were stored at -40 °C prior to phytohormone extraction and analyses.

3.3.3 Extraction of ABA and its metabolites

ABA and its metabolites (ABA-GE, 7′-OH-ABA, PA and DPA) were extracted and quantified as previously described by Müller and Munné-Bosch (2011) with some modifications. Briefly, 100 mg of freeze-dried samples were extracted using 3 mL of 75% HPLC grade methanol, 20% HPLC grade water and 5% formic acid. A 30 µL internal standards mixture at 1 µg mL^{-1} (Table 3-1) were added to each sample at the beginning of extraction process. A double blank and a blank were prepared such that the double blank contained only the extraction solvent and the blank contained the extraction solvent and the internal standard mix. The sample mixtures were vortexed for two minutes (at 2000 rpm) and kept in the dark on a shaker at 4 °C for one hour. The sample mixtures were then centrifuged for five minutes (at 4500 rpm) at 4°C. The supernatants were collected, snap-frozen in liquid nitrogen and dried overnight at -105°C using a solvent freeze-dryer (Scanavac Cool Safe, Denmark) connected to a 0.45 kw vacuum pump (Edwards, RV3, Czech). The dried samples were reconstituted with 400 µL of 90% HPLC water, 9.9% LC-MS grade acetonitrile and 0.1% formic acid. The resuspended samples were vortexed (at 2000 rpm) for two minutes, centrifuged for two minutes (at 12000 rpm) at 4°C, and filtered through a 13 mm diameter x 0.2 µm PTFE filter unit (Jaytee Biosciences Ltd, Kent, UK) into 1.5 mL HPLC salinized vials (Agilent Technologies, Cheshire, UK).

Table 3-1. List of internal (deuterated) and calibration (non-deuterated) standards used for the hormone analyses of onion bulbs (cv. 'Sherpa') grown under full or deficit irrigation, harvest at full maturity and cured under glass for six weeks

Compounds	Deuterated (internal) standards	Non-deuterated (calibration) standards
ABA	[2H^4]abscisic acid (–)-5, 8' 8' 8' (d^4-ABA)	(±)-ABA
ABA-GE	[2H^5]abscisic acid glucose ester (+)-4,5,8',8',8'-(d^5-ABA-GE)	(+)-ABA-GE
7′-OH ABA	[2H^4]7'-hydroxy-abscisic acid; (±)-5,8',8',8'-d4-7'-hydroxy-ABA (d^4-OH-ABA)	(±)-7'-hydroxy-ABA
PA	[2H^3]phaseic acid (–)-7',7',7' (d^3-PA)	(–)-PA
DPA	[2H^3]dihydrophaseic acid (–)-7',7',7'-d^3 dihydrophaseic acid (d^3-DPA)	(–)-DPA

3.3.4 Quantification of ABA and its metabolites

Phytohormone quantification was performed according to the methods described by Morris *et al*. (2018). Briefly, 10 times dilution was performed on the extracts, after which 10 µL was injected into a Luna column 3µm C18 (2) 100 Å (100 x 2 mm, 1.8 µm; Phenomenex) equipped with a ZORBAX Eclipse plus-C18 UHPLC Guard 3pk (2.1 x 5 mm 1.8 µm; Agilent Technologies) on the tandem mass spectroscopy (SCIEX TripleQuad 6500+, AB Sciex, Framingham, MA, USA). The mobile phases consisted of solvent A (0.01 M ammonium formate, 40% LC-MS grade acetonitrile and 60% HPLC grade water) and solvent B (95% LC-MS grade acetonitrile, 4.9% HPLC grade water, and 0.1% formic acid). The gradient involved a linear increase/decrease of acetonitrile content: 0-1.71 minutes, 96-87.4%; 1.71-7.00 minutes, 87-74.0%; 7-10 minutes, 74-60%; 10-10.55 minutes, 60-50%; 10.55-11.65 minutes, 50-0%; 11.65-12.70 minutes, 0-96%. The flow rate was 0.6 mL min^{-1} with a maximum pressure of 800 psi and an oven temperature 25°C. The presence and quantification of ABA and its metabolites were calculated from comparing peak areas in each sample to the calibration standards at 0.5 ng mL^{-1} – 30 ng mL^{-1} (Table 3-1) using the MultiQuant software (AB Sciex, Framingham, MA, USA) as described in Morris *et al*. (2018). Except for (±)-ABA purchased from Sigma-Aldrich (Dorset, UK), all other deuterated and non-deuterated standards used as internal and calibration standards, respectively, were purchased from the National Research Council of Canada-Plant Biotechnology Institute.

3.3.5 Non-structural carbohydrates extraction and quantification

Sugars (fructose, glucose and sucrose) were extracted from freeze-dried and pulverised samples from the top, bottom and baseplate sections and quantified using Agilent 1260 Infinity HPLC system coupled to an Evaporative Light-Scattering Detector (Agilent Technologies LDA UK Ltd., Cheshire, UK) as described elsewhere (Amoah *et al*., 2016). The presence and concentration of fructose, glucose and sucrose were calculated by comparing the peak areas in each sample to standards (0.05 – 5 mg mL^{-1}) using Agilent ChemStation software version 4 (Agilent Technologies, CA, USA). Fructose, glucose and sucrose standards were obtained from Sigma-Aldrich Co. (Dorset, UK).

3.3.6 Statistical analyses and plots

All statistical analyses were conducted using Genstat for Windows 12th Edition (VSN International Ltd, Herts., UK). Analysis of variance (ANOVA) was performed to identify factors that significantly affected variance in the physiological and biochemical data collected. ANOVA was performed on the data specifying a nested treatment structure of a common baseline (observation before postharvest treatments to ensure that any differences were due to the postharvest treatment). Least significant difference (LSD) values were calculated from each analysis, for comparison of appropriate treatment means, using general analysis of variance. Pearson's correlation between ABA and each of its metabolites and sugars were conducted (shown in 5.9J.1.1). A significance threshold of $p < 0.05$ was adopted, for all analyses. SigmaPlot for Windows SPW13 (Systat Software, Inc., London, UK) was used for all plots.

3.4 Results

3.4.1 Pre-storage accumulation and distribution of hormones in relation to pre-harvest deficit irrigation

At pre-harvest, the only significant differences in ABA content was in the baseplate where it was significantly higher in the DI compared to the FI treatment (Figure 3-1 A, B and C). Whereas the differences in DPA content was significantly higher in the DI compared to the FI across all sections (Figure 3-2 D, E and F). There were no significant differences in ABA-GE (Figure 3-1 D, E and F); 7′-OH-ABA (Figure E-1 A, B and C); and PA (Figure 3-2 A, B and C) contents between the DI and FI treatments pre-harvest.

At harvest, the differences in ABA content found in the baseplate were no longer significant, while the DPA contents in the top and bottom wedge, which were initially higher in the DI bulbs had become significantly higher in the FI treaments. No significant differences in ABA-GE, 7′-OH-ABA and PA contents were found between DI and FI bulbs at harvest. Although no differences were found in ABA and 7′-OH-ABA contents between the DI and the FI treatments at mid-curing, there was a significant spike in ABA-GE content of DI bulbs across all sections compared to FI. At the end of curing, while no significant differences in the ABA content was found in the top and bottom wedges, the ABA content for baseplate had become significantly higher for the DI bulbs compared to FI treatments. PA content was significantly higher in DI compared to FI bulbs across all sections. In contrast, the DPA content in the top and bottom wedges were significantly higher in the FI bulbs compared to the DI treatments, while no significant differences were found in the baseplate. There were no significant differences in ABA-GE and 7′-OH-ABA contents between the DI and FI treatments across all sections. Overall, ABA was distributed more in the baseplate by at least, 3.5-fold when compared to the top and bottom wedges.

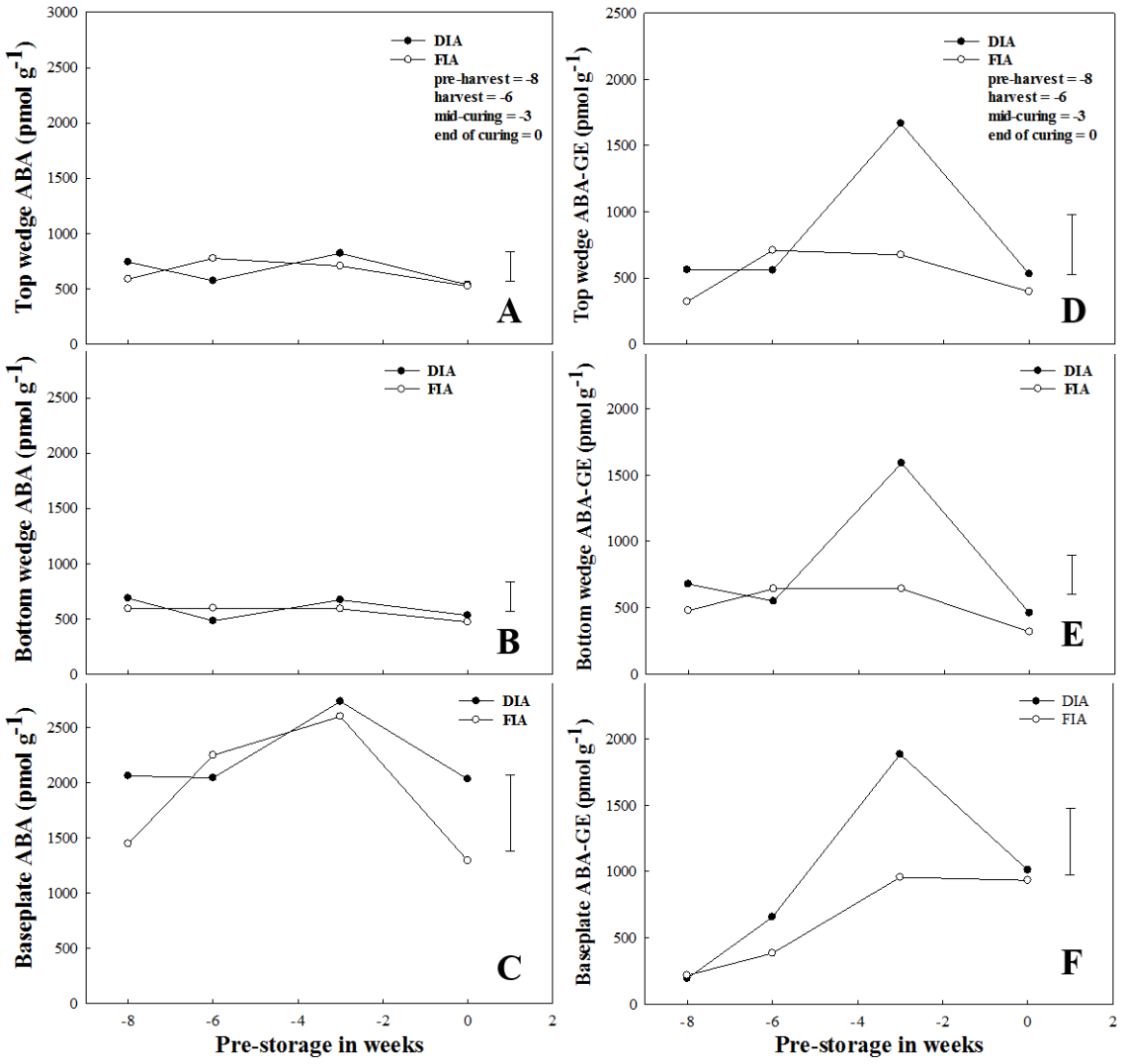

Figure 3-1. Pre-storage abscisic acid (ABA) and abscisic acid glucose ester (ABA-GE) and contents in the top wedge (A and D), bottom wedge (B and E) and baseplate (C and F) of onion bulbs (cv. 'Sherpa') grown under full and deficit irrigation (FIA and DIA, respectively). ABA and ABA-GE contents per dry weight for the top wedge, bottom wedge and baseplate of onion bulbs (cv. 'Sherpa') grown under full irrigation (FIA) or deficit irrigation (DIA); where FIA defines 100% replenishment of crop evapotranspiration (ET_c) and DI 50% replenishment of ET_c. Bulbs were harvest at full maturity (i.e. 100% fall-down) and cured under glass for six weeks. LSD bar at 95% confidence shown for interactions between treatments and time in weeks.

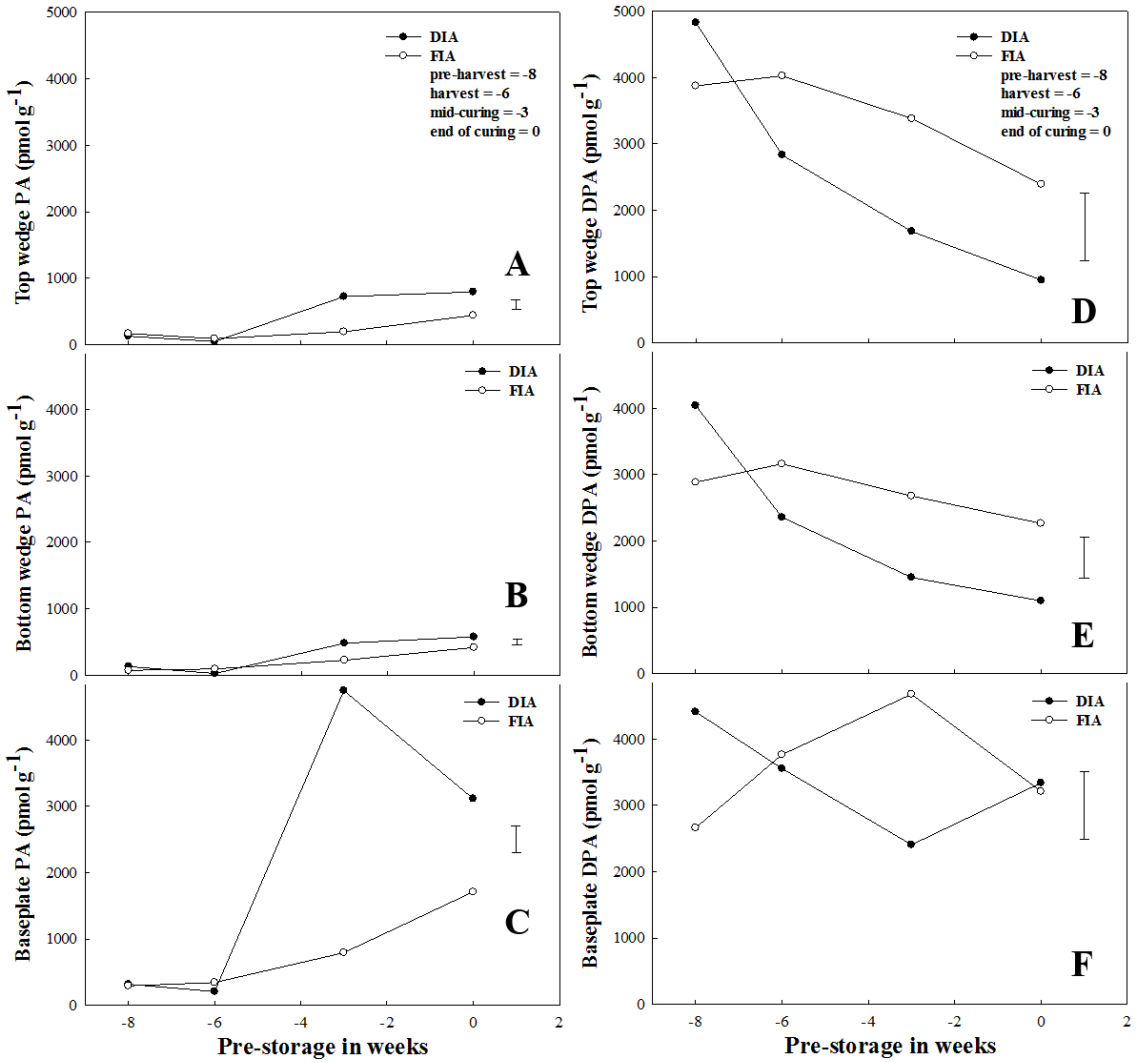

Figure 3-2. Pre-storage Phaseic acid (PA) and dihyrophaseic acid (DPA) contents in the top wedge (A and D), bottom wedge (B and E) and baseplate (C and F) of onion bulbs (cv. 'Sherpa') grown under full and deficit irrigation (FIA and DIA, respectively). PA and DPA contents per dry weight for the top wedge, bottom wedge and baseplate of onion bulbs (cv. 'Sherpa') grown under full irrigation (FIA) or deficit irrigation (DIA); where FIA defines 100% replenishment of crop evapotranspiration (ET_c) and DI 50% replenishment of ET_c. Bulbs were harvest at full maturity (i.e. 100% fall-down) and cured under glass for six weeks. LSD bar at 95% confidence shown for interactions between treatments and time in weeks.

3.4.2 Postharvest effects of ethylene on the accumulation and distribution of hormones in relation onion bulb dormancy

Prior to sprouting (four weeks storage), ABA (Figure 3-3 A, B and C), PA (Figure 3-4 A, B and C) and DPA (Figure 3-4 D, E and F) contents declined significantly to a minimum across all sections for both DI and FI bulbs. Changes in ABA-GE (Figure 3-3 D, E and F) and 7′-OH-ABA (Figure F-1 D, E and F) contents were not significant. Following this decline, the onion bulbs stored in air and under ethylene sprouted two and six weeks later, respectively. Despite the significant ($p=0.010$) decline in ABA content prior to sprouting, there were no significant differences between bulbs stored under ethylene when compared to bulbs stored in air. Interestingly, after the pre-sprouting decline in ABA, the differences found in the baseplate between the DI (2361.7 pmol g^{-1}) and the FI (1295.1 pmol g^{-1}) bulbs at pre-storage were no longer significant (645.8 and 590.2 pmol g^{-1} for DI and FI, respectively). When analysed as a whole bulb, the ABA contents showed that the overall ABA content was significantly ($p=0.010$) higher for bulbs stored under ethylene when compared to bulbs stored in air Figure 3-7. This differences in ABA content between bulbs stored in air and under ethylene remained significantly ($p=0.017$) higher for bulbs stored in the latter treatment until end of the storage period. The decline in DPA content, as with ABA, was was significantly ($p=0.007$) higher for bulbs stored under ethylene when compared to bulbs stored in air. There were no clear responses in ABA-GE, 7′-OH-ABA and PA contents at sprout emergence, irrespective of pre- or postharvest treatments.

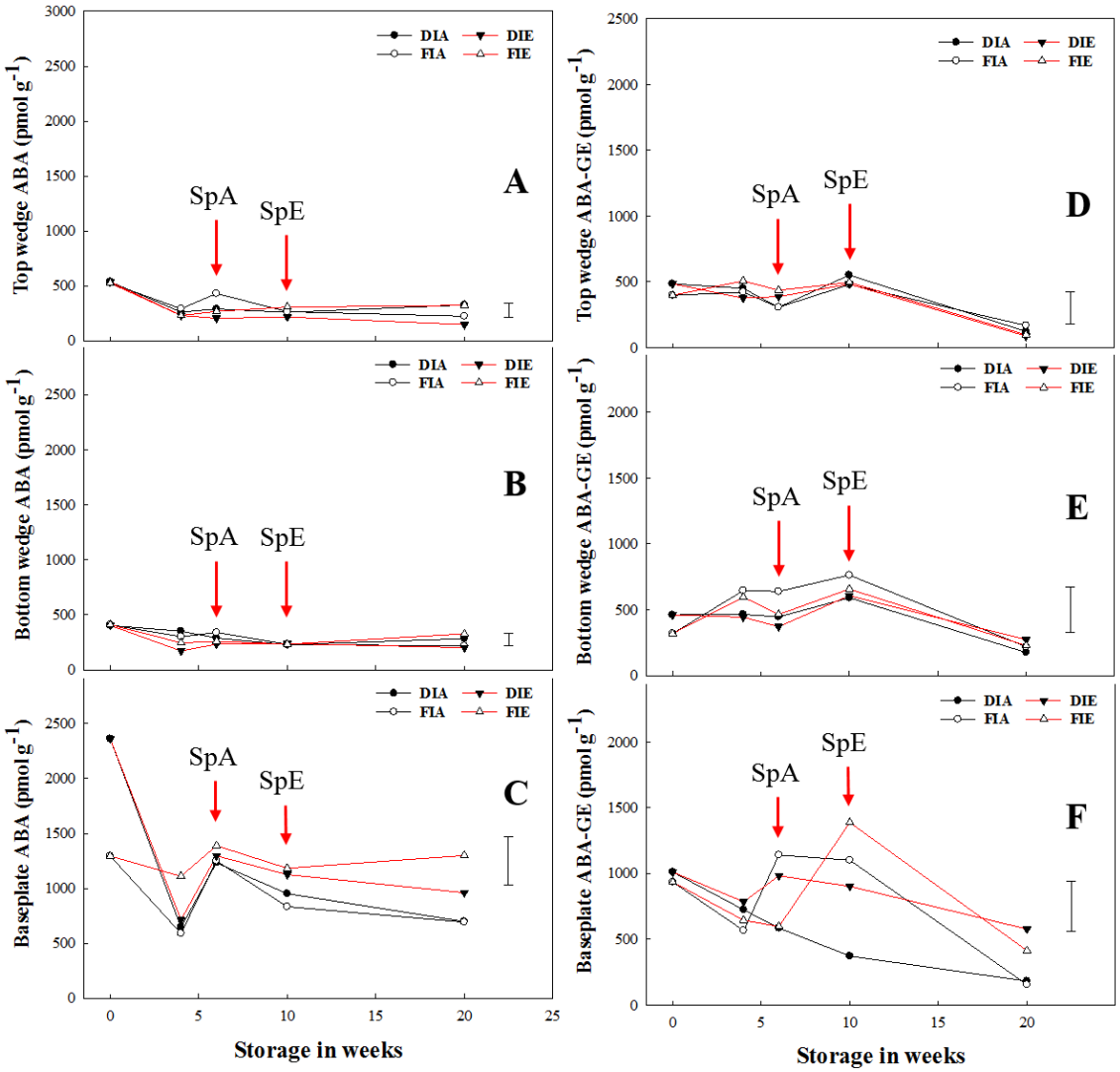

Figure 3-3. Abscisic acid (ABA) and abscisic acid glucose ester (ABA-GE) contents in the top wedge (A and D), bottom wedge (B and E) and baseplate (C and F) of stored onion bulbs (cv. 'Sherpa') grown under full and deficit irrigation and stored under air or ethylene. ABA (A, B and C) and ABA-GE (D, E and F) contents per dry weight for the top wedge, bottom wedge and baseplate of onion bulbs (cv. 'Sherpa') grown under full irrigation (FI) or deficit irrigation (DI); where FI defines 100% replenishment of crop evapotranspiration (ET_c) and DI 50% replenishment of ET_c. Bulbs were harvest at full maturity (i.e. 100% fall-down) and cured under glass for six weeks. Post-curing, bulbs were stored at 1 °C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIA and FIA are DI and FI bulbs stored in air and DIE and FIE are DI and FI bulbs stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95 % confidence shown for interactions between treatments and storage in weeks.

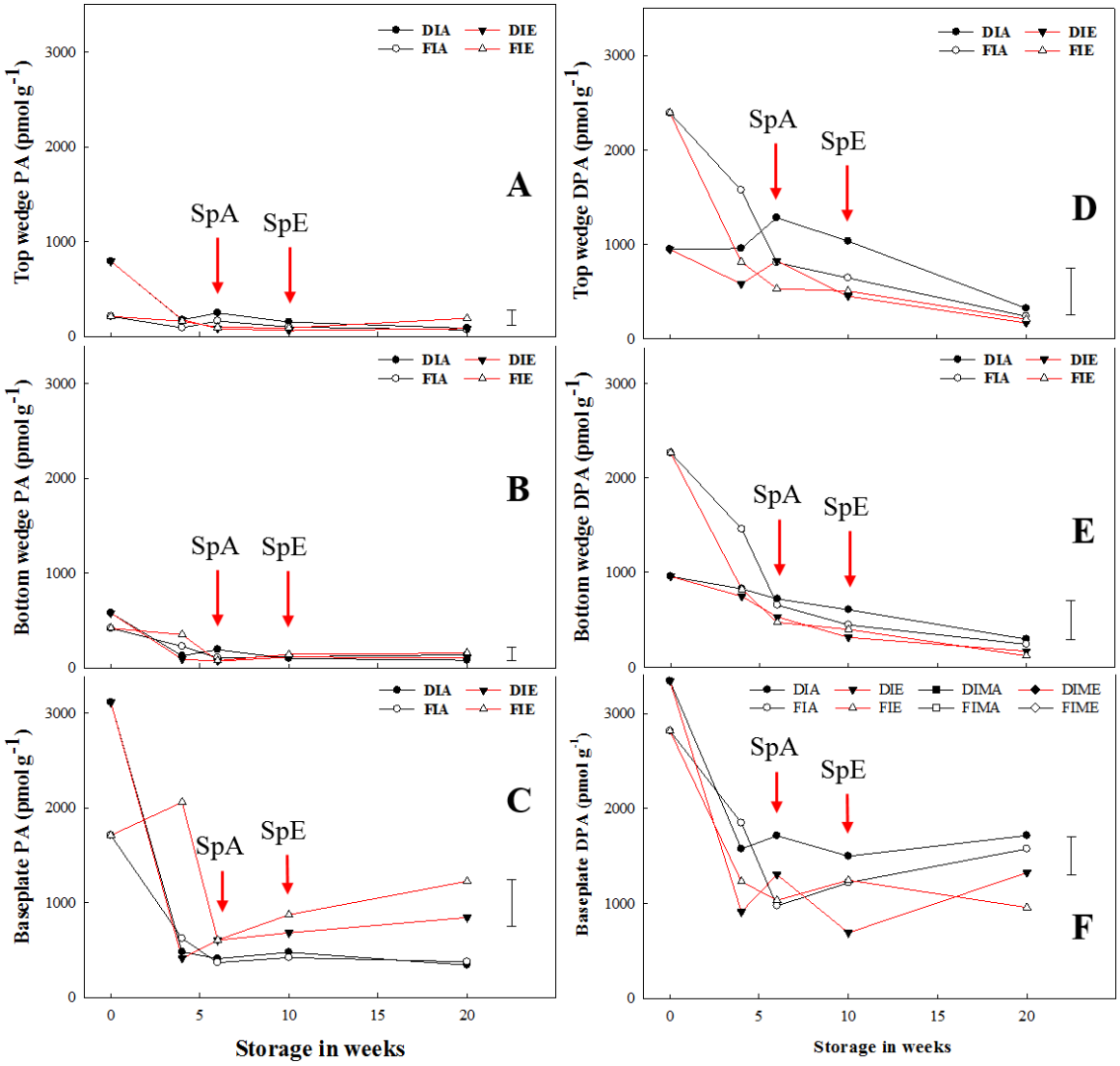

Figure 3-4. Phaseic acid (PA) and dihyrophaseic acid (DPA) contents in the top wedge (A and D), bottom wedge (B and E) and baseplate (C and F) of stored onion bulbs (cv. 'Sherpa') grown under full and deficit irrigation and stored under air or ethylene. PA (A, B and C), and DPA (D, E and F) contents per dry weight for the top wedge, bottom wedge and baseplate of onion bulbs (cv. 'Sherpa') grown under full irrigation (FI) or deficit irrigation (DI); where FI defines 100% replenishment of crop evapotranspiration (ET_c) and DI 50% replenishment of ET_c. Bulbs were harvest at full maturity (i.e. 100% fall-down) and cured under glass for six weeks. Post-curing, bulbs were stored at 1 °C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIA and FIA are DI and FI bulbs stored in air and DIE and FIE are DI and FI bulbs stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95 % confidence shown for interactions between treatments and storage in weeks.

3.4.3 Postharvest effects of 1-methylcyclopropene (1-MCP) on the accumulation and distribution of hormones in relation onion bulb dormancy

There were no significant differences in the ABA content between 1-MCP-treated and untreated bulbs, irrespective of section (Figure 3-5 A, B and C). Similar to ABA for the whole bulb, the overall ABA content was significantly ($p=0.024$) higher for 1-MCP-treated bulbs when compared to untreated onion bulbs (Figure 3-7 C and D). In contrast to ethylene, there were no significant differences in sprouting between 1-MCP-treated and untreated bulbs. There were also no significant differences in ABA-GE content (Figure 3-5 D, E and F) between 1-MCP-treated and untreated bulbs. Irrespective of the variations in 7′-OH-ABA (Figure G-1), PA (Figure 3-6 A, B and C) and DPA (Figure 3-6 D, E and F) contents in relation to 1-MCP treatment. Overall, 1-MCP treatment as an independent factor had no influence on sprouting time.

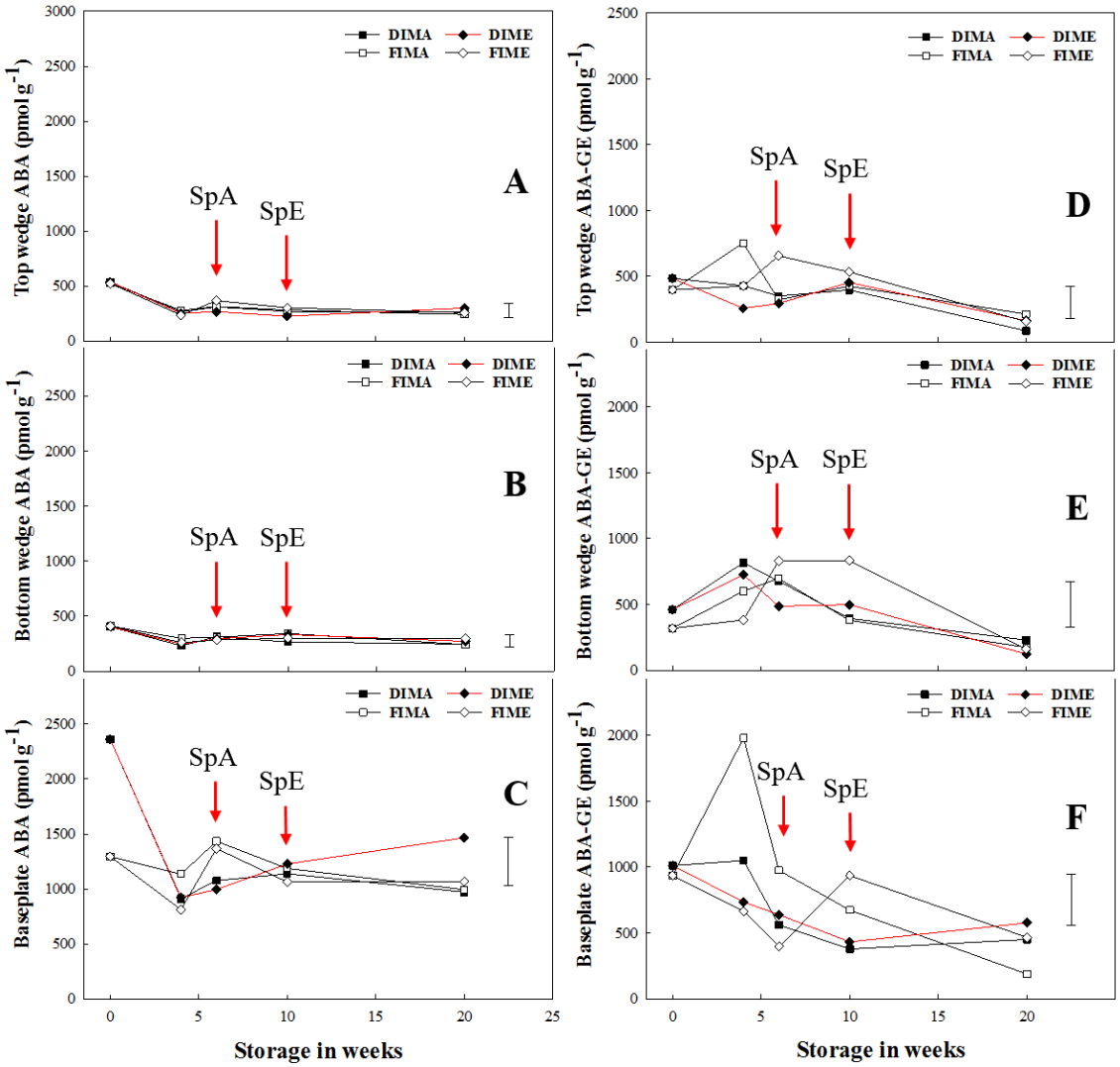

Figure 3-5. Abscisic acid (ABA) and abscisic acid glucose ester (ABA-GE) contents in the top wedge (A and D), bottom wedge (B and E) and baseplate (C and F) of stored onion bulbs (cv. 'Sherpa') grown under full and deficit irrigation, treated with or without 1-methylcyclopropene (1-MCP) and stored under air or ethylene. These contents per dry weight for the top wedge (A and D), bottom wedge (B and E) and baseplate (C and F) of onion bulbs (cv. 'Sherpa') grown under full irrigation (FI) or deficit irrigation (DI); where FI defines 100% replenishment of crop evapotranspiration (ET_c) and DI 50% replenishment of ET_c. Bulbs were harvested at full maturity (i.e. 100% fall-down) and cured under glass for six weeks. Post-curing, bulbs were treated with or without 1-MCP at 1 µL L^{-1} for 24 h before storage and stored at 1 °C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene,

respectively. LSD bar at 95 % confidence shown for interactions between treatments and storage in weeks.

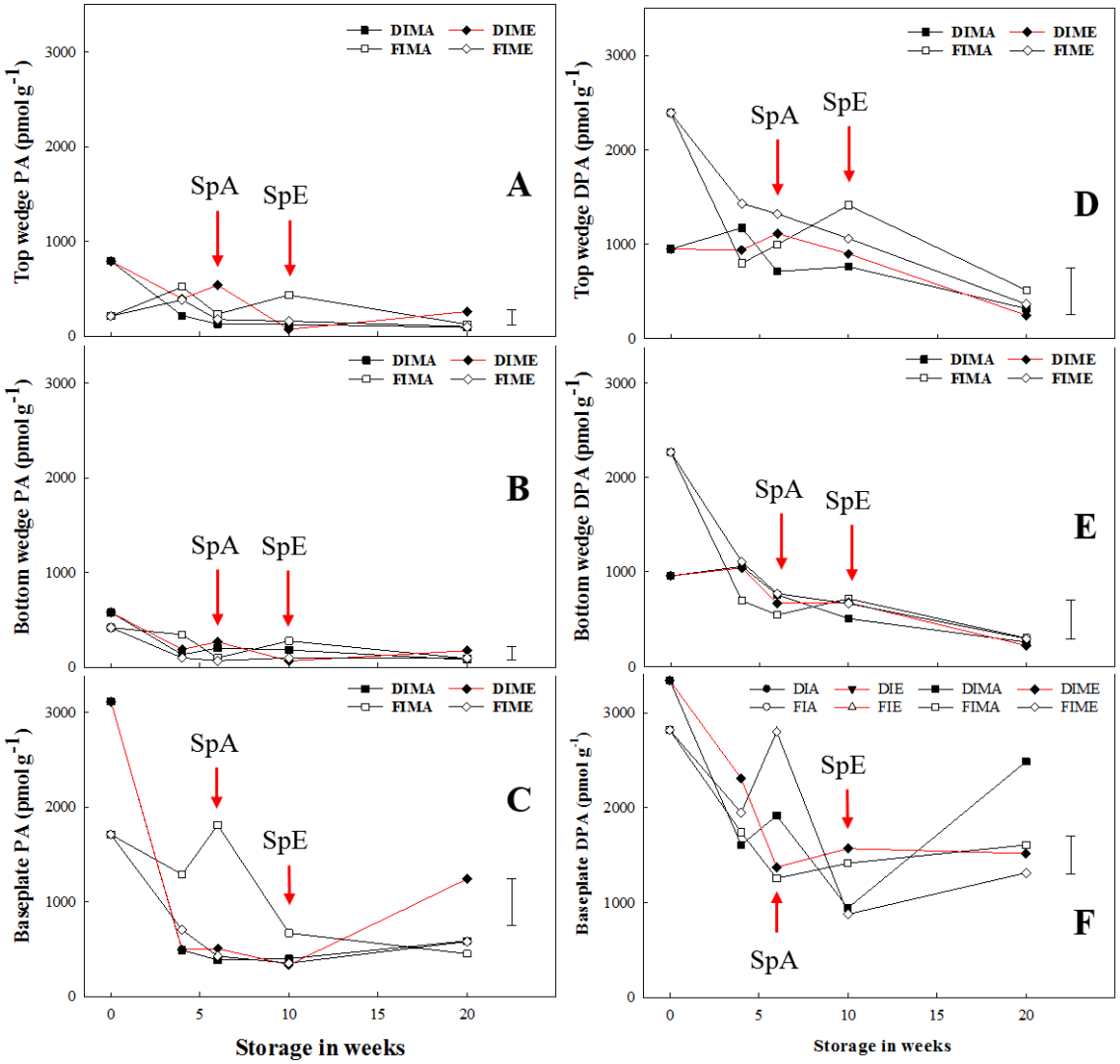

Figure 3-6. Phaseic acid (PA) and dihyrophaseic acid (DPA) contents in the top wedge (A and D), bottom wedge (B and E) and baseplate (C and F) of stored onion bulbs (cv. 'Sherpa') grown under full and deficit irrigation, treated with or without 1-methylcyclopropene (1-MCP) and stored under air or ethylene. These contents per dry weight for the top wedge (A and D), bottom wedge (B and E) and baseplate (C and F) of onion bulbs (cv. 'Sherpa') grown under full irrigation (FI) or deficit irrigation (DI); where FI defines 100% replenishment of crop evapotranspiration (ET_c) and DI 50% replenishment of ET_c. Bulbs were harvested at full maturity (i.e. 100% fall-down) and cured under glass for six weeks. Post-curing, bulbs were treated with or without 1-MCP at 1 µL L^{-1} for 24 h before storage and stored at 1 °C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI

bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95 % confidence shown for interactions between treatments and storage in weeks.

3.4.4 Effects of ethylene and 1-MCP interactions on the contents of hormones in relation to sprouting

Independently, ethylene (Figure 3-7 A and B) and 1-MCP (Figure 3-7 C and D) significantly reduced the overall decline of ABA in the whole onion bulb during storage (p=0.10 and 0.024, respectively). However, the interactions between ethylene and 1-MCP on ABA content was not significantly different (p=0.082); and neither were the interactions between ethylene and 1-MCP on ABA-GE and 7′-OH-ABA contents. In contrast, the interaction between ethylene and 1-MCP significantly (p=0.017) reduced the PA content (331.0 pmol g^{-1}) compared to treated bulbs stored in air (415.0 pmol g^{-1}). The interactions between pre- and postharvest treatments on ABA content was not significant (p=0.439) (Figure 3-7 B and D). Overall, the interaction between ethylene and 1-MCP produced the shortest sprouts when compared to the other treatments.

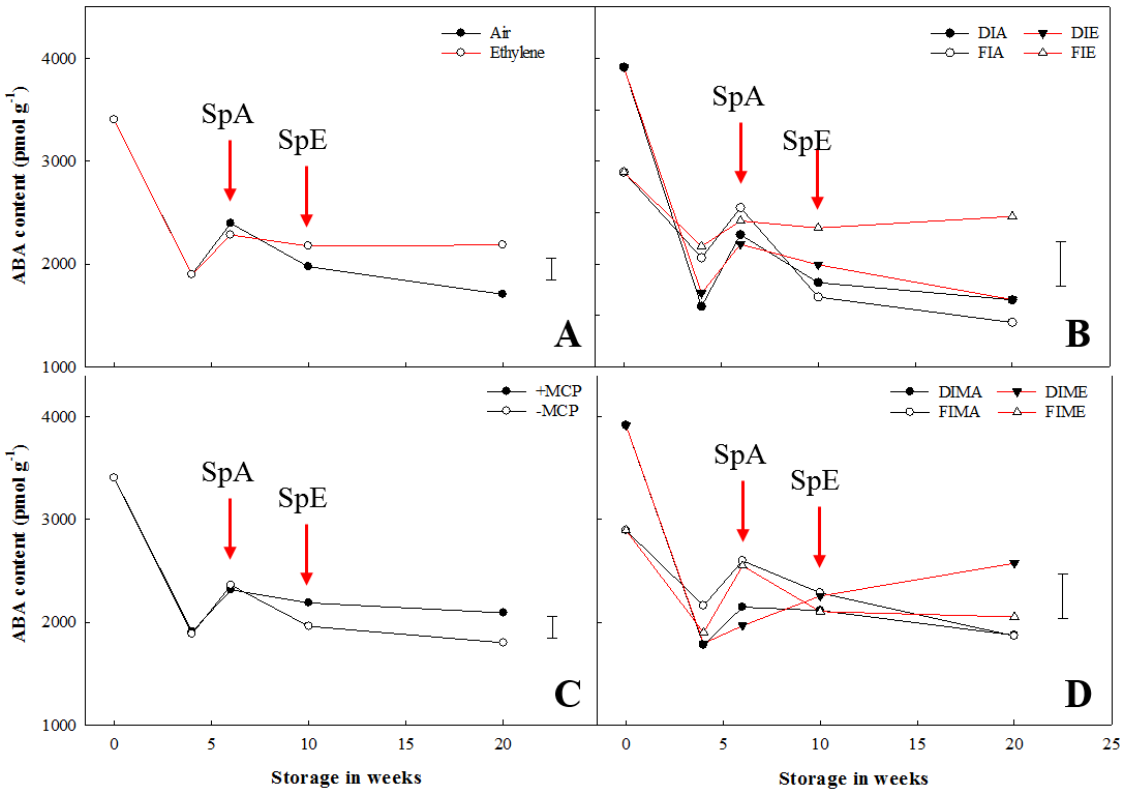

Figure 3-7. Abscisic acid content in stored onion bulbs as influenced by pre-harvest deficit irrigation and postharvest ethylene and 1-methylcyclopropene treatments (1-MCP). ABA content per dry weight (A and B) of onion bulbs (cv. 'Sherpa') grown under full irrigation (FI) or deficit irrigation (DI); where FIA defines 100% replenishment of crop evapotranspiration (ET_c) and DI 50% replenishment of ET_c. Bulbs were harvest at full maturity (i.e. 100% fall-down) and cured under glass for six weeks. Post-curing, bulbs were treated with 1-MCP at 1 µL L^{-1} for 24 h before storage (C and D) or untreated with 1-MCP and were stored at 1 °C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIA and FIA and untreated bulbs DI and FI bulbs stored in air, DIE and FIE are untreated bulbs DI and FI bulbs stored under ethylene, DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95 % confidence shown for interactions between treatments and storage in weeks.

3.4.5 Correlations between hormones and sugars of onion bulbs at pre- and postharvest stages

The correlation between ABA and all of its metabolites was positive at pre- and postharvest (Table 3-2). ABA showed the strongest correlation (0.81) with 7′-OH-ABA at pre-harvest; however, during storage this had diminished by at least 10-fold (-0.08) with a strongest correlation (0.60) established between ABA and DPA. The hormones showed significant negative correlations with the sugars; except for ABA-GE, which had no significant correlation with sucrose. At the pre-storage stage, the best correlation (-0.83) was between glucose and ABA and the least correlation (0.27) between DPA and glucose. During storage, the correlation between ABA and glucose had diminished slightly (-0.70); while the ABA correlation with fructose had become slightly stronger (-72); in contrast to pre-storage treatments.

Table 3-2. Pre- and storage correlation coefficients between ABA metabolites and sugars (fructose, glucose and sucrose) in onion bulbs (cv. 'Sherpa') grown under full or deficit irrigation, harvest at full maturity and cured under glass for six weeks.

	Pre-storage				
	ABA	**ABA-GE**	**7′-OH-ABA**	**PA**	**DPA**
ABA	1.00	0.35	0.81	0.40	0.40
ABAGE	0.35	1.00	0.33	0.76	-0.16
7′-OH-ABA	0.81	0.33	1.00	0.38	0.35
PA	0.40	0.76	0.38	1.00	-0.12
DPA	0.40	-0.16	0.35	-0.12	1.00
Fructose	-0.58	-0.13	-0.52	-0.28	-0.41
Glucose	-0.83	-0.33	-0.68	-0.45	-0.27
Sucrose	-0.52	0.00	-0.55	-0.26	-0.49
	Storage				
ABA	1.00	0.43	-0.08	0.54	0.60
ABA_GE	0.43	1.00	-0.02	0.22	0.33
7′-OH-ABA	-0.08	-0.02	1.00	0.50	0.32
PA	0.54	0.22	0.50	1.00	0.37
DPA	0.60	0.33	0.32	0.37	1.00
Fructose	-0.72	-0.35	-0.35	-0.40	-0.52
Glucose	-0.70	-0.31	-0.50	-0.43	-0.34
Sucrose	-0.31	0.11	-0.49	-0.26	-0.09

3.5 Discussion

The present study investigated if DI treatment would increase onion bulb ABA content. Ethylene and 1-MCP were used to test whether the widely reported ethylene-related sprout suppressions was mediated through ABA and its metabolites. The data showed that DI treatment caused a significant increase in the ABA content of the baseplate (where the meristematic tissue is found) at the end of curing. Despite this, there were no significant differences in sprout emergence between DI and FI bulbs. This contrasts with previous finding that an increase in the endogenous ABA content in onion bulbs may delay sprouting in stored onion bulbs. Nevertheless, while Chope *et al*. (2007) sampled the whole bulb, the differences in the present study were found in the baseplate. This highlights the importance of the differences in the tissues sampled.

Sprout growth in stored onion bulbs has previously been linked to ABA content within the bulb prior to storage such that a higher content will delay sprouting. Thus, Chope *et al*. (2007) postulated that increasing the endogenous ABA content may delay sprout emergence. To increase onion bulb ABA content, they dipped onion bulbs in ABA or ABA analogue (8'-methylene ABA methyl ester; PBI-365) solutions or applied them as a foliar spray. The aim was to enhance absorption and translocation to the bulbs. However, neither of these treatments caused an increase in the endogenous ABA content nor delayed sprouting. They concluded that exogenous application of ABA is of little benefit to increasing onion bulb endogenous ABA content. While this may be true, the accumulation of ABA in different plant tissues grown under water-stress such as deficit irrigation (DI) has been widely reported. This present study represents the first trial of the effects of DI treatments on the endogenous ABA content of onion bulb in relation to storage purposes.

During storage, the endogenous ABA content of onion bulb is known to decline continuously to a minimum at which point sprouting occurs (Chope and Terry, 2008). This suggests that delaying the endogenous ABA decline might also delay sprout emergence. However, ABA-induced dormancy is through its perception by its receptors (the protein complex PYR/PYL/RCAR) w, which triggers its signalling pathway activating the positive regulators (e.g. the plant-specific sugar non-fermenting related protein kinases SnRK2s) and hindering the negative regulators (e.g. the protein

phosphatase type-C - PP2C). Therefore, the decline in ABA due to catabolism to release dormancy may have led to a reduction in their perception and signalling. Ethylene delayed sprout emergence for four weeks compared to bulbs stored in air; irrespective of 1-MCP treatment for both DI and FI bulbs as previously reported (Ohanenye *et al.*, 2019). Continuous ethylene supplementation was found to reduce the decline in ABA, an indication that ethylene-related sprout suppression may be mediated through ABA catabolic pathways. ABA 8′-hydroxylases (*CYP707A*) is the central to, therefore, it will be interesting in the future to investigate if ethylene interferes with the *CYP707A* activities, especially in onion bulbs. ABA catabolism. Contrastingly, 1-MCP, on its own, had no influence on sprouting despite eliciting a similar effect on the endogenous ABA content. This disagreed with the findings of Chope *et al*. (2006a), where it was reported that 1-MCP treatment inhibited sprouting and that 1-MCP does not influence the endogenous ABA content in stored onion bulbs. Alternatively, the present results could mean that despite being low ethylene producers, endogenous ethylene in the onion may be required in concert with exogenous ethylene or ABA in the regulation of bulb dormancy and sprouting. 1-MCP may have inhibited the endogenous ethylene from interacting with ABA. These taken together, raises further questions as to the actual role of ABA in the regulation of onion dormancy.

The present study showed that the differences between DI and FI bulbs found in the baseplate prior to storage had diminished during storage before the bulbs sprouted. This therefore leads to the conclusion that stress-induced ABA accumulation in onion is not incorporated into the regulation of bulb dormancy. In additon, the decline in ABA content across all sections, which occurred four weeks into storage irrespective of pre- or postharvest treatments, may have identified a dormancy break. However, whether the decline resulted from or caused dormancy-break is not clear. Chope *et al*. (2006c) studied the ABA content in relation to dormancy among long-, mid- and poor-storing onion bulbs and found that the ABA concentration decreased from long- to poor-storing onion bulbs. Thus, suggesting that the differential accumulation of endogenous ABA content in onion bulbs in relation to dormancy may be typical of cultivars. Interestingly, they reported that the decline in ABA content followed a similar pattern, irrespective of whether long- or poor-storing onion bulbs.

There were no distinct changes in the concentrations of ABA metabolites in relation to sprout emergence, an indication that they may not interfere with onion bulb dormancy. Having said that, ABA showed strong positive correlation (0.81) with 7′-OH-ABA prior to storage; however, during storage this had declined to (-0.08). Similarly, the correlation with ABA-GE declined (from 0.33 to -0.02). These taken together suggested a change in the pathway of ABA regulation between dormant and non-dormant bulbs. Hydroxylation was the major pathway for ABA regulation in dormant bulbs until the break of dormancy when the route through 7′-OH-ABA ceases. Alternatively, it could be that 7′-OH-ABA elicited some ABA-like activities in dormant bulbs, which ceased after dormancy-break. Interestingly, Hill *et al*. (1992) and Zhou *et al*. (2004) had previously reported that 7′-OH-ABA possessed some hormonal activities similar to ABA; nevertheless, the activities of 7′-OH-ABA was 10-timess lesser compared to ABA.

Furthermore, the data showed that the endogenous DPA content was significantly higher in DI bulbs across all sections compared to FI at pre-harvest. However, from harvest until the end of curing the endogenous DPA content of the FI bulbs had significantly overtaken those of the DI bulbs. Notably, irrigation was switched off two weeks prior to harvest; thus, the changes in DPA content was indicative of a response to water stress. Understandably, DPA was higher in DI at pre-harvest while the higher accumulation at harvest until the end of curing may have been due to the unexpected exposure of FI bulbs to water stress for the first time. A similar accumulation of DPA was previously reported for barley seeds grown under drought stress (Seiler *et al.*, 2011). Moreover, DPA showed a strong negative correlation with sucrose in the top and bottom sections of onion bulbs at pre-storage. During storage the correlation had become significantly positive. A crosstalk between ABA and sugars has recently been highlighted by Han *et al*. (2018) and supported by Dekkers *et al*. (2008) who found the existence of a strong overlap between ABA and sugar signalling in *Arabidopsis* seedlings. ABA and sugars have been shown to exert concerted or antagonist influences on each other in relation to the plants developmental process (Finkelstein and Gibson, 2002; Rolland *et al*., 2006; Gambetta *et al.*, 2010), such that the exogenous application of sugars delayed seed germination, similar to exogenous ABA application (Pego *et al*., 1999; Price, 2003; Dekkers *et al*., 2004). While exogenous sugars application caused germination in *Arabidopsis* seeds, which were inhibited by the exogenous application of ABA (Finkelstein and Lynch, 2000;

Garciarrubio *et al*., 2015). Considering that ABA metabolites were previously thought to possess no biological activities until recent findings by Weng *et al*. (2016), the possibility that DPA may possess some biological functions in onion and other plants exists. Nevertheless, a clearer understanding of the roles of ABA and its metabolites on onion bulb dormancy will require more studies in the future.

3.6 Conclusions

Deficit irrigation increased the endogenous accumulation of ABA in the onion baseplate. However, this had no effect on sprouting, an indication that stress-induced accumulation of ABA content may not be incorporated into the regulation of onion bulb dormancy. ABA is distributed more to the baseplate compared to the rest of the onion bulbs, irrespective of pre- or postharvest treatments. Ethylene and 1-MCP when applied alone reduced the overall decline in ABA content during storage, however, only ethylene delayed sprouting. This indicated that while the possibility of the ethylene-related sprout suppression being mediated through the ABA catabolic pathway, there may be a role for endogenous produced ethylene in the regulation of onion dormancy. In addition, the changes in correlations between ABA and 7′-OH-ABA at pre-storage and during storage suggested that the ABA catabolic pathway in onion bulbs may differ depending on the state of dormancy. This changes in regulation could be the key to understanding the mechanisms and the role of ABA in the regulation of onion bulb dormancy. The clear role of ABA in onion bulb dormancy and sprouting remains unclear. Given this, a comprehensive understanding of the roles and mechanisms of ABA and its metabolites on the regulation of onion bulb dormancy and sprouting is needed in the future.

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4 Chapter Four: Fructans redistribution prior to sprouting in stored onion bulbs is a potential marker for dormancy break

4.1 Abstract

Continuous supply of high-quality onion bulbs to meet year-round demand is dependent on maintaining dormancy and bulb quality during storage. Sprouting impacts negatively on the storage quality of onion bulbs. Ethylene supplementation has previously been revealed to inhibit sprout growth in stored onion bulbs. Fructans content, especially those at a higher degree of polymerisation (DP), are reported to positively correlate with delayed sprouting. However, little is known about the impact of pre-harvest irrigation regimes on fructans accumulation and redistribution in relation to onion bulb dormancy and quality in store. Furthermore, the expression analyses of the genes involved in the biosynthesis of fructans and sucrose (the initial substrate for fructan biosynthesis) was conducted to understand the molecular basis of fructans metabolism in stored onion bulbs. Across two seasons, onion plants of cultivars 'Red Baron' and 'Sherpa' were subjected to full irrigation (FI) (100% replacement of crop evapotranspiration) or deficit irrigation (DI) (50% of FI treatment) from bulb initiation to harvest. Bulbs were harvested at full maturity and stored at 1°C for five months. Bulbs were treated with or without 1-MCP (1 µL L^{-1}) for 24 h before storage under continuous ethylene supplementation (10 µL L^{-1}) or air. DI had no effect on dormancy-break, sprout emergence, total fructans content and total sugar content. In contrast, ethylene delayed sprout emergence and suppressed sprout growth; added 1-MCP enhanced this effect. Genes involved in sucrose and fructans biosynthesis were significantly upregulated in sprouted bulbs, yet, neither sucrose nor fructans contents increased; which indicated posttranslational modifications or the preponderance of catabolism over biosynthesis. The concentration of DP3-8 fructans were higher in the top and bottom sections compared to the baseplate. Before sprout emergence, fructans of DPs 7-8 were no longer present in the top and bottom wedges, while they accumulated in the baseplate, irrespective of pre- or postharvest treatments. This redistribution of fructans within the bulb suggested a transition in the dormancy state and could be used as a predictive marker for sprouting in stored onion bulbs.

4.2 Introduction

Despite being a seasonal crop, the demand for onion bulbs is all-year-round, therefore, the onion industry relies on innate dormancy and storage treatments to extend availability. Dormancy break elicits various changes including increased weight-loss, sprouting and breakdown of sugars and fructans. Currently, the industrial standard sprout suppressant regime involves the use of maleic hydrazide (MH) - applied as a foliar spray - in conjunction with low temperature storage and, occasionally controlled atmosphere (CA).

Ethylene supplementation has previously been shown to delay and suppress sprouting in stored onion bulbs. Onion bulbs are low endogenous ethylene producers (Cools *et al.*, 2011; Chope *et al*., 2012); nonetheless, continuous exogenous ethylene supplementation at 10 µL L^{-1} during storage suppresses sprout growth (Briddon and Sbeu, 2006; Bufler, 2009; Cools *et al*., 2011; Chope *et al*., 2012). Further evidence of ethylene sprout suppression in other crops was revealed by Foukaraki *et al*. (2014) (potatoes) and Amoah *et al*. (2016) (sweet potatoes). Perhaps unexpectedly, the treatment of onion bulbs with 1-methylcyclopropene (1-MCP) - a known inhibitor of ethylene activities – also suppressed sprout growth and caused the accumulation of sugars (Chope *et al*., 2006b); and this sprout suppression was further enhanced when applied in combination with ethylene supplementation (Cools *et al.*, 2011).

Fructans are polymers of fructose and the major reserve carbohydrates in onion bulbs, which vary in their degree of polymerisation (DP) (Valluru and Van Den Ende, 2008). Fructans are synthesised during photosynthesis, and accumulation starts when the rate of sugar production becomes higher than the demand for energy (Pollock, 1984); in onion, this coincides with bulb initiation. The metabolism of fructans is catalysed by fructosyltransferases (FTs) and fructans exohydrolase (*FEH*) (Abeynayake *et al.*, 2015); while the FTs catalyse fructan biosynthesis, FEH catalyses fructans catabolism. Several FTs have been identified in various plants *viz*. sucrose:sucrose 1-fructosyltransferase (*1-SST*), sucrose:fructan fructosyltransferase (*SFT*), fructan:fructan 1-fructosyltransferase (*1-FFT*) and fructan:fructan *6G-FFT* (Van den Ende *et al*., 1996; Vijn *et al*., 1997, 1998; Lasseur *et al*., 2011, 2006; Abeynayake *et al*., 2015). In onions, *1-SST* and *6G-FFT* have been cloned and characterised as the only genes in onion fructans biosynthesis (Vijn *et al.*, 1997, 1998). *1-SST* was proposed to be key to fructan biosynthesis, since it catalyses

the first step in *de novo* synthesis of fructan from sucrose (Vijn *et al.,* 1998), which involves the transfer of fructosyl residue from one sucrose to another sucrose to form 1-kestose and glucose molecules as the only products (Livingston *et al*., 2009). Thus, making sucrose pivotal to fructans biosynthesis. The elongation of fructans to generate the structural DP diversity in onions (up to DP15 have been found in onions (Ernst *et al.*, 1998)) is catalysed by 6G-FFT; this enzyme catalyses the transfer fructosyl residue from one fructan to another (Vijn *et al*., 1997; Abeynayake *et al*., 2015;). Despite these, the study of *1-SST*, *6G-FFT* and sucrose phosphate synthase (*SPS*) – the key gene in sucrose biosynthesis (Huber and Huber, 1996), in relation to fructans biosynthesis in stored onion is scarce.

Dormancy transition in onion bulbs is characterised by a decrease in fructans and simple sugars (Chope *et al.*, 2012). The concentration of fructans at harvest was reported to positively correlate with long storage and delayed sprouting in onion bulbs (Suzuki and Cutcliffe, 1989; Jaime *et al*., 2001; Benkeblia *et al*., 2006). Furthermore, Jaime *et al*. (2001), reported a positive correlation between long storing onion bulbs and higher DP fructans content compared to poorer storing onion bulbs. However, the responses of the genes involved in fructans biosynthesis is unknown and the mechanism of fructans mobilisation and metabolism in relation to onion dormancy, is still unclear.

Previous studies on the role of deficit irrigation (DI) on the postharvest qualities of onion bulbs are contradictory. Rattin *et al*. (2011) and Vickers *et al*. (2015) reported that DI caused early sprouting and greater postharvest losses; Leskovar *et al*. (2012) reported an increase in postharvest storage life; while Martín de Santa Olalla *et al*. (2004) and Enciso *et al*. (2009) found no such differences. Notably, none of these earlier studies combined DI with any postharvest sprout mitigation treatments. Moreover, DI had previously been shown to cause the accumulation of non-structural carbohydrates such as sugars in strawberry (Bordonaba and Terry, 2010) and tomato fruits (Kirda *et al.*, 2004). In *Aloe vera* leaves, DI treatments caused up to 60% increase in the fructans content when compared to the fully irrigated plants (Salinas *et al.*, 2016). In the leaves of transgenic tobacco, Pilon-Smits *et al*. (1995a) reported that polyethylene glycol-mediated drought stress caused a 7-fold increase in fructans concentration and overall biomass, while starch level was not affected. Similarly, drought stress caused the accumulation of fructans in

the shoots and roots of transgenic sugar beets (Pilon-Smits *et al.*, 1999) and in the rhizophores (the storage organ) of *Vernonia herbacea* (Garcia *et al.*, 2011). Salinas *et al.* (2016) further reported that DI caused the accumulation of fructans with higher DP when compared to fully irrigated leaves of *Aloe vera* plants. Despite these reports, and the implication that fructans may regulate onion dormancy (Suzuki and Cutcliffe, 1989; Jaime *et al.*, 2001; Benkeblia *et al.*, 2006; Chope *et al.*, 2012), it remains unknown if fructans content can be manipulated through DI and the responses of genes involved in fructans biosynthesis in stored onion bulbs.

The aim of this study was to investigate the influences of pre-harvest DI and postharvest ethylene supplementation on the accumulation and distribution of fructans; as well as the expression of the genes involved in sucrose and fructans biosynthesis in relation to dormancy-break and sprouting in stored onion bulbs.

4.3 Materials and methods

4.3.1 Plant material

In this study, two different onion cultivars ('Red Baron' and 'Sherpa') were used in 2015 and only 'Sherpa' in 2016. In 2015, 'Red Baron' sets and 'Sherpa' seeds were sourced from Elsom Seeds (Lincs., UK), and Steve Howe Seeds (Lincs., UK), respectively; while in 2016, 'Sherpa' seeds were sourced from Limagrain (Lincs., UK). For both years, 'Sherpa' seeds were planted into trays with John Innes No 1 seed media and seedlings were transplanted into pots six weeks after germination, while 'Red Baron' were planted directly into pots. Final growth media was John Innes Compost No 3. Equal weights of the growth media (7.3 kg) were measured into 96 pots of 8 L capacity per cultivar for 2015 and 264 pots for 2016 experiments. Plants were transplanted at the rate of three plants per pot (used as pseudo-reps) for both years. Plants were split into three completely randomised blocks (replicates) formed across two benches in the glasshouse. The first and last rows of plants in blocks 1 and 3, respectively, served as guard plants (not included in the analysis) for both years (Figure 2-1). Plants were fertilised with 800 mL of Hoagland's solution as two single 400 mL applications per treatment. For both years, bulbs were harvested manually at full maturity when all plant foliage had lodged (100% fall-down) and were cured under glass for six weeks (August - September).

4.3.2 Experimental design

Plants were subjected to two pre-harvest treatments: full irrigation (FI) and deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c), and DI corresponded to 50% of the FI treatment. Irrigation was uniformly applied using an automated irrigation system (AC4, Hozelock, Warwickshire, UK). Polyvinyl tubing (20 mm diameter) were fitted with pressure-compensating emitters of 1.2 L min^{-1} flow rate, which were connected to polyvinyl tubes (5 mm diameter) to the pots (one emitter per pot). Soil moisture content was monitored weekly both gravimetrically (based on individual pot weight) and volumetrically using soil moisture probes (HH2 and ML2x, Delta-T, Cambs., UK); three pots per treatment per block were assessed. The onion plants were subjected to differential pre-harvest irrigation treatments for seven weeks - from bulb initiation stage until two weeks before harvest, when no more irrigation was applied thereafter.

At harvest, all three bulbs per pot were collected, tagged and weighed together. Afterwards, the bulbs were spread out in a single layer on the benches in the glasshouse for curing (18–35 °C and 40–90% relative humidity). After six weeks curing, bulbs were transferred to 100 L storage boxes and stored at 1 °C for 18 and 20 weeks for 2015 and 2016, respectively. For 2015, bulbs were subjected to two treatments: continuous ethylene supplementation at 10 µL L^{-1} or continuous air, as described elsewhere (Chope *et al*., 2006a; Amoah *et al*., 2017). For 2016, bulbs were treated with or without 1-MCP at 1 µL L^{-1} for 24 h before storage; and then stored in air or under continuous ethylene supplementation at 10 µL L^{-1} (Cools *et al*., 2011). Thus, the postharvest treatments were: air, air + ethylene, 1-MCP + air, and 1-MCP + ethylene. At each sampling point, three bulbs were collected in triplicate, per treatment and throughout the storage duration (3 bulbs x 3 replicates x treatments). All experiments (pre- and postharvest) were arranged in a completely randomised design.

Bulbs were stored for 18 weeks in 2015 with six postharvest sampling points, while in 2016, bulbs were stored for 20 weeks with seven postharvest sampling points with two pre-storage sampling points (pre-harvest and mid-curing) added. The pre-harvest samples for 2016 were collected at the termination of irrigation at 100% fall-down (two weeks before harvest), while the mid-curing samples were collected three weeks into curing.

Time 0 (week 0) samples for both years were collected at the end of curing, prior to bulbs being transferred to the cold room. Afterwards, sampling was conducted bi-weekly until sprout length was 40% in proportion to bulb height (Chope, 2006). At each sampling point, sprout assessment was conducted (sprout length as a percentage of total bulb height) and recorded (Figure 2-3). Bulbs were sliced in two equal parts from top to bottom with a sharp stainless-steel knife; then, the individual baseplates were excised, after which the remaining section (including the skin) was divided equally into top and bottom (Figure D-1). All samples were immediately snap-frozen in liquid nitrogen and stored at -80 °C before lyophilisation at -55 °C in a freeze-drier (Scanvac, Lynge, Denmark) in the dark for 7 days. Freeze-dried samples were stored at -40 °C prior to biochemical and molecular analyses.

4.3.3 Sprout assessment

Sprout incidence were assessed as previously described elsewhere (Chope *et al*., 2006b, 2006a). Onion bulbs were cut in half (vertically from top to bottom) and internal sprout length was presented as a percentage of the total bulb height (Figure 2-3**Error! Reference source not found.**).

4.3.4 Non-structural carbohydrates extraction and quantification

Freeze-dried samples (top wedge, bottom wedge and baseplate [Figure D-1]) were ground into fine powder using metallic ball bearings (size: 5 mm) placed in each tube with freeze-dried samples and placed into a Star-beater (VWR International bvba, Leuven, Belgium). Non-structural carbohydrates (NSCs) from the powdered samples were extracted and quantified as described elsewhere, with modifications. Briefly, 150 mg of pulverised samples were thoroughly vortexed in 3 ml of 62.5% (v/v) aqueous HPLC grade methanol, incubated in a shaking bath for 15 mins at 55 °C and filtered through a 13mm diameter X 0.2 µm PTFE filter unit (Jaytee Biosciences Ltd, Kent, UK). Extracts (10 µL) were injected into a Prevail Carbohydrate ES (250 X 4.6 mm, 5 µm particle size; Grace Discovery Sciences, IL, USA) with a guard column (ZORBAX Eclipse Plus-C18, 2.1 X 12.5 mm, 5 µm particle size) in Agilent 1260 Infinity HPLC system coupled to an Evaporative Light-Scattering Detector (Agilent Technologies LDA UK Limited, Cheshire, UK). The mobile phase was a linear increase/decrease amount of water in acetonitrile (% water): 15 minutes, 20-50%; 15-20 minutes, 50-20%; 20-23 minutes, 20-

15%; 23-30 minutes, 15-10%. The flow rate and injection volumes were 0.8 mL min^{-1} and 5 µL, respectively, and the oven temperature was set at 30 °C. The presence and concentration of fructose, glucose, sucrose, kestose and nystose were calculated by comparing the peak areas in each sample to standards (0.05–5 mg mL^{-1}) using Agilent ChemStation software version 4 (Agilent Technologies, CA, USA). Fructose, glucose, sucrose, kestose and nystose standards were obtained from Sigma-Aldrich Co. (Dorset, UK). Fructans with degree of polymerisation higher than four were quantified using the standard curve for nystose as previously described by Cools (2010). Total sugar content was calculated as the sum of the concentrations of fructose glucose and sucrose while total fructans was calculated as the sum of the concentrations of fructans of DP3-8 on a per section and per sample bases.

4.3.5 Molecular analyses

There were no significant differences in the total fructans content prior to or during storage. Therefore, the molecular analysis was conducted on the FI bulbs only.

4.3.5.1 Total RNA extraction

Total RNA from 20 – 40 mg of pulverised freeze-dried samples from the top wedge, bottom wedge and baseplate of fully irrigated plants were isolated using RNeasy total plant kit (Qiagen, Manchester, UK) following the manufacturer's instructions, with modifications. Briefly, after 600 µL of Qiagen RLC lysis buffer solution was added into each tube and vortexed vigorously, the mixture was filtered using Qiagen filtration tubes into fresh 1.5 mL Eppendorf tubes to remove insoluble debris. For the RNA elution, 35 µL of RNase-free water was used. The RNA concentration prior to 2% gel electrophoresis was determined using a Genova Nano micro-volume spectroscopy (Jenway, Staffordshire, UK); and to confirm the quality of RNA for further processing, the integrity of total RNA samples was determined using Experion RNA StdSens chips on the Bio-Rad Experion Automated Electrophoresis Station (Bio-Rad Laboratories, USA) following the supplier's protocol. The RIN (RNA integrity number) values of these samples were above 7.5. The RNA samples were stored in a -80 °C freezer until needed for reverse transcription to cDNA.

4.3.5.2 Reverse transcription

All samples preparation was conducted on ice and cDNA was generated using QuantiTect Reverse Transcription Kit (Qiagen, Manchester, UK) with 1ug total RNA used according to manufacturer instructions. The reverse-transcription reaction samples were stored at -20 °C until needed for real-time qPCR.

4.3.5.3 Primers

Primers were designed for the following onion genes involved fructans and sucrose synthesis: sucrose:sucrose 1-fructosyltransferase (*1-SST*; Accession number: AJ006066.1), fructan:fructan 6G-fructosyltransferase (*6G-FFT*; Accession number: KT935444.1), and sucrose phosphate synthase (*SPS*; Accession number: EU164758.1); β-Actin and ubiquitin were tested as the reference (housekeeping) gene. However, the ubiquitin expression was erratic such that it was expressed differently across different sections of the onion bulbs and at different timepoints. Therefore, only β-Actin, which showed consistent expressions irrespective of section of the onion bulb or timepoint was used in the gene expression analyses. The primers were designed using the Primer-BLAST of NCBI (NCBI, Maryland, USA) and the genes specific to onions were selected from the Primer-Blast. The primer quality namely, uniqueness to bind only to the gene of interest, chances of self- and hetero-dimer formation, the melting and annealing temperatues were checked using the Netprimer software (Premier Biosoft International, CA, USA). The primer sequences used are shown in Table 4-1. All oligosaccharides used herein were purchased from Thermo Fisher Scientific (Thermo Fisher Scientific, Paisley, UK).

Table 4-1. List of forward and reverse primers for 1-SST, 6G-FFT, SPS and β-Actin onion genes.

Genes	*Forward primers*	*Reverse primers*
1-SST	GTTGGTGTCGGCTTGAGGTA	TTCTGAACCGAAGCCCATCC
6G-FFT	ACGATATGCTAGCTTGGCAG	TTGCCCCAATATGCAAAATC
SPS	CTTTGGTGGAGCCTTTTGGC	TTCGACATGCTGCCACTCTT
β-Actin	CACCGGTGTCATGGTTGGTA	TGCTCTTCTGGAGCAACTCG

4.3.5.4 Real-time PCR

The real-time PCR was conducted using QuantiTect SYBR Green PCR Kit (Qiagen, Manchester, UK). For the real-time reaction, 5 µL of QuantiTect SYBR Green PCR

Master Mix, 1 μM each for the forward and reverse primers and 1 μM of cDNA template were mixed; the total reaction volume was then made up to 10 μL with RNase-free water. The reaction was conducted using Touch Real-time PCR detection (type: CFX96, Bio-Rad Laboratories, USA). A total of 40 reaction cycles was used with each cycle setup as follows: denaturation temperature at 95 °C for 15 mins and then 94 °C for 15 s, annealing temperature at 55 – 65 °C for 30 s and the extension was 72 °C for 30 s; finally, the data acquisition was set for 15 s. The ΔΔCT method as described by Livak and Schmittgen (2001) was used to analyse the data for the relative expressions of 1-SST, 6G-FFT and SPS.

4.3.6 Statistical analyses and plots

All statistical analyses were conducted using Genstat for Windows 10th Edition (VSN International Ltd, Herts., UK). Analysis of variance (ANOVA) was performed to identify factors that significantly affected variance in the physiological and biochemical data collected. ANOVA was performed on the data specifying a nested treatment structure of a common baseline (observation before postharvest treatments). Least significant difference (LSD) values were calculated from each analysis, for comparison of appropriate treatment means, using general analysis of variance (shown in 5.9K.1.1). A significance threshold of $p < 0.05$ was adopted, for all analyses. SigmaPlot for Windows SPW13 (Systat Software, Inc., London, UK) was used for all plots.

4.4 Results

4.4.1 Sprout length

Sprout emergence was not significantly different between pre-harvest treatments, for all years (Figure 2-6). However, ethylene delayed sprout emergence by two and four weeks (2015 and 2016, respectively) compared to bulbs stored in air. As an independent treatment, 1-MCP did not delay sprout growth; however, 1-MCP-treated stored under ethylene produced the shortest sprouts when compared to the other treatments applied herein as previously reported (Ohanenye *et al.*, 2019).

4.4.2 Fructans content

Neither pre- nor postharvest treatments significantly influenced the total fructans content within the bulbs (Figure 4-1). The highest total fructans content (sum of DP3-8 fructans concentration) in the top (283–288.6 g kg^{-1}) and bottom (297–305.3 g kg^{-1}) sections were measured at pre-harvest and this declined continuously throughout the storage period; irrespective of pre- or postharvest treatments. In contrast, the total fructans content for the baseplate (128–141.7 g kg^{-1}) increased continuously, irrespective of ethylene treatment, from pre-harvest until six weeks of storage (at sprout emergence for bulbs stored in air), before gradually declining continuously thereafter. Nevertheless, there were no significant differences between pre-harvest treatments as independent factors ($p=0.365$), or with ethylene ($p=0.404$) and 1-MCP ($p=0.627$).

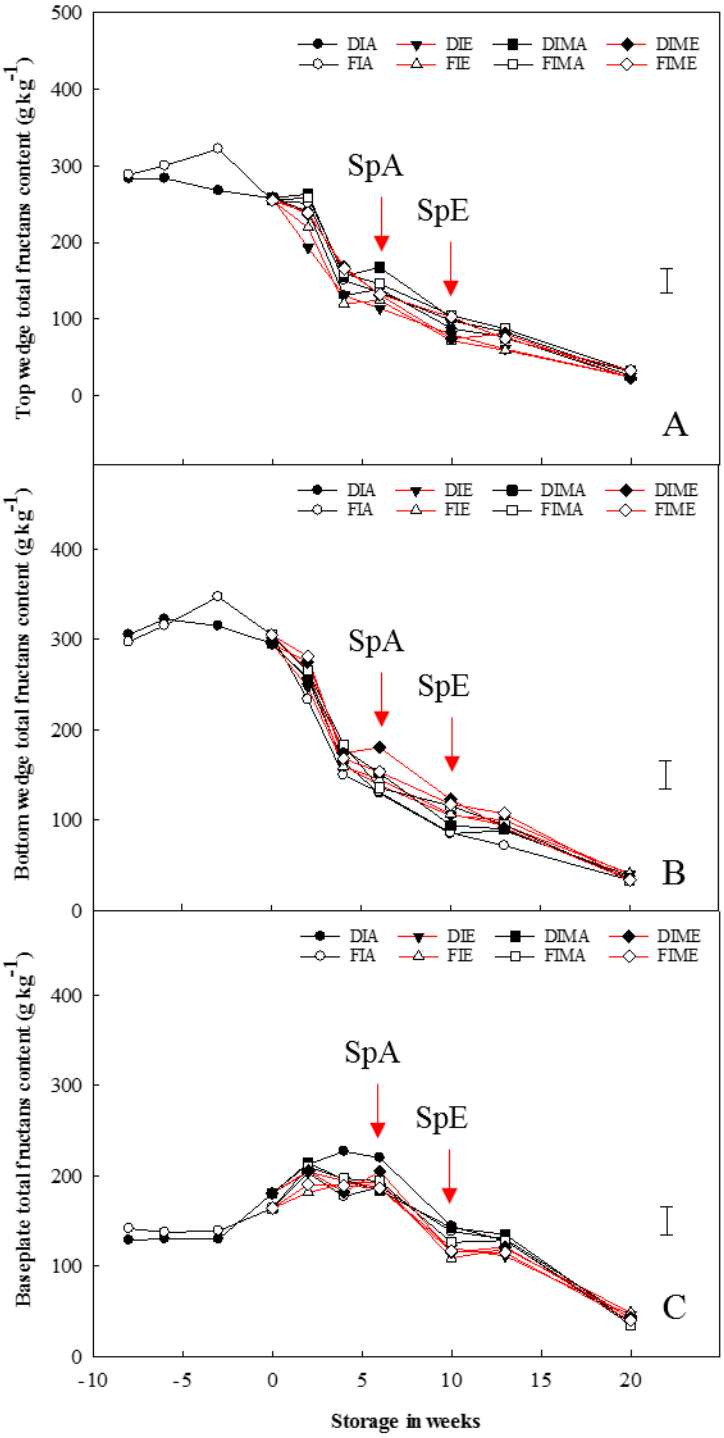

Figure 4-1. Pre- and postharvest total fructans content in the top wedge, bottom wedge and baseplate of stored onion bulbs treated or untreated with 1-MCP and ethylene. Total fructans contents (sum of fructans of DP3-8 content) per dry weight for the top wedge (A), bottom wedge (B) and baseplate (C) of onion bulbs of cultivar 'Sherpa' grown in 2016 under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested

at full maturity (100% fall-down) and cured under glass for six weeks for both years. Post-curing, bulbs were treated with 1-MCP at 1 µL L^{-1} for 24 h before storage or untreated with 1-MCP and were stored at 1 °C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. Where pre-harvest = -8, harvest = -6, mid-curing = -3 and end of curing = 0. LSD bar at 95% confidence shown for interactions between treatments and storage in weeks.

The kestose (DP3) content between harvest and mid-curing in the bottom wedge was significantly higher in DI compared to FI, while for the baseplate, DP3 was significantly higher in FI compared to DI bulbs. However, at the end of curing, these differences had disappeared. No similar differences were found for nystose (Figure 4-2) or other fructans of higher DP; irrespective of pre-harvest treatments or year. Moreover, by the end of curing and during storage, the differences in the accumulation of kestose between pre-harvest treatments had ceased to exist. Besides a significant decline in the nystose content for the bottom section and baseplates of 1-MCP-treated bulbs stored under ethylene at week 6, there was no consistent effect of ethylene or 1-MCP on kestose or nystose contents during storage.

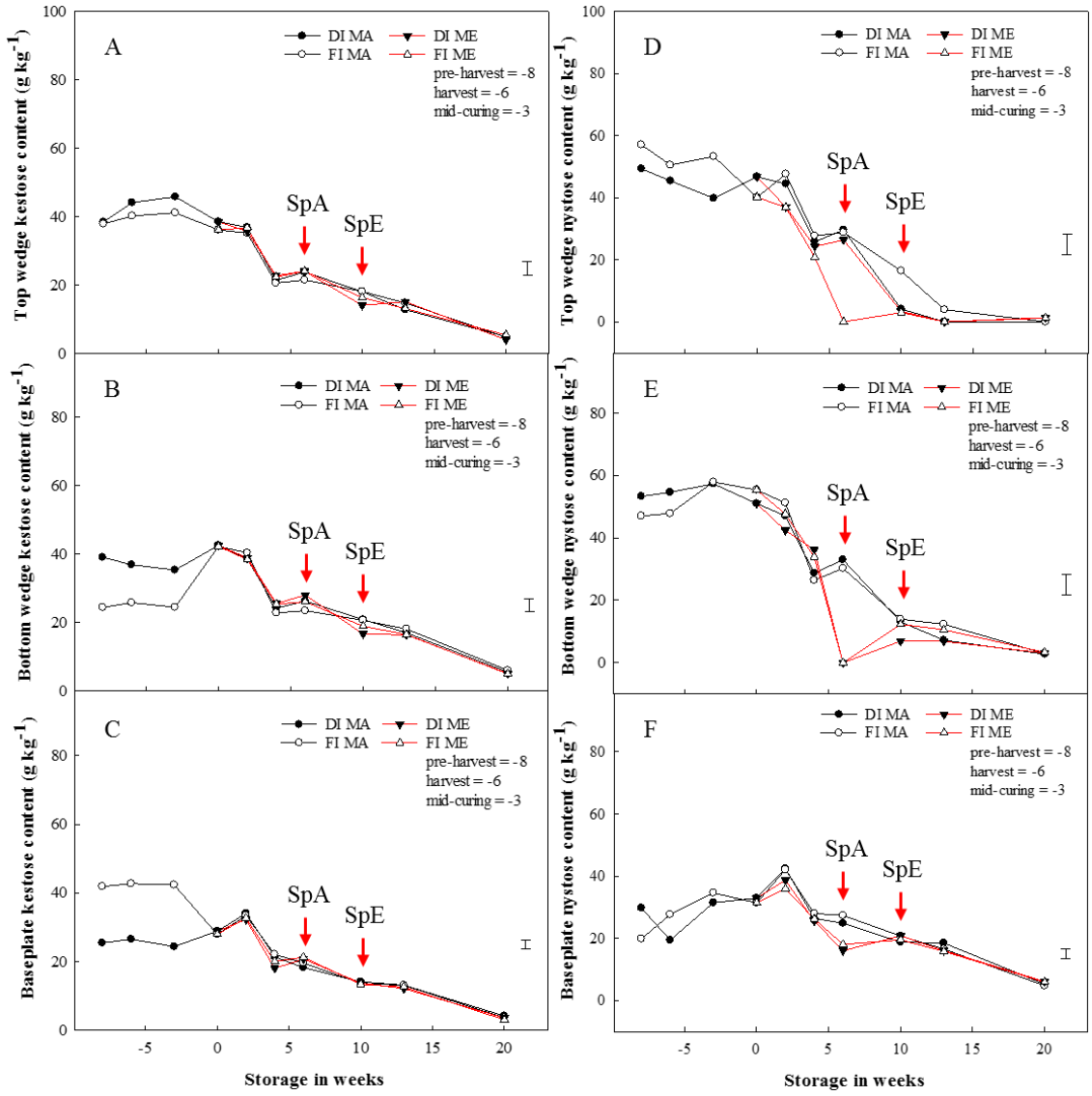

Figure 4-2. Pre- and postharvest kestose and nystose contents in the top section, bottom section and baseplate of stored onion bulbs treated with and without 1-MCP and ethylene. Kestose and nystose contents per dry weight for the top wedge (A and D), bottom wedge (B and E) and baseplate (C and F) of onion bulbs of cultivar 'Sherpa' grown in 2016 under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. Post-curing, bulbs were treated with 1-MCP at 1 µL L^{-1} for 24 h before storage and were stored at 1 °C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout

emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95% confidence shown for interactions between treatments and storage in weeks.

For 2015 onion bulbs, fructans of DP3-6 were present across all sections in the cured bulbs (DP6 shown in Figure H-1); while fructans of DP7 and 8 were present only in the baseplate. For 2016 onion bulbs, fructans of DP3-7 were present across all sections at pre-harvest, harvest, and mid-curing stages, with DP8 fructans being totally absent in the baseplate. However, at the end of curing, irrespective of pre-harvest treatments, DP8 fructans were no longer present in the top wedge (Figure 4-3).

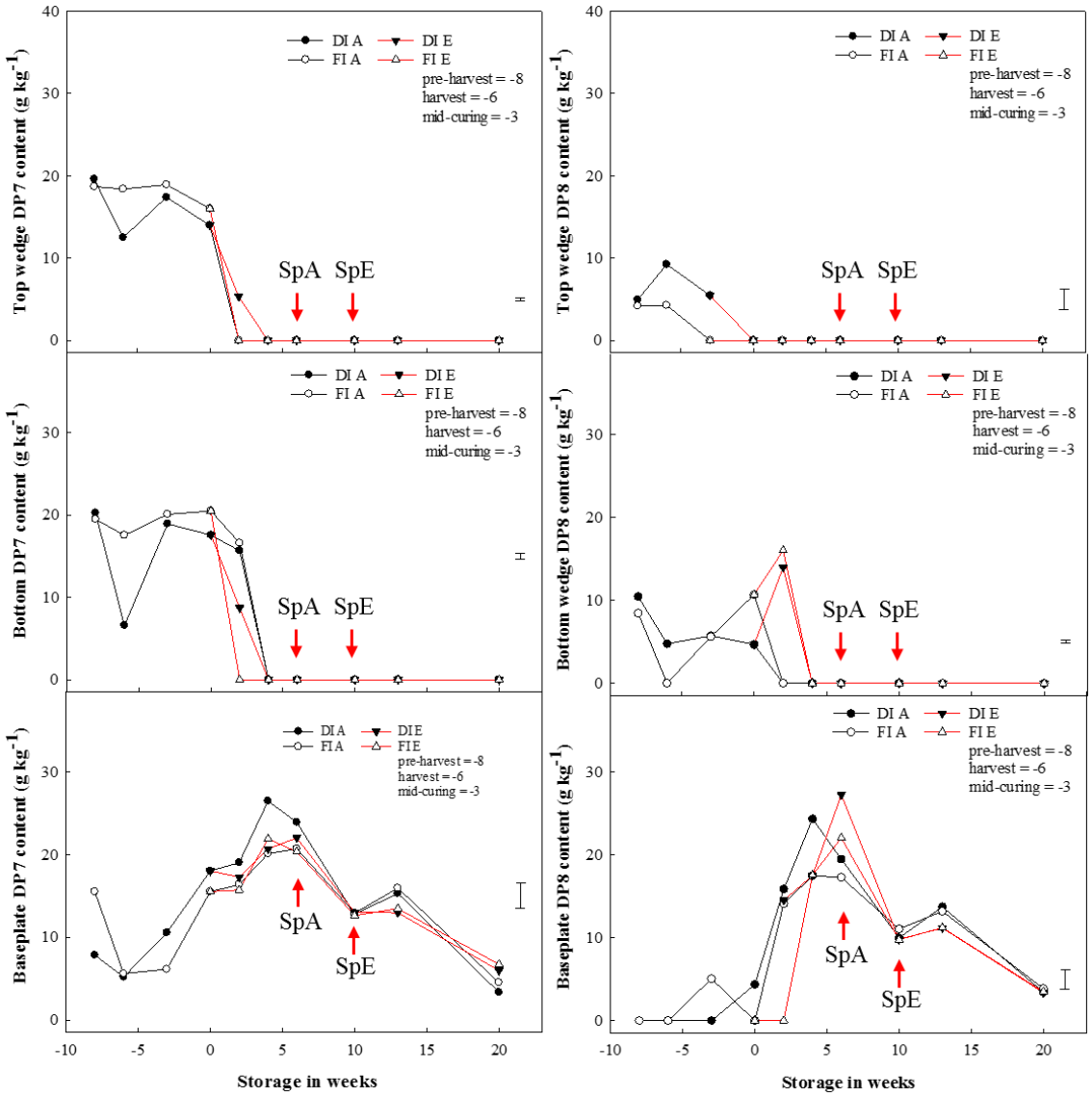

Figure 4-3. Pre- and postharvest fructans of DP7 and 8 contents for the top section, bottom section and baseplate of stored onion bulbs. Fructans of DP7 and 8 contents per dry weight

for the top wedge, bottom wedge and baseplate of onion bulbs of cultivar 'Sherpa' grown in 2016 under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. Post-curing, bulbs were stored at 1 °C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIA and FIA are DI and FI bulbs stored in air and DIE and FIE are DI and FI bulbs stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95% confidence shown for interactions between treatments and storage in weeks.

Prior to sprout emergence, there was a significant decline in fructans content, especially for fructans of DP6 and above. For 2015 bulbs, fructans of DP6 in the top and bottom wedges declined from *ca*. 47.7 to 0 g kg^{-1} (Figure H-1). A similar decline was found in 2016 bulbs. Fructans of DPs 7 and 8 (Figure 4-3) in the top and bottom wedges declined from 20.5 to 0 and 16 to 0 g kg^{-1} DW, for DPs 7 and 8, respectively. In contrast to the decline of higher DP fructans in the top and bottom wedges, they increased in the baseplate; irrespective of cultivar, pre- or postharvest treatments or year.

Correlations were found to be stronger between fructans of closer DPs (e.g. DP3 and DP4) when compared to fructans with DPs wider apart (e.g. DP3 and DP8) as shown in Table 4-2.

4.4.3 Total sugar (fructose, glucose, and sucrose) content

There were no significant differences in the total sugar content (the sum of fructose, glucose, and sucrose concentrations) between deficit and fully irrigated bulbs. Moreover, the total sugar content declined across all sections after four weeks of storage, with no significant differences between pre- and postharvest treatments (Figure 4-4); a similar trend was found in 2015.

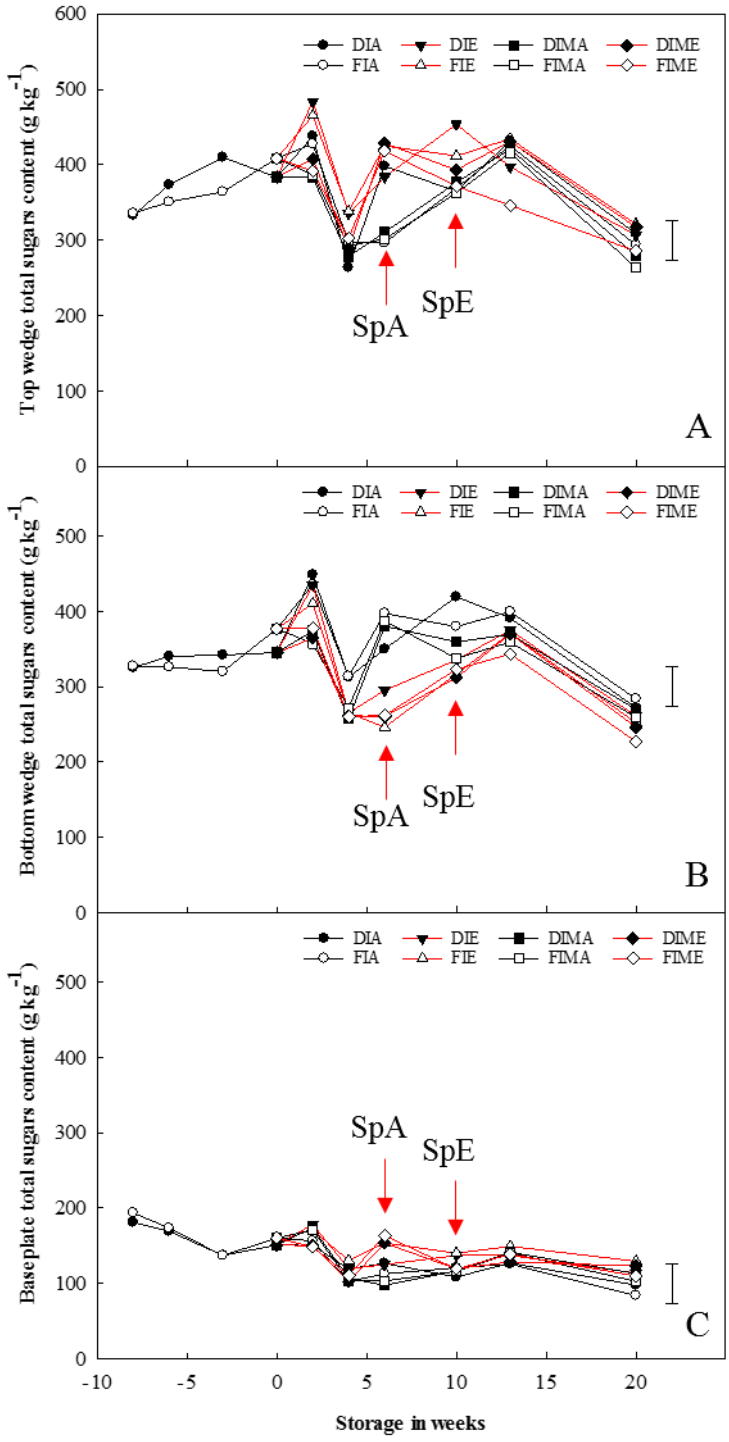

Figure 4-4. Pre- and postharvest total sugar content in the top section, bottom section and baseplate of stored onion bulbs treated with and without 1-MCP and ethylene. Total fructans contents (sum of fructose glucose and sucrose content) per dry weight for the top wedge (A), bottom wedge (B) and baseplate (C) of onion bulbs of cultivar 'Sherpa' grown in 2016 under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%.

Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. Post-curing, bulbs were treated with 1-MCP at 1 µL L^{-1} for 24 h before storage or untreated with 1-MCP and were stored at 1 °C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. Where pre-harvest = -8, harvest = -6, mid-curing = -3 and end of curing = 0. LSD bar at 95% confidence shown for interactions between treatments and storage in weeks.

Overall, fructose content increased continuously throughout the storage period. There were no significant differences in the fructose and sucrose contents for 1-MCP untreated bulbs stored under ethylene. In contrast, fructose and sucrose contents increased by 1.5-fold for 1-MCP treated bulbs stored under ethylene (Figure 4-5). Glucose and sucrose contents increased slightly in the first two weeks of storage before they declined by a half across all sections after four weeks of storage (prior to sprout emergence); nevertheless, there were no differences between pre-harvest treatments.

The strongest correlation between sugars were found between fructose and glucose (0.81) when compared to that between glucose and sucrose (0.77) and between fructose and sucrose (0.66) (Table 4-2).

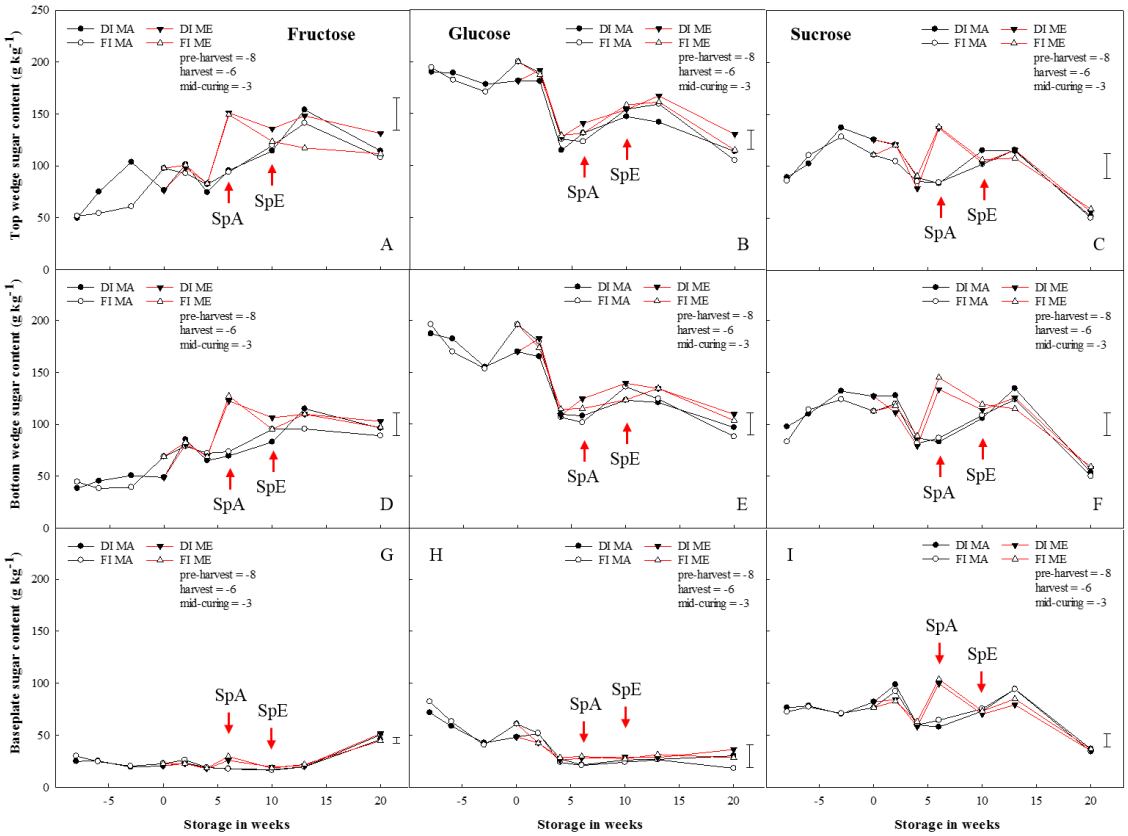

Figure 4-5. Pre- and postharvest sugar content in stored onion bulbs treated with 1-MCP. Fructose, glucose and sucrose contents per dry weight for the top wedge (A, B and C), bottom wedge (D, E and F) and baseplate (G, H and I) of onion bulbs of cultivar 'Sherpa' grown in 2016 under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. After curing, bulbs were treated with 1-MCP at 1 µL L^{-1} for 24 h before storage and were stored at 1 °C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95% confidence shown for interactions between treatments and storage in weeks.

4.4.4 Expressions of 1-SST, 6G-FFT and SPS in onion bulbs at mid-curing and during storage

4.4.4.1 Top wedge

The expression of *1-SST* peaked at the end of curing, and the expression at sprout emergence was at a similar level to mid-curing. There was a slight increase in *1-SST* expression at the end of the storage, which was not significantly different compared to the levels during storage. The overall expression of *1-SST* was stable during storage. *6G-FFT* expression was upregulated during the first weeks of storage; prior to sprouting. By the end of the trial, the expression of *6G-FFT* had increased significantly when compared to the levels during storage (Figure 4-6 A). Akin to *6G-FFT*, *SPS* was significantly upregulated in the first weeks of storage when compared to mid-curing and end of curing. *SPS* expression peaked after four weeks of storage just before sprout emergence, and at sprout emergence, this was significantly downregulated and remained stable afterwards until the end of the storage (Figure 4-6 D). In comparison, the expression of SPS was 10 and 100-folds higher when compared to *6G-FFT* and *1-SST*, respectively.

4.4.4.2 Bottom wedge

The only significant differences in *1-SST* expression was at the end of the storage when *1-SST* was significantly upregulated (*ca* 2-fold) compared to the levels at mid-curing and during storage (Figure 4-6 B). However, there was a continuous upregulation of *6G-FFT* expression throughout the storage period; more so, prior to sprout emergence and at the end of the trial (Figure 4-6 B). *SPS* exhibited a similar trend as found in the top wedge, with a significant (200-fold) higher expression prior to sprout emergence. Thereafter, the expression of *SPS* was not significantly different after sprouting; though there was a significant increase in expression at the end of storage (Figure 4-6 E).

4.4.4.3 Baseplate

1-SST expression was upregulated during curing and during storage, while a significant downregulation occurred prior to sprout emergence. Thereafter, the expression of *1-SST* increased continuously and at the end of the trial had become significantly higher (*ca* 1.5-fold) compared to the level at sprout emergence. Moreover, the expression of *6G-FFT*, which showed an increasing trend was only significantly up regulated at sprout

emergence (Figure 4-6 C). *SPS* expression followed a similar trend as found in the top and bottom wedges; however, the level of expression in the baseplate was significantly reduced by at least 10 and 20-folds when compared to the top and bottom wedges, respectively (Figure 4-6 F).

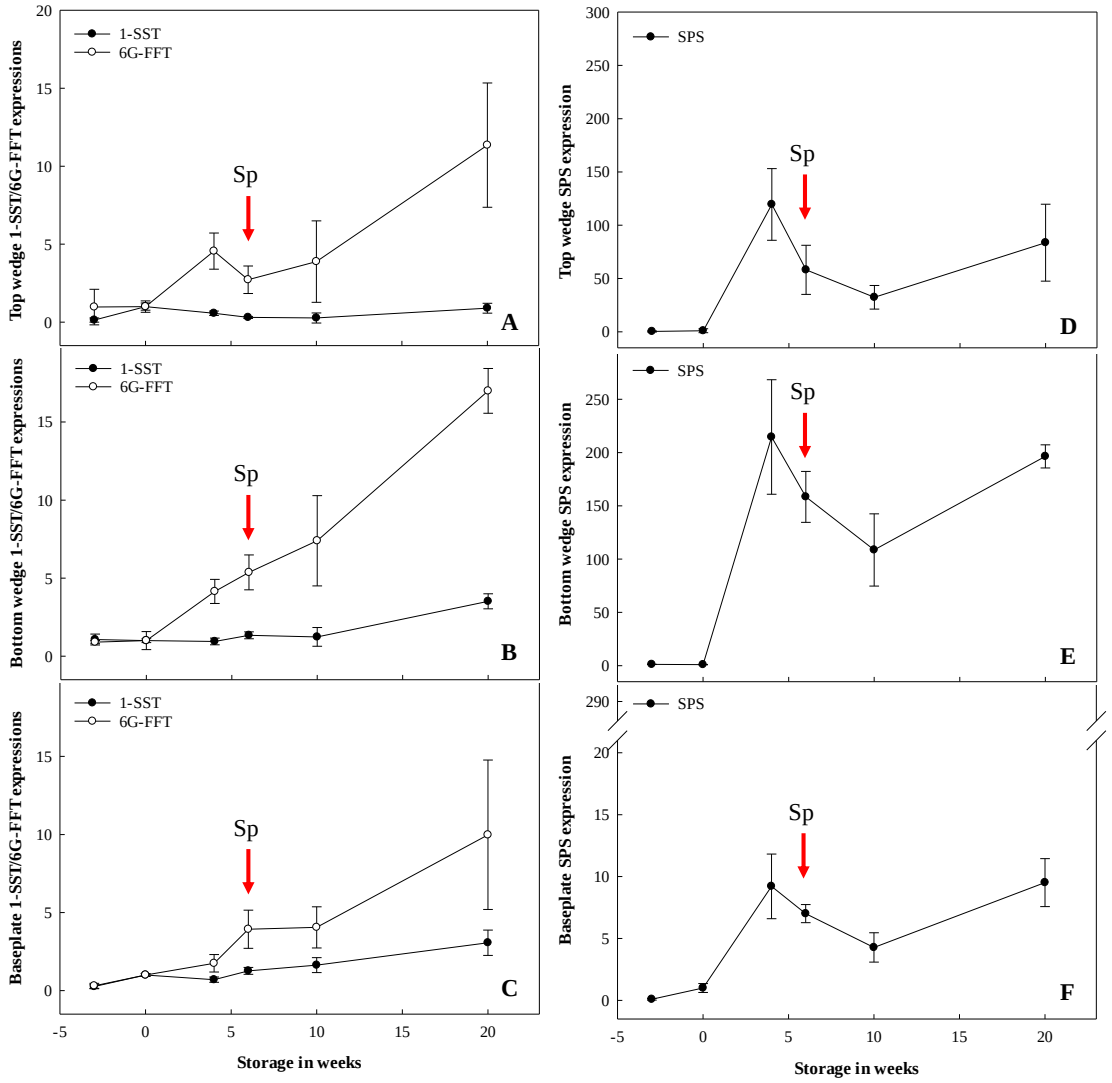

Figure 4-6. Sucrose:sucrose 1-fructosyltransferase (*1-SST*), fructan:fructan 6G-fructosyltransferase (*6G-FFT*) and sucrose phosphate synthase (*SPS*) genes expression levels in stored onion bulbs (cv. 'Sherpa'). 'Sherpa' seeds were grown under glasshouse conditions in 2016 and cured under glass for six weeks and stored at 1° C for 20 weeks. Relative *1-SST*, *6G-FFT* and *SPS* expressions in the top wedge (A and D), bottom wedge (B and E) and baseplate (C and F) were determined by RT-qPCR and normalised by comparison with the expression of β-actin, and analysed using the Livak and Schmittgen,

(2001) method. All RT-qPCRs for each gene used three biological replicates and the standard error bars are shown.

Table 4-2. Correlation between sugars, fructans, total sugars, total fructans and total non-structural carbohydrates for onion bulbs grown under deficit irrigation, treated with 1-methylecycloprpene (at 1 µL L^{-1}) 24 h prior to storage and stored under continuous ethylene (at 10 µL L^{-1}) or air.

Compounds	Fructose	Glucose	Sucrose	DP3	DP4	DP5	DP6	DP7	DP8	Total Sugars	Total fructans	Total NSc
Fructose	1.00	0.81	0.66	0.44	0.07	-0.05	-0.19	-0.33	-0.33	0.90	0.12	0.68
Glucose	0.81	1.00	0.77	0.77	0.51	0.37	0.17	-0.08	-0.28	0.96	0.53	0.91
Sucrose	0.66	0.77	1.00	0.83	0.60	0.54	0.39	0.20	0.05	0.87	0.68	0.92
DP3	0.44	0.77	0.83	1.00	0.83	0.77	0.58	0.31	0.02	0.75	0.89	0.93
DP4	0.07	0.51	0.60	0.83	1.00	0.88	0.73	0.58	0.25	0.43	0.95	0.73
DP5	-0.05	0.37	0.54	0.77	0.88	1.00	0.86	0.70	0.41	0.31	0.95	0.64
DP6	-0.19	0.17	0.39	0.58	0.73	0.86	1.00	0.81	0.57	0.13	0.84	0.47
DP7	-0.33	-0.08	0.20	0.31	0.58	0.70	0.81	1.00	0.71	-0.09	0.66	0.23
DP8	-0.33	-0.28	0.05	0.02	0.25	0.41	0.57	0.71	1.00	-0.22	0.35	0.00
Total Sugars	0.90	0.96	0.87	0.75	0.43	0.31	0.13	-0.09	-0.22	1.00	0.48	0.92
Total fructans	0.12	0.53	0.68	0.89	0.95	0.95	0.84	0.66	0.35	0.48	1.00	0.79
Total NSC	0.68	0.91	0.92	0.93	0.73	0.64	0.47	0.23	0.00	0.92	0.79	1.00

4.5 Discussion

4.5.1 Fructans redistribution prior to sprouting may predict dormancy-break

Fructans of higher DP were initially present across all bulb sections at the pre-storage stage could only be detected in the baseplate prior to sprouting. Fructans are polymers of fructose and the major reserve carbohydrates of onion. The concentration of fructans was reported to be highest at harvest, but decreased continuously to a minimum at dormancy-break and sprouting during storage (Suzuki and Cutcliffe, 1989; Jaime *et al*., 2001). The present study showed that whilst this is true for the fructans content in the top and bottom sections, the opposite was true for the fructan content in the baseplate region, where the fructans content increased continuously until sprout emergence after which it declined (see Figure 4-1). However, previous studies on onion fructans sampled either the whole bulb (Suzuki and Cutcliffe, 1989), equatorial sections (Darbyshire and Henry, 1978) or the inner scales (Jaime *et al.*, 2001) only. This study represents the first investigation of fructans content in baseplate of onion bulbs. The reasons behind this accumulation of fructans in the baseplate prior to sprouting is unclear, although they quickly declined after sprout emergence. Notably, neither ethylene nor 1-MCP affected this decline. Notwithstanding this, specific accumulation of fructans in the baseplate prior to sprouting may have been to ensure adequate reserve energy was available for the successful initiation of sprouting whilst also offering osmo-protection to the meristematic tissues.

Fructans vary in their degree of polymerisation (DP) and are known to possess some osmo-regulation activities (Asega and Machado De Carvalho, 2004; Garcia *et al.*, 2011). Drought stress increases the accumulation of sugars content, which could cause damage to the plant cells. To avoid this consequence, the excess sugars are used in the biosynthesis of fructans. Therefore, the higher accumulation of kestose (a DP3 fructan) content in the bottom section of DI and in the baseplates of FI bulbs between pre-harvest and mid-curing shown in this study (see Figure 4-2) was thought to be in response to water-stress. More so, these differences occurred in the sections closer to the roots where water availability differed between DI and FI plants. Interestingly, the differences in kestose content had disappeared at the end of curing, which suggested that this accumulation of kestose was

not related to dormancy. In addition, the higher DP fructans content did not vary according to the imposed irrigation regime. Since, kestose is the first and last product of fructan biosynthesis from sugars and fructan catabolism to sugars, respectively, their biosynthesis and catabolism may be central to osmoregulation in onion plants under water-stress. This is supported by the equal and strong positive correlations of 0.83 shown by kestose to sucrose and nystose (see Table 4-2). Nevertheless, the differences in kestose content between pre-harvest treatments were no longer significant in cured bulbs prior to storage. Overall, correlations between fructans diminished with increasing differences in DP as shown in Table 4-2. Fructans accumulation had previously been reported in transgenic tobacco leaves and roots (Pilon-Smits *et al*., 1995; Li *et al*., 2007), rice leaves (Kawakami *et al*., 2008) and sugar beet roots and shoots (Pilon-Smits *et al.*, 1999) when grown under drought-stress. While these allude to a role for kestose content prior to end of curing, it contrasts with data on total fructan content. This then suggests that fructans may be recycled in relation to the plants' responses to certain stimuli (in this case drought stress) without affecting total fructan content. Therefore, the accumulation of fructans in response to drought-stress may be typical of fructan type, plant tissues and species.

A positive relationship between onion bulb higher DP fructans content and delayed sprouting has been previously reported, such that long storing onion bulbs are known to typically have higher accumulation of higher DP fructans while poorer storing onion bulbs tend to accumulate low DP fructans (Jaime *et al.,* 2001). While this suggests that the accumulation of higher DP fructans may extend dormancy, the data presented in this study is at odds with this understanding. 'Red Baron' had a higher fructans content, including those of higher DP, when compared to 'Sherpa'. However, 'Red Baron' sprouted two weeks earlier. This suggests that the accumulation of higher DP fructans in the whole bulb alone is not sufficient to predict dormancy-break or length of storage of onion bulbs.

Fructans of DP3-7 were present across all sections of the onion bulb before harvest, while fructans of DP8 also present in the top and bottom wedges, was conspicuously absent from the baseplate. Previous authors Suzuki and Cutcliffe, (1989), Jaime *et al*. (2001), Benkeblia *et al*. (2006), and Chope *et al*. (2012) reported that the concentration of fructans declined to a minimum, which coincided with dormancy-break and sprout emergence.

Understandably, dormancy-break and sprout emergence had not been clearly differentiated in previous onion studies, thus, they are used interchangeably. Dormancy-break is the release of meristematic tissues for regrowth, at which point there might not be any physical signs of sprouting. Sprout emergence is when there is a physical appearance of a sprout. Furthermore, considering the bulb sections sampled, it becomes apparent why previous studies reported a decline in fructans content at dormancy-break and sprout emergence. The data in this study has shown for the first time that: (1) fructans of DP7 and 8 became conspicuously absent from the top and bottom wedges two and six weeks prior to sprout emergence (for bulbs stored in air and ethylene, respectively); and (2) the decline of fructans from the top and bottom wedges coincided with the accumulation of higher DP in the baseplate. This phenomenon suggests a top-to-bottom remobilisation of higher DP fructans. Notably, even though ethylene and 1-MCP treatments delayed and suppressed sprout growth, neither treatments affected the decline of these fructans. This suggests that ethylene-related sprout suppression, while effective in the regulation of eco-dormancy, may not be involved in mediating endo-dormancy. Nevertheless, it was unclear if this redistribution of fructans of DP7 and 8 resulted from or was caused by dormancy-break.

4.5.2 Total sugar content declined across all sections at dormancy-break irrespective of pre- or postharvest treatments

The total sugar content data showed an increase in the first two weeks of storage across all sections. Thereafter, the total sugar content declined prior to sprout emergence at which point there was an increase in respiration rate. This suggested that the decline in sugars was in response to the energy demand for sprouting to occur. Individual sugar (*viz.* fructose, glucose and sucrose) contents varied during storage and more so at sprout emergence. Pre-harvest fructose content increased continuously during storage, irrespective of a steep decline in RR from pre-harvest to end of curing; and this coincided with the decline in fructans as reported here and previously (Suzuki and Cutcliffe, 1989; Jaime *et al*., 2001; Benkeblia *et al*., 2006). This corresponded to the continuous decline in the total fructans content, which is consistent with the continuous hydrolysis of fructans to release fructose molecules. Furthermore, there was a spike in the concentration of fructose and sucrose across all sections for bulbs stored in ethylene, which was clearer

for 1-MCP-treated bulbs stored in ethylene. The reason behind this transient increase is unclear, since there was also a spike in the RR for these bulbs. However, this finding is also supported by those of Chope *et al*. (2006a) who reported an increased accumulation of sugars in 1-MCP treated onion bulbs. In contrast to fructose, glucose content declined by 50% after four weeks of storage irrespective of pre- or postharvest treatments. This could be because there is no direct link between glucose and fructan hydrolysis, until the catalyses of the sucrose moiety of the fructans, or that the glucose released are used in the generation of energy through the glycolytic pathway and Kreb's cycle. Following this decline, sprouts were recorded two and six weeks later for bulbs stored in air and ethylene, respectively and coincided with the disappearance of DP7 and 8 fructans from the top and bottom wedges and concomitant accumulation in the baseplate. This observation suggests that glucose may be the preferred source of energy at dormancy-break, where glucose is used for glycolysis to generate pyruvate fed into the Kreb's cycle to produce the required energy. Interestingly, neither pre- nor postharvest treatments influenced the decline of glucose prior to sprouting, suggesting that neither ethylene nor 1-MCP is involved in endodormancy break but instead perhaps in eco-dormancy (sprout suppression).

Three classes of dormancy exist *viz*. endo-dormancy, para-dormancy and eco-dormancy; where endo-dormancy is time-dependent, irrespective of the environmental condition, para-dormancy is dependent on the transfer of biochemical compounds, while eco-dormancy is dependent on environmental conditions (Campbell, 2006). Endo-dormancy is thought to occur at around bulb initiation, when the growth of all meristematic tissues is arrested (Terry *et al*., 2015). Notably, significant changes in sugar concentration occurred only after sprout emergence.

4.5.3 The upregulations of *1-SST*, *6G-FFT* and *SPS* in sprouted bulbs neither increased sucrose nor fructans contents

Sugars are substrates for fructans biosynthesis where the first step in the fructans biosynthesis pathway involves the transfer of the fructosyl group from one sucrose to another sucrose creating kestose, a DP3 fructan. This first step is catalysed by sucrose:sucrose 1-fructosyltranferase (*1-SST*); while the elongation of the fructan chain (increase in degree of polymerisation) is catalysed by fructan:fructan 6G-fructosyltranferase (*6G-FFT*) (Vijn *et al*., 1998; Lüscher *et al*., 2000). Catabolism of

fructans into its individual sugar components is by hydrolysis, which releases the fructose moiety, which is catalysed by fructan 1-exohydrolase (*1-FEH*) (W. Van Den Ende *et al.*, 2003). It has previously been reported that the fructans content in stored onion bulbs declined continuously, and was lowest at sprouting (Suzuki and Cutcliffe, 1989; Jaime *et al*., 2001; Chope *et al*., 2012). This decline will be consistent with the catabolic activities of *1-FEH*. Zhang *et al*. (2016) reported an increase in the expression of the genes involved in sucrose biosynthesis during the early stages of bulb development but declined thereafter with bulb progression to maturity. Herein, reported for the first time is the expression of the genes *SPS*, *1-SST* and *6G-FFT* in stored onion bulbs. The results showed there was a spike in the upregulation of *SPS* prior to sprouting, which corresponded with the continuous decline in total fructans content. This suggested that due to the release of fructose from the catabolism of fructans there was need to regulate fructose content through sucrose biosynthesis. It was not clear if this was in response to osmoregulation or in relation to sprouting; however, there was a slight decline in *SPS* at sprout emergence. Furthermore, while *SPS* and *6G-FFT* were upregulated across all sections prior to sprout emergence, *1-SST* was either downregulated or remained stable across all sections, more so in the baseplate. These suggested that the accumulation of higher DP fructans in the baseplate prior to sprout was mainly due to translocation. It has become common knowledge that fructans are synthesised, stored and catabolised in vacuole. However, the transport of fructans within the plant system is not fully understood. For instance, the discovery of fructans and *FEH* in the apoplast of cold-stressed plants questions fructans localisation to the vacuole, which led to the proposition of a vesicle-mediated fructans transport system (Valluru *et al.*, 2008). Having said that, the upregulations of all three genes during storage, especially at the end of storage, had no effect on sucrose or total fructans contents. It is therefore hypothesised that the genes may have been translated into the corresponding proteins (the synthase and fructosyltransferases enzymes), yet no actual sucrose or fructans biosynthesis occurred due to posttranslational modifications. Posttranslational modifications such as phosphorylation, methylation, glycosylation proteolysis, and others (listed and detailed here: https://www.uniprot.org/docs/ptmlist) can inhibit protein functions (Deribe *et al*., 2010; The UniProt Consortium, 2010). Alternatively, the energy demands from sucrose and fructans catabolism may have outweighed their corresponding biosynthesis. Understandably, the resources for sucrose

and fructans biosynthesis may be scarce due to the energy requirements as the onion bulbs progressed to sprout growth. This is supported by Pollock (1984) who reported that fructans accumulation in onion bulbs occurs when the biosynthesis of sugars outweighs the energy demands. These taken together highlights the possibility that *FEH* expression may have outweighed those of *1-SST* and *6G-FFT*. Nevertheless, this will need confirmation in the future.

4.6 Conclusions

Deficit irrigation had no effect on sprout emergence, or the accumulation or distribution of total fructans content within the onion bulbs under cold storage. Therefore, the accumulation of fructans and the degrees of polymerisation thereof in onion bulbs may be genetically driven rather than being dependent on pre-harvest irrigation regimes. The upregulations of *SPS, 1-SST* and *6G-FFT* genes when sprout length was longest did not inhibit the declines in sucrose and total fructans contents. An indication that posttranslational modifications may have inhibited the actual synthesis, or that the energy demand from catabolism of sucrose and fructans outweighed the quantities synthesised. Ethylene as an independent factor delayed sprout emergence and when combined with 1-MCP produced the shortest sprouts as reported by (Cools *et al.*, 2011). However, neither of them significantly influenced the fructans content within the onion bulbs in relation to dormancy-break. This suggests that the mechanism by which ethylene and 1-MCP reduce sprouting may not be through fructans remobilisation. Prior to sprout emergence and regardless of pre- or postharvest treatments, fructans of DP7 and 8 were redistributed from the top and bottom sections of the bulb to the baseplate - in a para-dormancy-like remobilisation. It is unclear whether this redistribution resulted from or was caused by dormancy-break – a question not clearly answered by the expressions analyses herein of the genes involved in sucrose and fructans biosynthesis. Nonetheless, these redistribution of fructans occurred prior to sprout emergence; irrespective of ethylene and 1-MCP treatments. As such, although ethylene and 1-MCP treatments influenced eco-dormancy through delaying sprouting, neither seemed to affect endo-dormancy. Furthermore, neither the interactions between pre-harvest irrigation, postharvest ethylene nor 1-MCP treatments significantly influenced the accumulation and redistribution of fructans within the onion bulb in relation to dormancy-break and sprouting. Given these findings, the

redistribution of fructans prior to sprouting could serve as a potential marker to predict the transition from endo-dormancy.

4.7 Acknowledgement

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4.8 References

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5 Chapter Five: General discussion and recommendations for future research

5.1 Background

The effect of climate change on freshwater availability for crop production is changing the way onion and other crops are cultivated. Consequently, there is a greater dependence on irrigation (blue water) for food crop production; such that on a global scale, the average irrigation water applied per hectare stood at 7 700 m^3 (FAO AQUASTAT, 2016). Thus, 75% of the total water extraction by humans are used for agricultural crop production. Extraction at this magnitude is unsustainable in the future. It becomes apparent that the implementation of certain water management strategies is needed. In response, various water saving strategies have been trialled and deficit irrigation (DI), the application of blue water below the crop evapotranspiration becomes a preferred water saving strategy. Although DI has become the most common water saving strategy in crop production, previous DI studies on onions had only focused on its impact on yield. Onion DI treatments have often been associated with some levels of yield deficit (Kadayifci *et al*., 2005; Ayas and Demirtaş, 2009) depending on the severity of treatment. The universal adoption of DI has been slow probably due to breeders aim to increase yield and profit; and perhaps, because in temperate regions freshwater availability is not presently a hindrance to onion production.

Onion bulbs are stored for up to nine months depending on cultivar, and thus, the storage qualities of onion bulbs are equally as important as yield. Premature sprouting in stored onion bulb is a major contributor to the UK bulb losses postharvest (Terry *et al.*, 2011). The current industrial sprout suppressant is maleic hydrazide (MH), however, continued use of MH in the future is uncertain (see Chapter Two for details). Recent studies have revealed ethylene and its antagonist 1-methylcyclopropene (1-MCP) as potential alternatives to MH, but their interactions with DI has not been reported. These taken together explains why the study reported in this thesis investigated how sprouting in cold stored onion bulbs is influenced by pre-harvest DI as an independent factor and in combination with postharvest ethylene and 1-MCP treatments.

5.2 Deficit irrigation had no influence on sprout emergence but postharvest treatments with ethylene and 1-methylcyclopropene delayed and suppressed sprout growth

Previous studies on the effect of DI on onion bulb sprouting during storage are confounding. For example, Sorensen and Grevsen (2001) and Biswas *et al*. (2010) reported that DI delayed and reduced sprouting, while Vickers *et al*. (2015) reported that DI enhanced sprouting. In contrast to these, the present study showed that DI as an independent factor had no deleterious or beneficial impact on sprouting (Chapter Two; Ohanenye *et al*., (2019)). However, certain factors may have influenced these discrepancies in findings. Sorensen and Grevsen (2001) applied DI just before harvest and used shelf-life temperatures of 15 and 25 °C to initiate sprout growth; Biswas *et al*. (2010) stored onion bulbs under room temperature, while Vickers *et al*. (2010) withheld irrigation for up to 36 days as part of the deficit irrigation treatment. Collectively, these studies suggested that the timing and severity of DI treatment and the storage conditions may be key factors in the manipulation of sprouting in stored onion bulbs.

Ethylene is the simplest known plant growth hormone and the first gaseous signalling molecule found in any organism (Abeles *et al*., 1992; Chang, 2016). Despite its structural simplicity and gaseous state, ethylene is involved in the regulation of complex plant growth and developmental activities such as, seed germination, root growth and development, shoot growth, flowering, abscission of leaves and fruits, sex determination, senescence of flowers and leaves, and fruit ripening (Abeles *et al*., 1992; Kende, 1993; Wang *et al*., 2002; McManus, 2012). These activities of ethylene are regulated by the five known ethylene receptors LeETR1, LeETR2, NR, LeETR4, and LeETR5 found in tomato (Gallie, 2015). Similar homologues have been reported in Arabidopsis *viz*., ETR1, ERS1, ETR2, EIN4, and ERS2 (Hua *et al.*, 1995, 1998; Sakai *et al.*, 1998; Tieman *et al.*, 2000). Ethylene receptors are specialised proteins localised in the endoplasmic reticulum, which in the absence of ethylene act as negative regulators of ethylene signalling (Gallie, 2015). However, ethylene is autocatalytic (i.e. the binding of the first ethylene catalyses the binding of the ones thereafter) and is perceived only when it binds to its receptors. Despite the interactions of ethylene with its receptors, it is not known whether ethylene binding to its receptors stimulates or inhibits some biochemical activities in the receptor (Chang,

2016). Of these reported ethylene activities, the most explored is its regulation of ripening in climacteric fruits. Climacteric fruits show increases in respiration rate, significant production of endogenous ethylene during ripening and the exogenous application of ethylene hastens the ripening process. In contrast, non-climacteric fruits during ripening produce basal amounts of endogenous ethylene and exogenous ethylene application does not hasten ripening. Based on ethylene production, onion is regarded as a non-climacteric (Downes *et al.*, 2010). Climacteric fruit ripening hastened by ethylene is slowed down by the application of 1-MCP. Thus, 1-MCP is perceived as an ethylene antagonist. Further antagonism of ethylene and 1-MCP have been reported in potato. Potatoes treated with ethylene produce brown colouration when fried (due to sugar accumulation), but treatments with 1-MCP alone or combination with ethylene inhibited this frying-related discolouration (Prange *et al*., 2005; Daniels-Lake *et al*., 2008). Moreover, although 1-MCP antagonised ethylene darkening activity in fried potatoes, it did not interfere with sprout suppression activities of ethylene (Daniels-Lake *et al.*, 2008). This is an indication that 1-MCP antagonism of ethylene activities may be selective, and further highlights the possibility that 1-MCP and ethylene may have differential affinity for each of the five ethylene receptors, where each receptor may elicit distinct and varying responses when expressed individually. Gallie (2015) has previously highlighted the need to determine if major differences existed in ethylene receptors' signalling activities. These taken together would suggest that while the five known receptors may collectively antagonise ethylene activity, they may exhibit varying individual responses.

Recently, studies into ethylene and 1-MCP activities on non-climacteric fruits and other fresh produce is gaining traction. In onion, ethylene supplementation and treatment of bulbs with 1-MCP have previously been reported to delay and suppress sprout growth during storage, whether applied independently or in combination (Chope *et al*., 2006a; Chope and Terry, 2008; Bufler, 2009; Downes *et al*., 2010). The revelation that onion bulbs (cv. SS1) treated with 1-MCP alone (at 1 µL L-1 for 24h post-curing) elicited an ethylene-like sprout suppression activity in stored onion bulb, a non-climacteric crop (Chope *et al*., 2006b), further complicates the understanding of the 1-MCP/ethylene antagonism. Sprout growth was also suppressed in onion bulbs treated with 1-MCP at 1 µL L^{-1} for 24 h, pre- or post-curing, and stored under continuous ethylene at 10 µL L^{-1} (Cools *et al.*, 2011). Cools *et al*. (2011) further reported that sprout growth was

suppressed by 2-fold in onion bulbs treated with 1-MCP and ethylene at precuring stage, when compared to bulbs treated with 1-MCP alone at the stage; suggesting an additive sprout suppression activity. They also investigated the molecular responses in 1-MCP/ethylene treated samples, which revealed that probes annotated as ethylene receptors were down-regulated in onion bulbs under combined 1-MCP and ethhylene treatments. While these responses are expected in ethylene-treated samples, that this occurred in bulbs under combined treatment with 1-MCP/ethylene suggested these combined treatments amplified the ethylene activity. Cools *et al*. (2011) further showed that a cluster of probes representing transcripts were up-regulated by 1-MCP alone, were down-regulated by ethylene, either alone or in combination with 1-MCP. Thus, the enhanced sprout suppression reported for 1-MCP-treated onion bulbs stored under ethylene may be due to the enhancement of ethylene sprout suppression by 1-MCP. Having said this, the combined effects of pre-harvest DI and postharvest ethylene and 1-MCP treatments have not been reported previously. As described in Chapter Two, continuous ethylene supplementation at 10 µL L^{-1} delayed sprout emergence for four weeks, irrespective of the pre-harvest treatments for the onion cultivars 'Red Baron' and 'Sherpa'. While treatments with 1-MCP at 1 µL L^{-1} for 24h prior to storage neither delayed nor suppressed sprout growth for both DI and fully irrigated (FI) bulbs. This contrasts with the findings of Chope *et al*. (2006). However, 1-MCP-treated onion bulbs stored under continuous ethylene produced the shortest sprouts, in accordance with previous reports (Cools *et al.*, 2011). The present study, suggests that in the presence of ethylene, the ethylene receptors 1-MCP exhibits stronger affinities for may not elicit sprout regulation activities, while ethylene binds to the sprout regulating recptors.

Sprout delay and sprout suppression are often used interchangeably by most authors when describing the effects of ethylene on stored onion bulbs. As such, previous authors have reported that ethylene suppressed sprout growth (Chope *et al*., 2006a; Chope and Terry, 2008; Bufler, 2009; Downes *et al*., 2010). For clarity, sprout delay is an extension of time to sprouting while sprout suppression is a reduction in the rate of sprout elongation (vigour); whether ethylene does both is not known. To test this, the analysis of sprout vigour was conducted in this study and it revealed that ethylene delayed sprout emergence but had no significant effect on sprout elongation (see Chapter Two). On the other hand, the combined treatments of bulbs with 1-MCP and ethylene delayed and reduced sprout

elongation, irrespective of pre-harvest irrigation treatments. Taken together, these results suggested that ethylene may have delayed the meristematic tissues cell division associated with sprouting but was no more effective after mitosis had started, while 1-MCP/ethylene combined treatments interference continued after mitosis. Despite this, the overall mechanism underlying the 1-MCP/ethylene synergistic sprout suppression activities remain poorly understood. This study has shown that 1-MCP and ethylene could be a robust alternative to MH, even in areas where water availability may be a constraint to onion production.

5.3 Variations in the metabolic rates prior to and during storage may have reflected different states of onion bulb dormancy

Harvested onion bulbs are assumed to be in a dormancy state. Dormancy in onion bulbs was defined as when the growth of all meristematic tissues is arrested (Terry *et al*., 2015). This is often associated with a reduced metabolic and respiration rate (RR). Endo-dormancy is thought to occur when onion bulbs are still in the field, while eco-dormancy is dependent on the postharvest regime. Indeed, Chope (2006) suggested that endo-dormancy is the true dormancy. During onion bulb storage, the meristematic tissues are released for regrowth otherwise known as dormancy-break. This is usually associated with an increase in the respiration rate (RR). Nonetheless, the response of RR from onion bulb progression to dormancy is not known. The results described in Chapter Two showed that the RR was highest in onion bulbs at full maturity prior to harvest (pre-harvest) but declined continuously from harvest to end of curing. Notably, irrigation was stopped immediately after the pre-harvest samples were collected. Since this continuous decline in RR coincided with the termination of irrigation – an environmental cue, it may have indicated the bulbs' progression into eco-dormancy. At that stage, the onion plant has stopped producing fresh leaves, an indication that cell division may have ceased, and thus, the bulbs were already in endo-dormancy state. Moreover, the higher RR at pre-harvest compared to end curing showed an active metabolic rate, which may be due to continued cell expansion despite the arrest of cell division. Thus, the termination of irrigation may have stopped cell expansion and enhanced the onion bulb transition into eco-dormancy.

5.4 Abscisic acid (ABA) accumulated under water-stress may not play a role in the regulation of onion bulb dormancy and sprouting

Abscisic acid (ABA) is a phytohormone known to be involved in various plant growth and development stages; including but not restricted to seed germination (Nambara *et al*., 2010; Kang *et al*., 2015), vegetative growth (Finkelstein and Lynch, 2000; Finkelstein *et al*., 2002; Garciarrubio *et al*., 2015), senescence (Suttle *et al*., 2012) and water-stress tolerance in plants grown under water deficit (Kuromori *et al*., 2018). ABA has been demonstrated to regulate sprouting in *Allium wakegi* – a relative of onion, where ABA treatment (at 500 μM) delayed time to sprouting while treatment with fluridone (an ABA inhibitor) enhanced sprouting (Yamazaki *et al.*, 1999). In onion, a higher endogenous ABA content was found in long-storing bulbs when compared to poor-storing onion bulbs (Chope *et al*., 2006b; Chope and Terry, 2008). Thus, Chope *et al*. (2007) postulated that increasing the endogenous ABA content might delay sprouting and thus improve the storage potential of onion bulbs. To test this hypothesis, they dipped onion bulbs in ABA or ABA analogue (8'-methylene ABA methyl ester; PBI-365) solution or applied them as a foliar spray to onion plants to encourage absorption and translocation to the bulbs. However, neither of these treatments caused an increase in the endogenous ABA content or delayed sprouting. Thus, they concluded that exogenous application of ABA is of little benefit to increasing onion bulb endogenous ABA content.

Moreover, the major role of ABA is in the regulation of plant response to water-stress such as deficit irrigation; and the accumulation of ABA has previously been reported in plants grown under DI. The experiment described in Chapter Three herein showed that there was a significant accumulation of ABA in the baseplate (which contains the meristematic tissues) of DI when compared to FI bulbs. This, according to Chope *et al*. (2007)'s postulation, would have been expected to cause a delay in sprouting for the DI bulbs; more so, since the differential accumulation occurred in the baseplate. However, there were no significant differences in the sprout emergences between DI and FI bulbs during storage. This contrasts with the Chope *et al*. (2007) postulation, although it is unclear whether these differences found in the baseplate would have remained significant had the analyses been conducted on the whole bulb as with Chope *et al*. (2007). This, thus, highlights the importance of the tissues selected for sampling on analyses.

Notwithstanding, the differential accumulation of ABA between the DI and FI bulbs was no longer significant after four weeks of storage. This is supported by Chope *et al*. (2007) who reported that the decline in the ABA content followed a similar pattern; irrespective of the pre-storage ABA content. These taken together leads to the hypothesis that ABA accumulated in response to drought-stress may not be involved in the regulation of onion bulb dormancy.

Ethylene supplementation is known to delay sprout growth in stored onion bulbs as reported herein and elsewhere (Bufler, 2009; Downes *et al*., 2010). However, it is not known if this is mediated through ABA metabolism, considering that ethylene was previously reported to counteract ABA activities in seed germination (Corbineau *et al.*, 2014). To test this, the analyses of the interaction between ABA, ethylene and 1-MCP was conducted, which revealed that ethylene and 1-MCP reduced the rate of ABA decline in stored onion bulbs (see Chapter Three). This suggested that the widely reported ethylene-related sprout suppression in stored onion bulbs may be through ethylene interaction with ABA catabolic pathway. However, it is unclear whether the reduction in ABA decline in bulbs stored under ethylene resulted from ethylene antagonism of ABA catabolism, which will be supported by Corbineau *et al*. (2014) or through the other reported ABA/ethylene complex interactions (Brady and McCourt, 2003; Matilla and Matilla-Vázquez, 2008; Arc *et al.*, 2013). Notwithstanding, a similar reduction in ABA decline was found for bulbs treated with 1-MCP, but sprout emergence was not delayed, which questions the role of ABA in regulation of onion bulb dormancy. That said, 1-MCP may have inhibited bulb endogenous ethylene production which in combination with exogenous ethylene and ABA may work in concert to elicit the sprout delay reported for bulbs stored under ethylene. This would then suggest that despite onion bulbs being low ethylene producers, this low ethylene concentration may be necessary for the widely reported ethylene-related sprout suppression. Given, ethylene production in onion bulb is at a basal level below the detection limits of gas chromatography. However, high sensitivity laser photo acoustic spectroscopy (Cristescu *et al.*, 2008) was used by (Morris *et al.*, 2018) to detect low ethylene production in transgenic potatoes. Whether this could be used to explain the differential sprout responses to ethylene and 1-MCP decline in ABA will depend on future research.

5.5 The metabolites of ABA responded to water-stress but not to sprouting

The catabolism of ABA is through glycosylation to abscisic acid glucose ester (ABA-GE) or hydroxylation at various Carbon positions to form 7′-OH-ABA, 8′-OH-ABA or 9′-OH-ABA. While glycosylation of ABA is often thought to be a deactivation step, hydroxylation of ABA (mainly through 8′-OH-ABA) is degraded to phaseic acid (PA) and further to dihydroxyphaseic acid (DPA). The results on the metabolites of ABA described in Chapter Three did not show any clear interactive response to ethylene or 1-MCP treatments, and no distinct response in relation to sprout emergence. As such, ABA metabolites may not play major roles in the regulation of onion dormancy or sprouting. Regardless, some metabolites of ABA, especially ABA-GE and DPA responded to the pre-harvest irrigation treatments. There was a significant spike in ABA-GE content across all section of DI when compared to FI bulbs during mid-curing. Although this spike had disappeared at the end of curing, it indicated that the spike in ABA-GE may have regulated ABA accumulation through a temporary deactivation to ABA-GE pending catabolism. Notably, curing is characterised by a significant amount of water-loss, which may have driven the accumulation of ABA in the DI bulbs where deactivation through glycosylation to ABA-GE became necessary. DPA content was at least 6-fold higher in the top and bottom sections of onion bulbs when compared to ABA content, which is an indication that DPA may truly be the major metabolite of ABA as previously suggested (Seiler *et al.*, 2011). Furthermore, DPA content was significantly higher in the DI when compared to FI onion bulbs, prior to the termination of irrigation. However, at harvest (two weeks after irrigation was stopped), the DPA content had become significantly higher in the top and bottom sections of the FI compared to DI bulbs. A suggestion that the unexpected exposure of the FI plants to water-stress may have caused an excessive accumulation of ABA, which was quickly catabolised to DPA. Notwithstanding, due to the magnitude of the differences between DPA and ABA contents it is not clear if the accumulation/retention of DPA was solely from ABA catabolism or in response to any biological activities. For instance, the switch in DPA contents between DI and FI plants depending on water-stress conditions and the 6-fold higher DPA content compared to ABA content reported herein suggested that a higher DPA content compared to ABA maybe required to elicit some water-stress tolerance. Notably, metabolites of ABA were

previously thought to be biologically inactive until Weng *et al*. (2016) *Arabidopsis* transcriptomics analyses showed that PA-responsive genes overlapped with the ABA-responsive genes. This suggested that PA possessed some ABA-like activities. However, Weng *et al*. (2016) reported that the expression of the ABA-responsive genes were 10-fold higher when compared to the PA-responsive genes. In agreement, an earlier work by (Hill *et al*., 1992) reported that ABA was 10-fold more effective than PA at inhibiting the germination of immature embryos of barley. Therefore, the possibility that DPA may possess some biological activities in relation to water-stress responses exists.

This study focused on ABA and its metabolites. However, other phytohormones such as gibberellin, cytokinins and auxin are known to play some key roles in the regulation of dormancy. Gibberellin is a known inhibitor of ABA activities in seed germination; such that, while ABA inhibits seed germination, gibberellin promotes seed germination (Khan, 1971). ABA and courmarin are known to work in concert as inhibitors of seed germination while gibberellin and cytokins (e.g. zeatin – a naturally occurring cytokinin) are known antagonise the ABA- and courmarin-induced dormancy in lettuce seeds (Khan, 1967). Later studies showed that zeatin antagonism of ABA activities stretched beyond the regulation of dormancy to drought responses (Hansen and Dörffling, 2003). Nevertheless, cytokinins on their own were reported to be ineffective in breaking seed dormancy in certain seeds while gibberellin alone did. Gibberellin was thus thought to be the main germination promoter (Khan, 1971). In onions, it has been reported that cytokinins and gibberellin cause cell division and elongation, respectively. Thus, cytokinin causes dormancy-break while gibberellin controls sprout length (Sharma *et al*., 2016). In relation to the results herein, the reduction in ABA decline as found in 1-MCP-treated onion bulbs stored in air where sprout was not delayed compared to untreated onion bulbs stored under ethylene suggested that the reduction in ABA decline may not be enough to delay sprouting. This is supported by Khan (1971), who suggested that dormancy may not be enhanced by the accumulation of germination inhibitors, instead may be due to the reduction in growth promoters. Interestingly, ethylene has previously been reported to modulate gibberellin activities (Pierik *et al.*, 2004). These taken together would suggest that a reduction in ABA catabolism (germination inhibitor) alone may not have been enough to elicit dormancy in stored onion bulbs, while ethylene-related sprout delay may be through the modulation of gibberellin activities (germination promoter).

This highlights the need to expand the studies of the hormonal regulation of onion dormancy beyond ABA as recently reported for potato (Morris *et al*., 2018).

5.6 Fructans accumulation and the regulation of onion bulb dormancy and sprouting

Fructans are polymers of fructose, the primary reserve carbohydrate and together with sucrose, glucose and fructose are the only non-structural carbohydrates contained in onions. Fructans are built up during photosynthesis (Ernst *et al.*, 1998) and vary in the degree of polymerisation (DP) (Vijn and Smeekens, 1999; Van Den Ende *et al.*, 2003). Akin to ABA, the content of fructans within the onion bulbs was reported to decline continuously during storage and sprouting occurred at a minimum fructans content (Suzuki and Cutcliffe, 1989; Benkeblia and Selselet-Attou, 1999; Jaime *et al*., 2001;Chope *et al*., 2012). These studies would suggest that onion bulbs with a higher fructans content may store longer before sprouting compared to those with lower fructans content. In contrast, 'Red Baron' had a higher amount of total fructans (166.7 g kg^{-1}) when compared to 'Sherpa' (144.9 g kg^{-1}). However, 'Red Baron' sprouted two weeks before 'Sherpa', an indication that total fructans content is not a good tool to predict the storage quality of onion bulb. A positive correlation was reported between fructans DP and time to sprout, such that long-storing onion bulbs contained more fructans of longer DP when compared to poor-storing onion bulbs (Jaime *et al.*, 2001). Although the total fructans content was higher in 'Red Baron', 'Sherpa' had a higher content of fructans with DP6-8 and the amount of DP7 and 8 were twice as higher in 'Sherpa' compared to 'Red Baron'. These could therefore explain why 'Sherpa' sprouted two weeks later than 'Red Baron' and in agreement with Jaime *et al*. (2001).

In addition to their sprout-related implication, fructans possess some osmoregulation properties (Vijn and Smeekens, 1999b; Valluru and Van Den Ende, 2008; Livingston *et al*., 2009) and their content was reported to increase in plants grown under water-stress (Pilon-Smits *et al*., 1995, 1999; De Roover *et al*., 2000; Garcia *et al*., 2011; Van den Ende and El-Esawe, 2014). The analyses of influence of DI on fructans accumulation and (longitudinal) distribution within the onion bulb showed that DI had no significant effect on the accumulation of total fructans (Chapter Four; Ohanenye *et al*. (2019)). This suggested that fructans accumulation in response to water-stress may be tissue, organ or

specie dependent. The total fructans content declined steeply and continuously in the top and bottom sections of onion bulbs from pre-storage to end of storage. In concomitance, the total fructans content in the baseplate (harbouring the meristematic tissues) increased significantly and continued until sprout emergence. Thereafter, the baseplate total fructans content in accordance with the other sections declined steeply and continuously until end of trial for both years – irrespective of cultivar. Whilst the response of the total fructans content in the top and bottom sections was concordant with previous reports (Suzuki and Cutcliffe, 1989; Benkeblia and Selselet-Attou, 1999; Jaime *et al*., 2001; Chope *et al*., 2012), the initial increase in the meristematic tissue section was not. The result herein highlighted the importance of the tissue selection for sampling. Notably, the fructans content in the baseplate of onion bulbs have been reported herein (Chapter Four) for the first time; previous studies collected samples from the inner scales (Jaime *et al*., 2001), equatorial sections (Darbyshire and Henry, 1978) or the whole bulb (Suzuki and Cutcliffe, 1989). Moreover, the timing of this accumulation of fructans in the baseplate (prior to sprout emergence), may be of biological importance. It was previously reported that fructans make up 30% of the total carbohydrate content of onion bulb young sprouts. Therefore, this accumulation of fructans in the baseplate prior to sprouting may have been to guarantee that the energy reserve was enough for sprout initiation to proceed. Notwithstanding, this will require investigations in the future to further the understanding of the roles of fructans metabolism in relation to onion dormancy.

Furthermore, it is not known whether the biological functions attributed to fructans in relation to sprouting and osmoregulation is through localised catabolism or remobilisation within the onion bulb. The longitudinal analyses of individual fructans revealed that fructans with DP7 and 8 were present in the top and bottom section of onion bulbs at pre-storage; while only traces could be detected in the meristematic tissue section (see Chapter Four; Ohanenye *et al*. 2019). However, four weeks into storage, fructans of DP7 and 8 were no longer present in either the top or bottom sections but had accumulated in the baseplate. Following this, the bulbs sprouted two and four weeks later for bulbs stored in air and under ethylene, respectively. Thus, the disappearance of these higher DP fructans from the top and bottom section may have indicated bulbs' endo-dormancy exit. Interestingly, ethylene delayed sprout emergence but had no effect on this redistribution of fructans; which suggested that exogenous ethylene application regulates eco-dormancy

but not endo-dormancy. Although it was unclear whether this fructans redistribution resulted from or initiated dormancy-break; it suggested that this redistribution is a potential marker for dormancy-break and sprouting in stored onion bulbs. Further exploration of this could reduce the storage losses of onion bulb due to unpredictable sprouting.

Moreover, it is not known whether these DP 7 and 8 fructans were translocated from the top to the baseplate section or synthesised in the baseplate. To understand the molecular mechanism behind the redistribution of fructans with higher DP, the fold expression of the genes involved in sucrose and fructans biosynthesis were conducted using ΔΔCT method (Livak and Schmittgen, 2001) as described in Chapter Three. In fructan biosynthesis, the first step involves the transfer of the fructosyl group from one sucrose to another to form the kestose – a fructan with DP3. This is followed elongation of the fructans chain creating the variations in the DP of fructans and this involves the transfer of fructosyl group from one fructan to another. The synthesis and elongation of fructans in onions are catalysed by sucrose:sucrose 1-fructosyltransferase (*1-SST*) and fructan:fructan 6G-fructosyltransferase (*6G-FFT*) (Vijn *et al.*, 1997, 1998). These taken together showed that fructans biosynthesis rely so much sucrose availability; hence, sucrose phosphate synthase (*SPS*) - the key gene in sucrose biosynthesis (Huber and Huber, 1996) was also studied. There was a spike in *SPS* expressions prior to sprouting across all sections; such that the upregulations were approximately 120, 200 and 9-folds for the top, bottom and baseplate, respectively. Concomitantly, the expression of *6G-FFT* was also upregulated – though by a much lesser fold - across all sections. In contrast, *1-SST* expression was significantly downregulated in top section and baseplate while it remained unchanged in the bottom wedge. These differential expressions of *1-SST* and *6G-FFT* suggested that while the polymerisation of fructans occurred across all sections, the synthesis did not. Therefore, it can be hypothesised that the accumulation of fructans with higher DP in the baseplate prior to sprouting was mainly due to translocation. At the end of storage (20 weeks), there was a significant upregulation of *SPS*, *1-SST* and *6G-FFT*, however, there were no corresponding increases in sucrose or fructans contents. Which then suggested that the expression of these genes may not have progressed to actual synthesis due to posttranslational modifications. Alternatively, the rate of fructans

catabolism (catalysed by fructan exohydrolases - *FEH*) may have outweighed the rate of synthesis - an avenue for future research.

5.7 The potential impact of this work to the onion industry

First, this study has shown that postharvest qualities (e.g. weight-loss and sprouting) of onion bulbs were not negatively impact by deficit irrigation. Therefore, in the UK where concerns over water use during hot summers is on the increase and in other regions where water is a major constraint and onion cultivation is dependent on irrigation, deficit irrigation can be applied without compromising the storage qualities of onion bulbs. Nevertheless, this may be accompanied by varying levels yield deficit as reported herein.

In the UK, continuous ethylene supplementation (10 µL L^{-1}) is currently adopted by some practitioners as a sprout suppressant in onion bulbs meant for long storage. The results described herein showed that the ethylene delayed time to sprouting in onion bulbs irrespective of a reduction in the amount of irrigation water used during the growing season. Nevertheless, once sprouted, ethylene was not found to suppress sprout growth. Sprout delay and sprout suppression were achieved by treating onion bulbs with 1-MCP at 1 µL L^{-1} for 24 h after curing before storage under ethylene. Understandably, 1-MCP application is not currently a commercial practice in onion storage in the UK, however, they are used in the storage of apples. In view of this, there is a potential to use 1-MCP and ethylene combined treatments to extend the storage qualities of onion bulbs.

In the UK, premature sprouting is a major contributor to onion bulb losses during storage. This study found that certain fructans (with DPs 7 and 8) which were initially present in the onion flesh at harvest and during early stages of storage. However, prior to sprout emergence, these fructans had disappeared from the onion flesh and have accumulated in the baseplate - where only traces were found at the time of storage. The bulbs sprouted after this occurrence; therefore, there is a potential to use these fructans redistribution as a marker to predict dormancy-break and sprouting in stored onion, which could inform decisions on what onion bulbs from what field or storage batch would be supplied first to reduce storage bulb losses due to unpredictable sprouting. Nevertheless, due to the cost of the equipment and skill sets needed for fructans analyses, this may not benefit medium/small scale industries. That said, the possibility of contracting these analyses or undertaken as a collective investment exists.

5.8 Project Conclusions

1. The results in Chapter Two showed that DI had no negative impact on the sprout incidence of cold stored onion bulbs. Therefore, DI could be adopted in places where water availability is a major restriction to onion production; nevertheless, there is a potential yield deficit associated with the adoption of DI.
2. Ethylene supplementation delays sprout growth in cold stored onion bulbs and when combined with 1-MCP causes both sprout delay and sprout growth suppression; irrespective of pre-harvest irrigation treatments as shown by the results in Chapter Two.
3. Abscisic acid accumulated in the baseplate of DI onion bulbs at the end of curing but did not delay sprout growth as shown by the results in Chapter Three, which suggested that ABA accumulated during water-stress may not be involved in the regulation of dormancy in onion bulbs.
4. The mechanism behind ethylene-related sprout delay in stored onion bulbs may be caused by the combined effects of endogenous and exogenous ethylene interactions with the ABA catabolic pathway; since 1-MCP elicited a similar reduction in ABA catabolism did not sprout emergence.
5. Kestose may be central to osmoregulation in onion bulbs, since their contents were higher in DI bulbs yet the total fructans content was not affected. Thus, suggesting that though total fructans content may be cultivar dependent, fructans may be recycled within DPs in response to biological needs.
6. The redistribution of fructans with higher DP from the top and bottom sections of the onion bulb to the baseplate, is a potential marker to predict dormancy-break and sprouting in stored onion bulbs. This result can be explored by the onion industry to reduce onion bulb losses to unpredictable sprouting; nevertheless, this may not be suitable for small scale businesses due to the overall cost of fructans analyses.
7. This redistribution of fructans prior to sprouting may be through translocation as shown by the gene expression analyses; however, the upregulations of these genes involved in sucrose and fructans biosynthesis had no concomitant effect on the fructans content. These taken together indicated that the genes involved in

fructans biosynthesis underwent posttranslational regulation or highlighted the superiority of fructans catabolism to synthesis in stored onion bulbs.

8. Further understanding of the metabolic and molecular regulations of onion bulb dormancy will depend on more investigations in the future.

5.9 Recommendations for future works

Ethylene supplementation is now widely accepted to delay sprouting in stored onion bulbs and recently the combined treatment of 1-MCP and ethylene has been reported to enhance this effect. Furthermore, the report herein revealed that combined 1-MCP and ethylene treatments besides delaying sprout emergence also reduced sprout vigour. As such, the speculations that 1-MCP/ethylene combined treatments could be an alternative replacement for maleic hydrazide (MH) is on the rise. That said, future studies should compare the sprout suppression activities of MH to that of combined 1-MCP/ethylene treatments to evaluate their robustness as MH replacement.

Despite recent attempts at understanding the regulation of onion bulb dormancy, the mechanism behind entrance and exit of dormancy is not fully understood. While some progress has been made at the regulation of onion bulb exit of dormancy, there paucity in knowledge as to bulb's entrance into dormancy. The study herein identified a continuous decline in metabolic rate from pre-harvest to end of curing, which suggested bulbs entrance into eco-dormancy. It is not known if eco-dormancy would have been delayed had irrigation continued; as such, the identification of other factors involved the regulation of eco-dormancy would enhance the potential manipulating of onion dormancy.

Onion bulbs are low ethylene producers, thus, the focus shifted to exogenous ethylene application due to its known benefits of sprout delay in stored onion bulbs. 1-MCP is a known ethylene inhibitor, which when combined with ethylene has been shown to suppress sprout growth. The study here showed that both ethylene and 1-MCP as individual treatments reduced the rate of ABA decline, however, sprout delay was only found in bulbs stored under ethylene. This suggested that the reduction of ABA decline alone was not enough to delay sprout growth and that the contribution of endogenous

ethylene may be required for sprout delay to occur. Understandably, the level of ethylene produced in onion bulbs is below the limits of gas chromatography identification levels. However, high sensitivity laser photo acoustic spectroscopy has been used to identify trends in ethylene production in potatoes – another low ethylene producer. Whether this could be used to identify if the ethylene production patterns in onion bulbs will enhance the identification of regrowth and thus explain the differential sprout responses to ethylene and 1-MCP decline in ABA will depend on future research.

Moreover, given that the reduction in ABA decline for bulbs stored under ethylene led to sprout delay but the same trend in 1-MCP-treated bulbs did not questions the role of ABA in onion dormancy. Having said that, ethylene is known to be involved in a complex regulation network with ABA and other phytohormones such as gibberellins and cytokinins involved in the regulation of dormancy will need further exploration in onion studies. In addition, the metabolites of ABA have been shown to possess some biological activities, however, these have not been proven in onions. Therefore, a more comprehensive investigation into the roles of ABA, its metabolites and other phytohormones will be a task for the future investigators.

The occurrence of para-dormancy has not been shown in onions. The results herein showed for the first time that fructans with higher degree of polymerisation (DP) that were present in the top and sections of onion but absent in the baseplate had disappeared from the top and bottom sections and at same time accumulated in the baseplate. This result suggested a para-dormancy-like transfer of these higher DP fructans in relation to sprouting and this redistribution if explored hold the potential to reduce the storage losses of onion bulb due to unpredictable sprouting. Moreover, due to the differential accumulation of kestose (the first fructan produced in fructans biosynthesis with DP 3) in response to deficit irrigation, kestose was suggested to be central to osmoregulation in onion bulbs herein. These taken together highlights the need for more investigations in the future to further the understanding of the roles of fructans metabolism in relation to onion osmoregulation and dormancy.

Onion genetics studies is hampered by the huge size of the onion genome, which was estimated to be 107 times larger than the genome of *Arabidopsis* (King *et al.*, 1998). However, the use of other molecular biology techniques such as cloning, gene expression

analyses and transcriptomics have enhanced the understanding of the functions of various genes in onion. The genes sucrose:sucrose 1-fructosyltransferase (*1-SST*) and fructan:fructan 6G-fructosyltransferase (*6G-FFT*) were cloned and identified as the genes involved in fructans biosynthesis in onion (Vijn *et al.*, 1997, 1998). It was not clear if the fructans redistribution reported herein was due to translocation from the top and bottom sections to baseplate of the onion or if they were synthesised in the baseplate. The molecular analyses of *1-SST* and *6G-FFT* suggested that these higher DP fructans were translocated from top to the bottom. But these genes were found to be significantly upregulated in already sprouted bulbs at the end of storage with no corresponding increase in fructans content. These were thought to be due to posttranslational modification of these genes. Moreover, as demonstrated herein and elsewhere, fructans content decline continuously in stored onion bulbs. This decline is due to fructans hydrolysis catalysed by fructan exohydrolase (*FEH*); therefore, posttranslational modification may not be the only reason why the upregulations of *1-SST* and *6G-FFT* did not increase fructans content. Alternatively, the rate of fructans catabolism (catalysed by fructan exohydrolases - *FEH*) may have outweighed the rate of synthesis – this is an avenue for future research.

Finally, future studies should focus on understanding the mechanisms of the changes occurring prior to or around dormancy-break (the cause of sprouting) to improve the storage qualities of onion bulbs; than the current focus on tackling sprout growth (the consequence of dormancy-break).

Chapter Six: Literature cited

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Appendix A

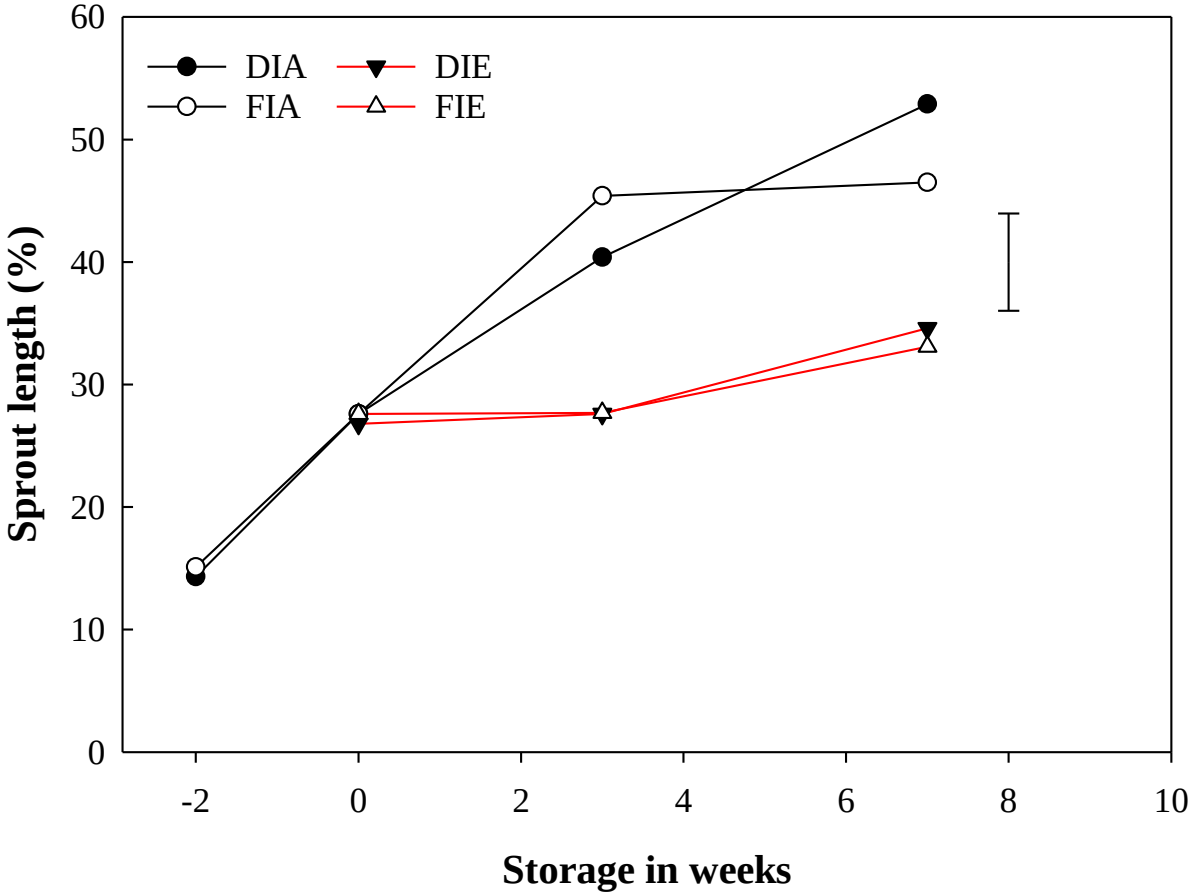


Figure A-1. Sprout length in stored onion bulbs. Sprout length was measured as sprout length in proportion to bulb height represented as a percentage for onion double haploid line (CUDH2107) bulbs grown in 2017 under full irrigation [FI] or deficit irrigation [DI]; where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for two weeks for both years. After curing, bulbs were stored at 1°C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIA and FIA are DI and FI bulbs in air, DIE and FIE are DI and FI bulbs stored under ethylene. LSD bar at 95% confidence shown for interactions between treatments and storage in weeks.

Appendix B

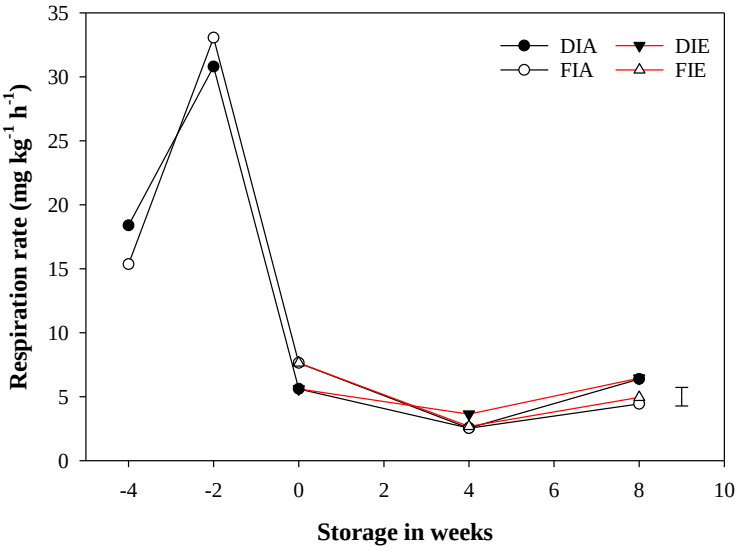


Figure B-1. Real-time respiration rates as CO_2 production (mg kg^{-1} h^{-1}) of stored onion bulbs. Onion double haploid line (CUDH2107) bulbs grown in 2017 under full irrigation [FI] or deficit irrigation [DI]; where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for two weeks for both years. After curing, bulbs were stored at 1°C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIA and FIA are DI and FI bulbs in air, DIE and FIE are DI and FI bulbs stored under ethylene. LSD bar at 95% confidence shown for interactions between treatments and storage in weeks.

Appendix C

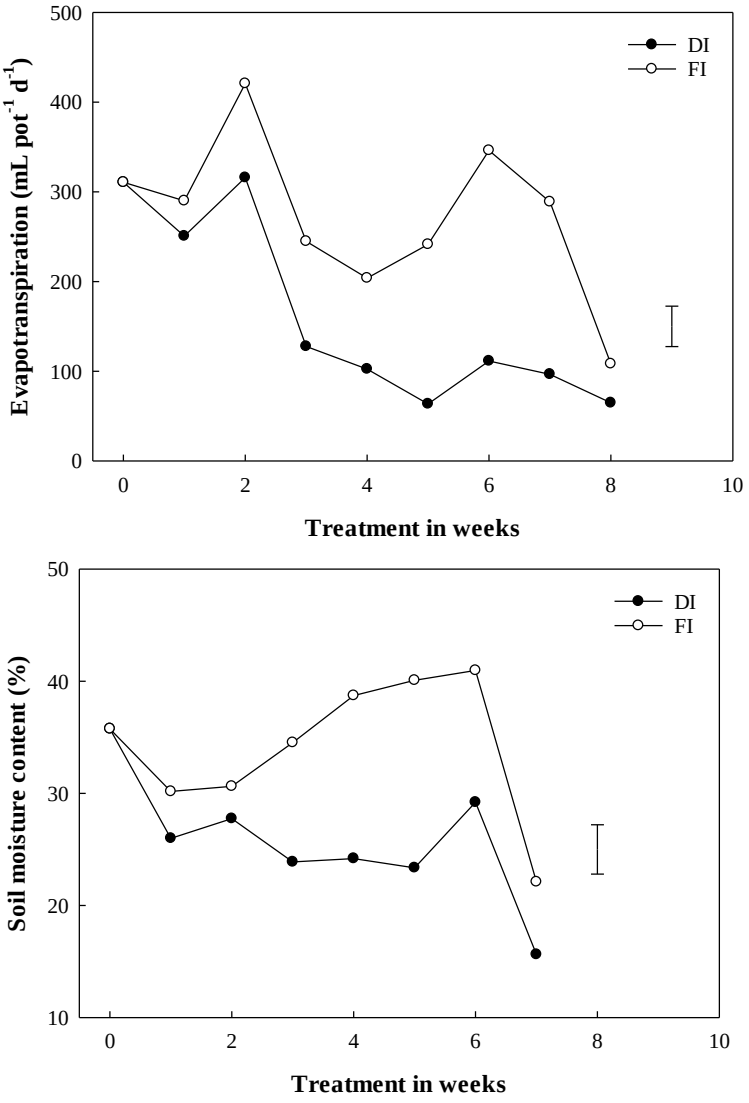


Figure C-1. Evapotranspiration and soil moisture content. Onion double haploid lines (CUDH2107) seeds were grown in 2017 under full irrigation (FI) or deficit irrigation (DI), where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. LSD bar at 95% confidence shown for interactions between treatments and time in weeks.

Appendix D

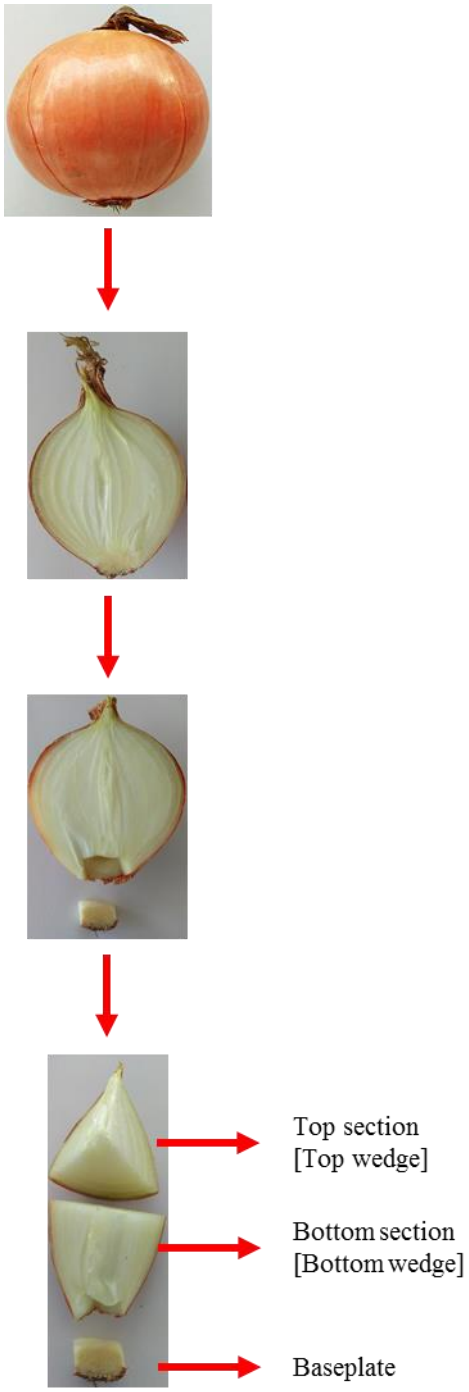


Figure D-1. Onion bulb cut into top wedge, bottom wedge and baseplate

Appendix E

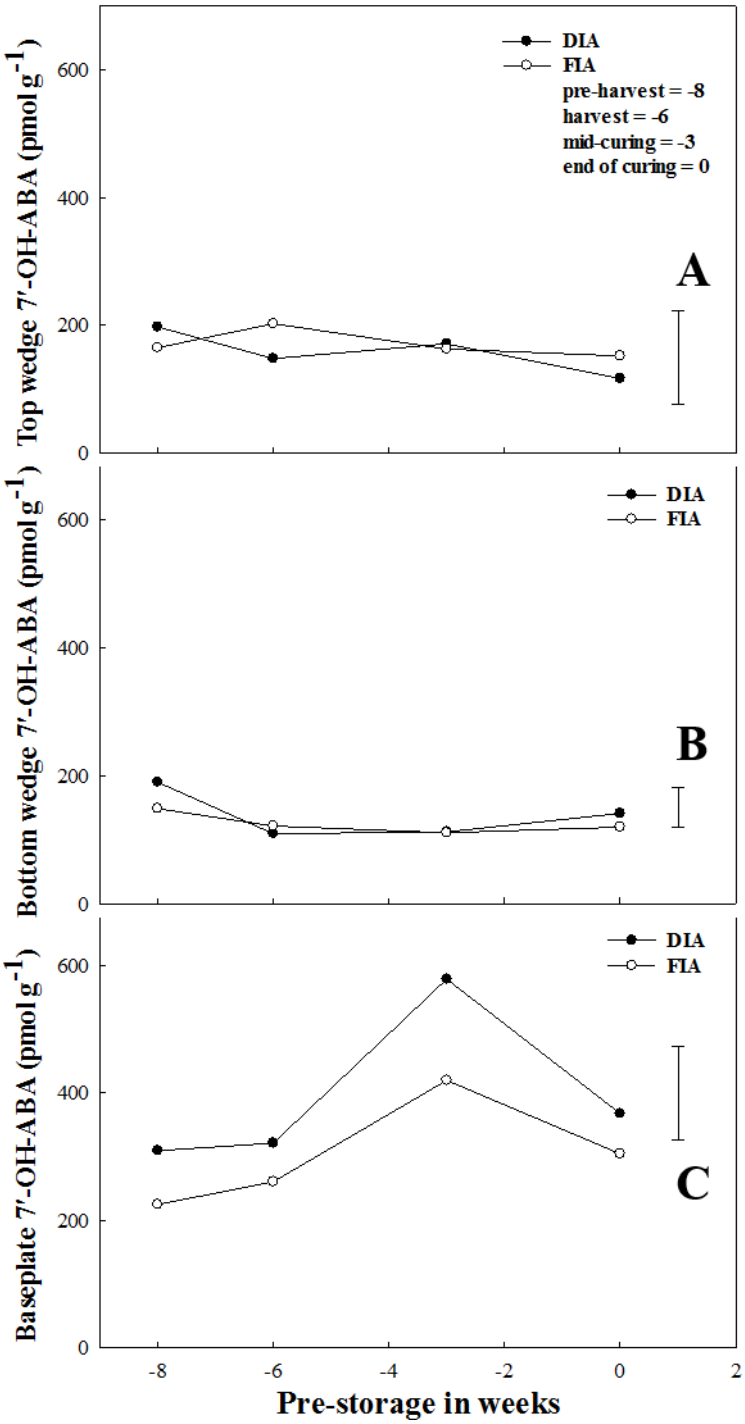


Figure E-1. 7′-hydroxy abscisic acid (7′-OH-ABA) contents in the top wedge (A), bottom wedge (B) and baseplate (C) of onion bulbs (cv. 'Sherpa') grown under full and deficit irrigation (FIA and DIA, respectively). 7′-OH-ABA contents per dry weight for the top wedge, bottom wedge and baseplate of onion bulbs (cv. 'Sherpa') grown under full irrigation (FIA) or deficit irrigation (DIA); where FIA defines 100% replenishment of crop

evapotranspiration (ET_c) and DI 50% replenishment of ET_c. Bulbs were harvest at full maturity (i.e. 100% fall-down) and cured under glass for six weeks. LSD bar at 95% confidence shown for interactions between treatments and time in weeks.

Appendix F

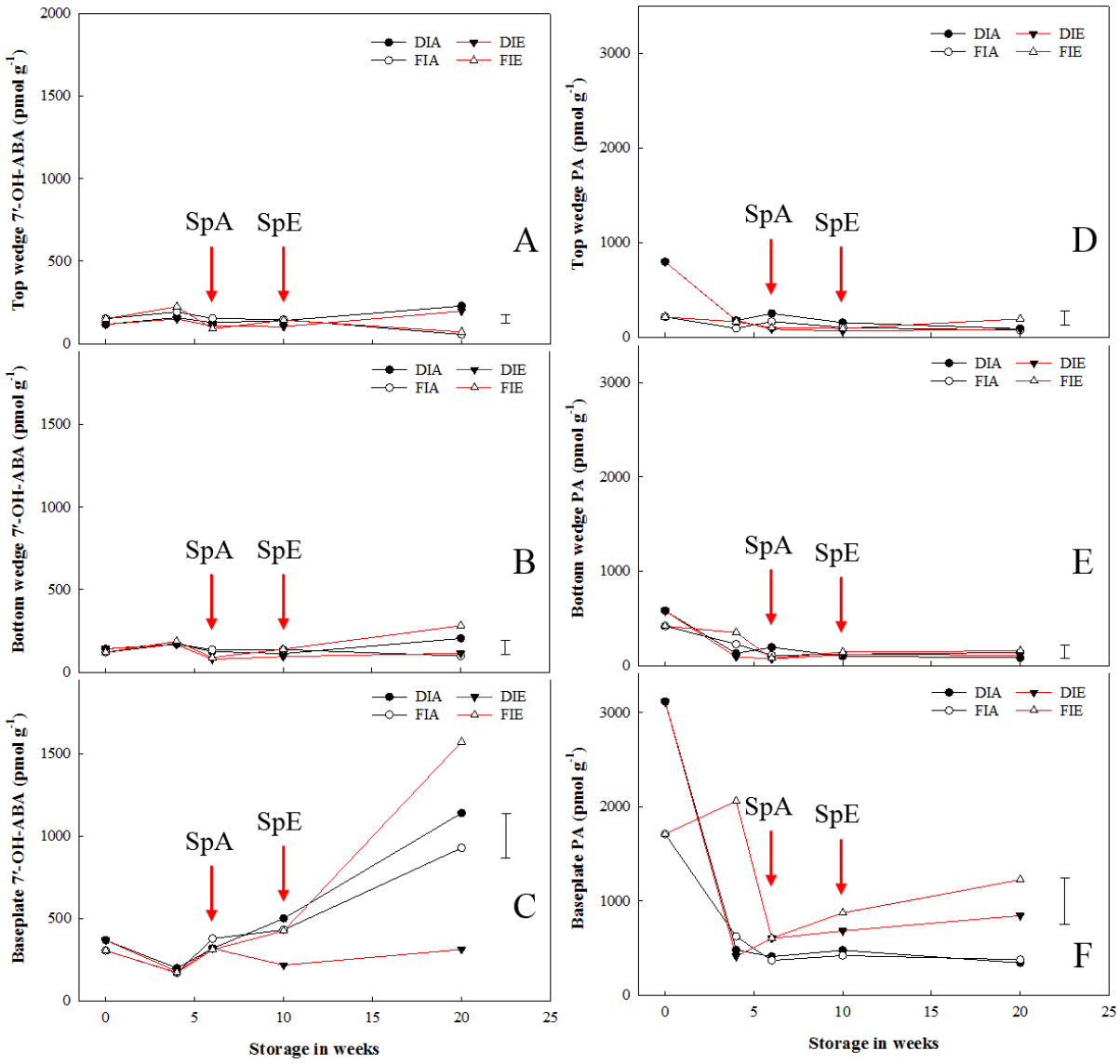


Figure F-1. 7′-hydroxy abscisic acid (7′-OH-ABA) and phaseic acid (PA) contents in the top wedge, bottom wedge and baseplate of stored onion bulbs (cv. 'Sherpa') grown under full and deficit irrigation and stored under air or ethylene. 7′-OH-ABA (A, B and C) and PA (D, E and F) contents per dry weight for the top wedge (A and D), bottom wedge (B and E) and baseplate (C and F) of onion bulbs (cv. 'Sherpa') grown under full irrigation (FI) or deficit irrigation (DI); where FI defines 100% replenishment of crop evapotranspiration (ET_c) and DI 50% replenishment of FI's ET_c. Bulbs were harvest at full maturity (i.e. 100% fall-down) and cured under glass for six weeks. Post-curing, bulbs were stored at 1 °C under continuous ethylene supplementation at 10 µL L-1 or air. DIA and FIA are DI and FI bulbs stored in air and DIE and FIE are DI and FI bulbs stored under ethylene. SpA and SpE are

sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95 % confidence shown for interactions between treatments and storage in weeks.

Appendix G

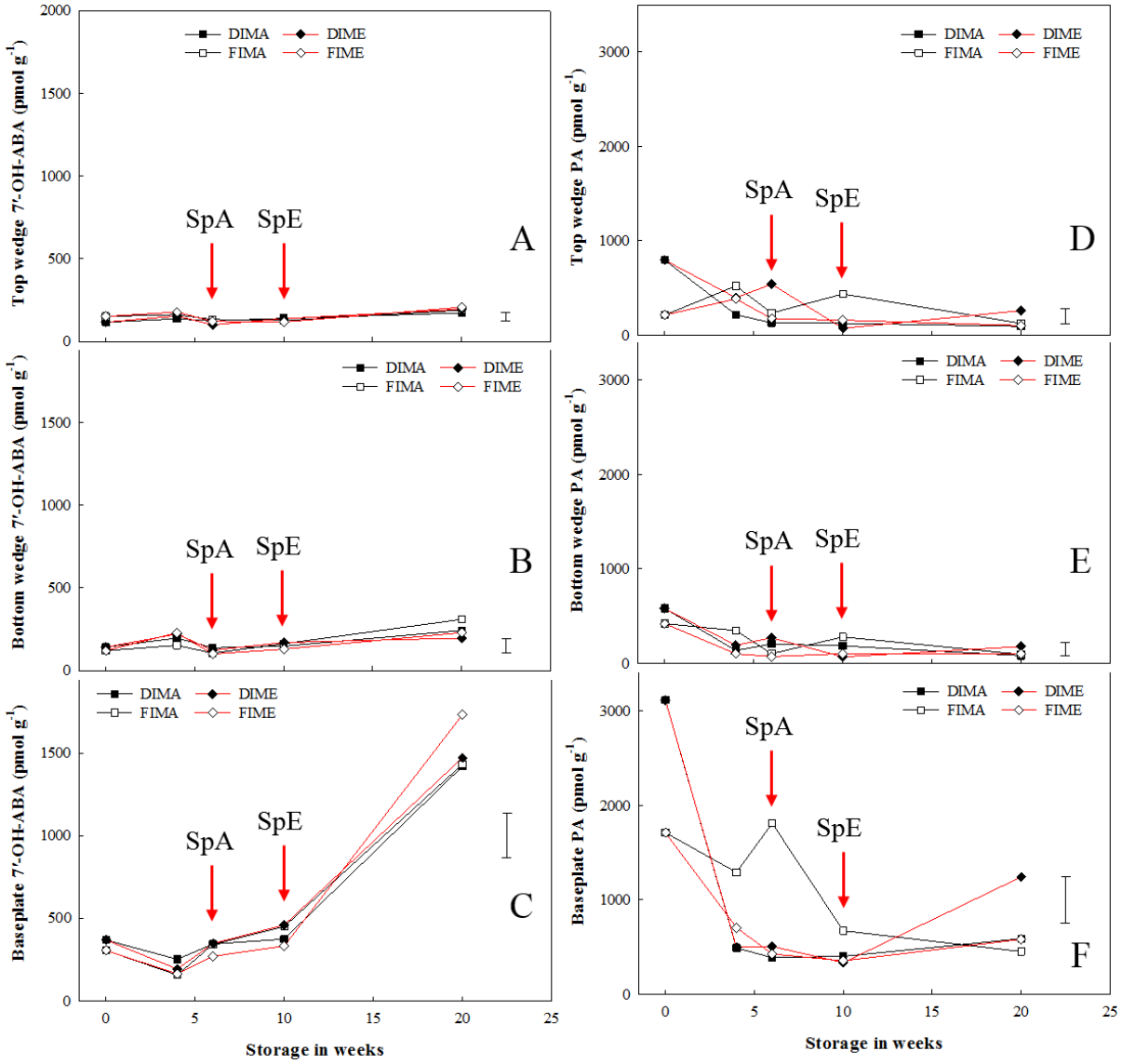


Figure G-1. 7′-hydroxy abscisic acid (7′-OH-ABA) and phaseic acid (PA) contents in the top wedge, bottom wedge and baseplate of stored onion bulbs (cv. 'Sherpa') grown under full and deficit irrigation, treated with or without 1-methylcyclopropene (1-MCP) and stored under air or ethylene. 7′-OH-ABA and PA contents per dry weight for the top wedge (A and D), bottom wedge (B and E) and baseplate (C and F) of onion bulbs (cv. 'Sherpa') grown under full irrigation (FI) or deficit irrigation (DI); where FI defines 100% replenishment of crop evapotranspiration (ET_c) and DI 50% replenishment of ET_c. Bulbs were harvested at full maturity (i.e. 100% fall-down) and cured under glass for six weeks. Post-curing, bulbs were treated with or without 1-MCP at 1 µL L^{-1} for 24hrs before storage and stored at 1 °C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIMA and FIMA are DI

and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95 % confidence shown for interactions between treatments and storage in weeks.

Appendix H

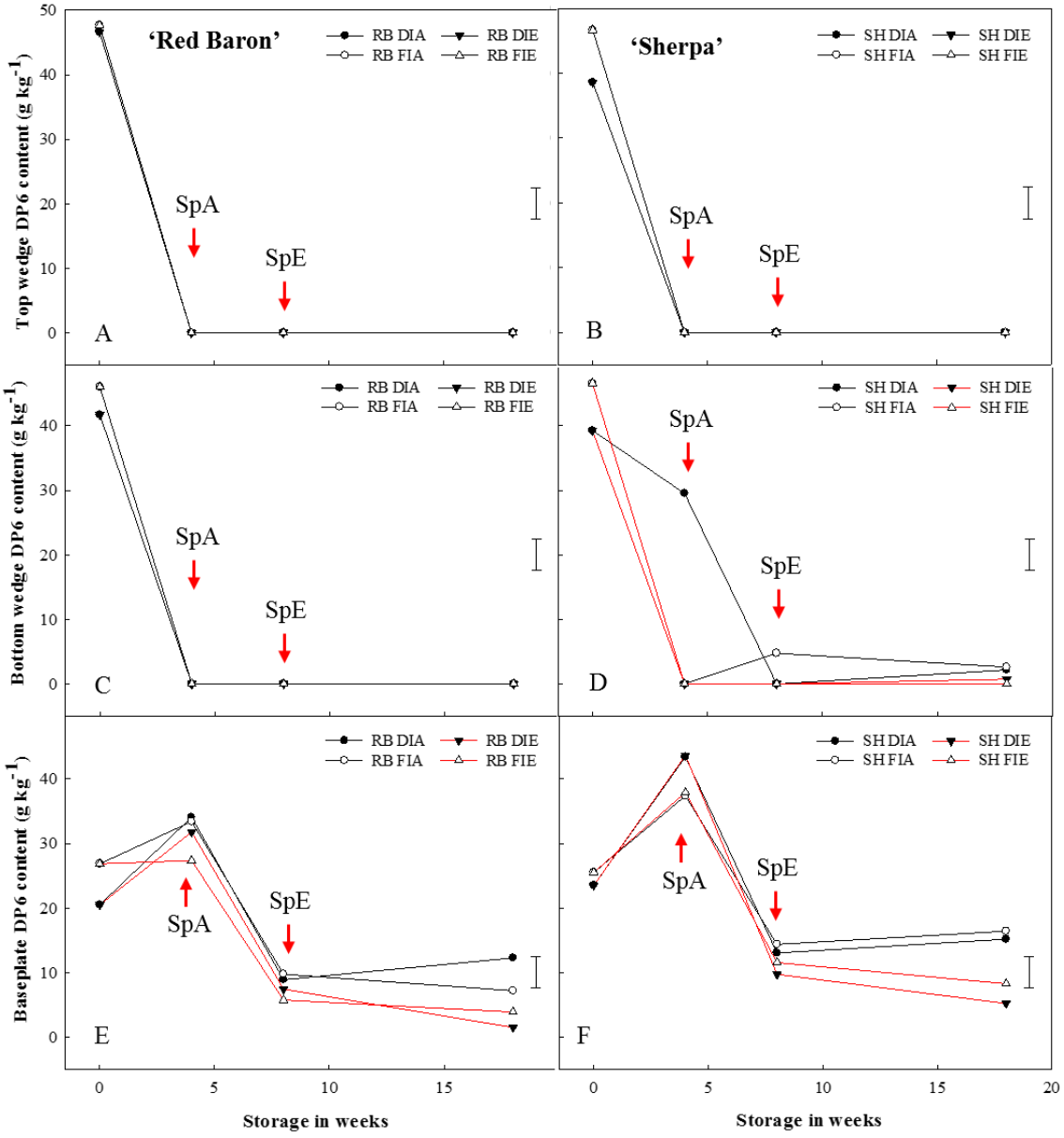


Figure H-1. Pre- and postharvest fructans DP6 content in stored onion bulbs. DP6 fructans contents per dry weight for the top wedge (A and B), bottom wedge (C and D) and baseplate (E and F) of onion bulbs of cultivar 'Sherpa' grown in 2015 under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. Post-curing, bulbs were stored at 1 °C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIA and FIA are DI and FI bulbs stored in air and DIE and FIE are DI and FI bulbs stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and

ethylene, respectively. LSD bar at 95% confidence shown for interactions between treatments and storage in weeks.

Appendix I

I.1.1 Introduction

The tables below were the analyses of variance (ANOVA) for the figures used in this thesis for onion cvs. 'Red Baron' (2015/16), 'Sherpa' (2015/16, 2016/17) and onion double haploid line CUDH2107 (2017/18). The treatments were divided into pre- and postharvest; represented in this ANOVA tables as preH and postH, respectively. For all analyses, baseline defines samples collected prior to the treatments under investigation, while outturn defines different sampling points. For the biochemical analyses (abscisic acid [ABA], ABA metabolites [ABA glucose ester – ABA-GE; 7′-hydroxy-ABA – 7′-OH-ABA; phaseic acid – PA; and dihydrophaseic acid – DPA], sugars [fructose, glucose and sucrose] and fructans [with degrees of polymerisation 3 – 8]) from the top, bottom or baseplate sections of the onion bulbs as shown in Figure D-1. For all years, the pre-harvest treatments were full or deficit irrigation (FI and DI, respectively); where FI defined the replacement of evaporation at 100% while DI was replacement at 50%. For all years, the postharvest treatments involved storing of onion bulbs under continuous ethylene (10 µL L^{-1}) or air. In 2016/17, additional postharvest treatments were added; such that after curing the onion bulbs for six weeks, they were treated with or without 1-methylcyclopropene (at 1 µL L^{-1}) for 24 h after which the bulbs were stored under ethylene or air as with 2015/16 while 2017/18 were the same treatments as in 2015/16.

I.1.2 Analyses of variance (ANOVA) for figures used in Chapter Two

Table I-1. ANOVA for sprouting for 'Red Baron' and 'Sherpa' grown under full or deficit irrigation, cured under glass for six weeks and stored at 1 °C for 18 weeks under ethylene (10 µL L^{-1}) or air 2015/16 (*n* = 9 $cultivar^{-1}$ $treatment^{-1}$ $outturn^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	1	3225.26	3225.26	99.53	<.001
PreH	1	8.02	8.02	0.25	0.62
Baseline	1	2996.33	2996.33	92.47	<.001
Cultivar.PreH	1	54.97	54.97	1.7	0.195
Cultivar.Baseline	1	537.54	537.54	16.59	<.001
PreH.Baseline	1	1.34	1.34	0.04	0.839
Baseline.PostH	1	1263.21	1263.21	38.98	<.001
Baseline.Outturn	5	15035.09	3007.02	92.8	<.001
Cultivar.PreH.Baseline	1	9.16	9.16	0.28	0.596
Cultivar.Baseline.PostH	1	314.18	314.18	9.7	0.002
PreH.Baseline.PostH	1	17.43	17.43	0.54	0.465
Cultivar.Baseline.Outturn	5	1602.98	320.6	9.89	<.001
PreH.Baseline.Outturn	5	136.06	27.21	0.84	0.524
Baseline.PostH.Outturn	5	495.96	99.19	3.06	0.012
Cultivar.PreH.Baseline.PostH	1	224.75	224.75	6.94	0.01
Cultivar.PreH.Baseline.Outturn	5	186.73	37.35	1.15	0.337
Cultivar.Baseline.PostH.Outturn	5	494.23	98.85	3.05	0.013
PreH.Baseline.PostH.Outturn	5	125.31	25.06	0.77	0.571
Cultivar.PreH.Baseline.PostH.Outturn	5	384.03	76.81	2.37	0.044
Residual	116	3758.89	32.4		
Total	167	30871.48			

Table I-2. ANOVA for sprouting for 'Sherpa' grown under full or deficit irrigation, cured under glass for six weeks, treated with or without 1-methylcyclopropene (1 µL L^{-1}) for 24 h and stored at 1 °C for 20 weeks under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 $cultivar^{-1}$ $treatment^{-1}$ $outturn^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	10.34	10.34	0.91	0.343
Baseline	1	1215.58	1215.58	106.74	<.001
PreH.Baseline	1	0.37	0.37	0.03	0.857
Baseline.PostH	3	1223.44	407.81	35.81	<.001
Baseline.Outturn	6	27283.73	4547.29	399.28	<.001
PreH.Baseline.PostH	3	29.98	9.99	0.88	0.455
PreH.Baseline.Outturn	6	186.33	31.05	2.73	0.016
Baseline.PostH.Outturn	18	1659.51	92.2	8.1	<.001
PreH.Baseline.PostH.Outturn	18	578.68	32.15	2.82	<.001
Residual	116	1321.08	11.39		
Total	173	33509.05			

Table I-3. ANOVA of the sprout vigour for 'Sherpa' grown under full or deficit irrigation, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (1 µL L^{-1}) for 24 h and stored at 1 °C for 20 weeks under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 $cultivar^{-1}$ $treatment^{-1}$ $outturn^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	8.99	8.99	0.73	0.396
Baseline	1	9799.93	9799.93	793.93	<.001
PreH.Baseline	1	2.25	2.25	0.18	0.671
Baseline.1MCP	1	115.93	115.93	9.39	0.003
Baseline.Ethyl	1	355.02	355.02	28.76	<.001
Baseline.Outturn	3	6579.44	2193.15	177.68	<.001
PreH.Baseline.1MCP	1	11.19	11.19	0.91	0.344
PreH.Baseline.Ethyl	1	29.24	29.24	2.37	0.127
Baseline.1MCP.Ethyl	1	186.96	186.96	15.15	<.001
PreH.Baseline.Outturn	3	13.51	4.5	0.36	0.778
Baseline.1MCP.Outturn	3	182.66	60.89	4.93	0.003
Baseline.Ethyl.Outturn	3	601.82	200.61	16.25	<.001
PreH.Baseline.1MCP.Ethyl	1	2.58	2.58	0.21	0.649
PreH.Baseline.1MCP.Outturn	3	497.97	165.99	13.45	<.001
PreH.Baseline.Ethyl.Outturn	3	165.17	55.06	4.46	0.006
Baseline.1MCP.Ethyl.Outturn	3	425.94	141.98	11.5	<.001
PreH.Baseline.1MCP.Ethyl.Outturn	3	66.78	22.26	1.8	0.153
Residual	86	1061.55	12.34		
Total	119	20106.94			

Table I-4. ANOVA for sprouting for CUDH2107 grown under full or deficit irrigation, cured under glass for two weeks and stored at 1 °C for 8 weeks under ethylene (10 µL L^{-1}) or air 2017/18 (*n* = 9 $cultivar^{-1}$ $treatment^{-1}$ $outturn^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	7.6	7.6	0.17	0.683
Baseline	1	2181.09	2181.09	49.06	<.001
PreH.Baseline	1	0.01	0.01	0	0.99
Baseline.PostH	2	2482.44	1241.22	27.92	<.001
Baseline.Outturn	2	211	105.5	2.37	0.115
PreH.Baseline.PostH	2	80.88	40.44	0.91	0.416
PreH.Baseline.Outturn	2	87.39	43.7	0.98	0.389
Residual	24	1066.89	44.45		
Total	35	6117.3			

Table I-5. ANOVA for respiration rate (CO_2 kg^{-1} h^{-1}) for 'Red Baron' and 'Sherpa' grown under full or deficit irrigation, cured under glass for six weeks and stored at 1 °C for 18 weeks under ethylene (10 µL L^{-1}) or air 2015/16 (*n* = 9 $cultivar^{-1}$ pre-/postharvest $treatment^{-1}$ $outturn^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	1	35.346	35.346	17.71	<.001
PreH	1	0.769	0.769	0.39	0.536
Baseline	1	1.143	1.143	0.57	0.451
Cultivar.PreH	1	1.135	1.135	0.57	0.452
Cultivar.Baseline	1	3.5	3.5	1.75	0.188
PreH.Baseline	1	4.967	4.967	2.49	0.118
Baseline.PostH	1	2.597	2.597	1.3	0.257
Baseline.Outturn	5	155.018	31.004	15.54	<.001
Cultivar.PreH.Baseline	1	0.172	0.172	0.09	0.77
Cultivar.Baseline.PostH	1	0.837	0.837	0.42	0.519
PreH.Baseline.PostH	1	3.052	3.052	1.53	0.219
Cultivar.Baseline.Outturn	5	14.77	2.954	1.48	0.203
PreH.Baseline.Outturn	5	4.985	0.997	0.5	0.776
Baseline.PostH.Outturn	5	157.977	31.595	15.83	<.001
Cultivar.PreH.Baseline.PostH	1	0.613	0.613	0.31	0.581
Cultivar.PreH.Baseline.Outturn	5	10.337	2.067	1.04	0.4
Cultivar.Baseline.PostH.Outturn	5	13.416	2.683	1.34	0.251
PreH.Baseline.PostH.Outturn	5	11.528	2.306	1.16	0.336
Cultivar.PreH.Baseline.PostH.Outturn	5	9.7	1.94	0.97	0.438
Residual	104	207.515	1.995		
Total	155	639.378			

Table I-6. ANOVA for respiration rate (CO_2 kg^{-1} h^{-1}) for CUDH2107 grown under full or deficit irrigation, cured under glass for two weeks and stored at 1 °C for 8 weeks under ethylene (10 µL L^{-1}) or air 2017/18 (*n* = 9 $cultivar^{-1}$ pre-/ $postharvest\ treatment^{-1}$ $outturn^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	0.063	0.063	0.02	0.88
Baseline	1	33.896	33.896	12.58	0.002
PreH.Baseline	1	8.459	8.459	3.14	0.092
Baseline.PostH	1	1.362	1.362	0.51	0.485
Baseline.Outturn	1	29.633	29.633	11	0.003
PreH.Baseline.PostH	1	0.132	0.132	0.05	0.827
PreH.Baseline.Outturn	1	0.114	0.114	0.04	0.839
Baseline.PostH.Outturn	1	0.12	0.12	0.04	0.835
PreH.Baseline.PostH.Outturn	1	0.641	0.641	0.24	0.631
Residual	20	53.896	2.695		
Total	29	128.318			

Table I-7. ANOVA for respiration respirate (CO_2 kg^{-1} h^{-1}) for 'Sherpa' grown under full or deficit irrigation, harvested at full maturity, cured for six weeks, treated with or without 1-methylcyclopropene (1 µL L^{-1}) and stored at 1 C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1} outturn^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	8.793	8.793	19.92	<.001
Baseline	1	284.5273	284.5273	644.43	<.001
PreH.Baseline	1	0.0963	0.0963	0.22	0.641
Baseline.PostH	4	79.5462	19.8865	45.04	<.001
Baseline.Outturn	9	471.1943	52.3549	118.58	<.001
PreH.Baseline.PostH	4	12.4209	3.1052	7.03	<.001
PreH.Baseline.Outturn	9	10.3854	1.1539	2.61	0.008
Baseline.PostH.Outturn	17	115.8424	6.8143	15.43	<.001
PreH.Baseline.PostH.Outturn	17	19.9752	1.175	2.66	<.001
Residual	128	56.5145	0.4415		
Total	191	1059.296			

Table I-8. ANOVA for crop evapotranspiration for 'Sherpa' grown under full or deficit irrigation 2015/16 (*n* = 12 treatment^{-1} outturn^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	2	186753	93377	31.04	<.001
Baseline.Outturn	8	1896988	237123	78.83	<.001
PreH.Baseline.Outturn	8	151872	18984	6.31	<.001
Residual	95	285747	3008		
Total	113	2521360			

Table I-9. ANOVA for crop evapotranspiration for 'Red Baron' grown under full or deficit irrigation 2015/16 (*n* = 12 treatment^{-1} outturn^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pre-harvest treatments (PreH)	2	55921	27961	11.73	<.001
Baseline.Outturn	8	1871177	233897	98.1	<.001
PreH.Baseline.Outturn	8	250744	31343	13.15	<.001
Residual	95	226508	2384		
Total	113	2404351			

Table I-10. ANOVA for soil moisture content for 'Sherpa' grown under full or deficit irrigation 2015/16 (*n* = 12 treatment^{-1} outturn^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	2	2051.37	1025.69	45.23	<.001
Baseline.Outturn	7	1683.31	240.47	10.61	<.001
PreH.Baseline.Outturn	7	406.04	58.01	2.56	0.019
Residual	85	1927.36	22.67		
Total	101	6068.09			

Table I-11. ANOVA for soil moisture content for 'Red Baron' grown under full or deficit irrigation 2015/16 (*n* = 12).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	2	2640.39	1320.19	97.25	<.001
Baseline.Outturn	6	1741	290.17	21.37	<.001
PreH.Baseline.Outturn	6	429.06	71.51	5.27	<.001
Residual	75	1018.15	13.58		
Total	89	5828.6			

Table I-12. ANOVA for crop evapotranspiration for 'Sherpa' grown under full or deficit irrigation 2016/17 (*n* = 12 pre-harvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	2	131847.7	65923.9	111.38	<.001
Baseline.Outturn	9	465114.1	51679.3	87.31	<.001
PreH.Baseline.Outturn	9	12255.6	1361.7	2.3	0.032
Residual	45	26635.8	591.9		
Total	65	635853.2			

Table I-13. ANOVA for soil moisture content for 'Sherpa' grown under full or deficit irrigation 2016/17 (*n* = 12 pre-harvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	2	8472	4236	3.14	0.053
Baseline.Outturn	9	14826	1647	1.22	0.306
PreH.Baseline.Outturn	9	13489	1499	1.11	0.374
Residual	45	60654	1348		
Total	65	97442			

Table I-14. ANOVA for curing weight-loss for 'Sherpa' grown under full or deficit irrigation 2016/17 (*n* = 12 pre-harvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	49.7129	49.7129	225.93	<.001
Baseline	1	80.3767	80.3767	365.29	<.001
PreH.Baseline	1	8.2855	8.2855	37.66	<.001
Baseline.Outturn	5	27.8168	5.5634	25.28	<.001
PreH.Baseline.Outturn	5	3.9462	0.7892	3.59	0.012
Residual	28	6.161	0.22		
Total	41	176.2992			

Table I-15. ANOVA for storage weight-loss for 'Sherpa' grown under full or deficit irrigation 2016/17 (*n* = 12 pre-harvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	2	226.3892	113.1946	290.61	<.001
Baseline	5	25.9026	5.1805	13.3	<.001
Baseline.PostH	7	4.0095	0.5728	1.47	0.185
Baseline.Outturn	5	191.9884	38.3977	98.58	<.001
PreH.Baseline.PostH	3	1.04	0.3467	0.89	0.449
PreH.Baseline.Outturn	5	0.5458	0.1092	0.28	0.923
Baseline.PostH.Outturn	15	1.8984	0.1266	0.32	0.992
PreH.Baseline.PostH.Outturn	15	8.4261	0.5617	1.44	0.139
Residual	116	45.1836	0.3895		
Total	173	505.3836			

Appendix J

J.1.1 Analyses of variance (ANOVA) for figures used in Chapter Three

Table J-1. ANOVA of pre-storage abscisic acid content (pmol g^{-1} DW) in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	29580	29580	3.28	0.076
Section	2	2095322	1047661	116.35	<.001
Baseline	1	2378	2378	0.26	0.61
PreH.Section	2	55920	27960	3.11	0.054
PreH.Baseline	1	2816	2816	0.31	0.579
Section.Baseline	2	34169	17085	1.9	0.161
Baseline.Outturn	2	87550	43775	4.86	0.012
PreH.Section.Baseline	2	1667	834	0.09	0.912
PreH.Baseline.Outturn	2	42148	21074	2.34	0.107
Section.Baseline.Outturn	4	49004	12251	1.36	0.262
PreH.Section.Baseline.Outturn	4	21958	5489	0.61	0.658
Residual	48	432218	9005		
Total	71	2854730			

Table J-2. ANOVA of pre-storage abscisic acid glucose ester content (pmol g^{-1} DW) in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	813130	813130	9.68	0.003
Section	2	683974	341987	4.07	0.023
Baseline	1	360084	360084	4.29	0.044
PreH.Section	2	44682	22341	0.27	0.768
PreH.Baseline	1	117974	117974	1.4	0.242
Section.Baseline	2	57049	28525	0.34	0.714
Baseline.Outturn	2	2140795	1070398	12.74	<.001
PreH.Section.Baseline	2	7114	3557	0.04	0.959
PreH.Baseline.Outturn	2	1219242	609621	7.26	0.002
Section.Baseline.Outturn	4	368134	92034	1.1	0.369
PreH.Section.Baseline.Outturn	4	192007	48002	0.57	0.685
Residual	48	4032071	84001		
Total	71	10036258			

Table J-3. ANOVA of pre-storage 7′-hydroxyabscisic acid (pmol g^{-1} DW) in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	1344	1344	2.17	0.147
Section	2	51269.2	25634.6	41.39	<.001
Baseline	1	148.4	148.4	0.24	0.627
PreH.Section	2	2776.6	1388.3	2.24	0.117
PreH.Baseline	1	227.6	227.6	0.37	0.547
Section.Baseline	2	5049.8	2524.9	4.08	0.023
Baseline.Outturn	2	3673.3	1836.7	2.97	0.061
PreH.Section.Baseline	2	223.2	111.6	0.18	0.836
PreH.Baseline.Outturn	2	625.8	312.9	0.51	0.607
Section.Baseline.Outturn	4	8192.6	2048.1	3.31	0.018
PreH.Section.Baseline.Outturn					
Residual	48	29728.1	619.3		
Total	71	103682.4			

Table J-4. ANOVA of pre-storage phaseic acid (pmol g^{-1} DW) in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	178715	178715	3.75	0.059
Section	2	1018550	509275	10.7	<.001
Baseline	1	213206	213206	4.48	0.04
PreH.Section	2	204706	102353	2.15	0.128
PreH.Baseline	1	65094	65094	1.37	0.248
Section.Baseline	2	223994	111997	2.35	0.106
Baseline.Outturn	2	552600	276300	5.8	0.006
PreH.Section.Baseline	2	58306	29153	0.61	0.546
PreH.Baseline.Outturn	2	226897	113449	2.38	0.103
Section.Baseline.Outturn	4	441959	110490	2.32	0.07
PreH.Section.Baseline.Outturn	4	269825	67456	1.42	0.243
Residual	48	2284713	47598		
Total	71	5738566			

Table J-5. ANOVA of pre-storage dihydrophaseic acid (pmol g^{-1} DW) in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	446364	446364	14.85	<.001
Section	2	1146663	573332	19.07	<.001
Baseline	1	1072005	1072005	35.66	<.001
PreH.Section	2	126286	63143	2.1	0.133
PreH.Baseline	1	1404500	1404500	46.72	<.001
Section.Baseline	2	569202	284601	9.47	<.001
Baseline.Outturn	2	864234	432117	14.38	<.001
PreH.Section.Baseline	2	18115	9058	0.3	0.741
PreH.Baseline.Outturn	2	230421	115210	3.83	0.029
Section.Baseline.Outturn	4	237947	59487	1.98	0.113
PreH.Section.Baseline.Outturn	4	209901	52475	1.75	0.155
Residual	48	1442837	30059		
Total	71	7768476			

Table J-6. ANOVA of abscisic acid content (pmol g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	902	902	0.31	0.577
Section	2	2882230	1441115	498.39	<.001
Baseline	1	151569	151569	52.42	<.001
PreH.Section	2	803	402	0.14	0.87
PreH.Baseline	1	26536	26536	9.18	0.003
Section.Baseline	2	40969	20485	7.08	0.001
Baseline.1MCP	1	5603	5603	1.94	0.165
Baseline.Ethylene	1	3054	3054	1.06	0.305
Baseline.Outturn	3	50237	16746	5.79	<.001
PreH.Section.Baseline	2	34709	17354	6	0.003
PreH.Baseline.1MCP	1	145	145	0.05	0.823
Section.Baseline.1MCP	2	26734	13367	4.62	0.011
PreH.Baseline.Ethylene	1	432	432	0.15	0.7
Section.Baseline.Ethylene	2	23342	11671	4.04	0.019
Baseline.1MCP.Ethylene	1	2146	2146	0.74	0.39
PreH.Baseline.Outturn	3	8338	2779	0.96	0.412
Section.Baseline.Outturn	6	132830	22138	7.66	<.001
Baseline.1MCP.Outturn	3	6649	2216	0.77	0.514
Baseline.Ethylene.Outturn	3	25082	8361	2.89	0.036
PreH.Section.Baseline.1MCP	2	428	214	0.07	0.929
PreH.Section.Baseline.Ethylene	2	1372	686	0.24	0.789
PreH.Baseline.1MCP.Ethylene	1	12495	12495	4.32	0.039
Section.Baseline.1MCP.Ethylene	2	20765	10382	3.59	0.029
PreH.Section.Baseline.Outturn	6	10733	1789	0.62	0.715
PreH.Baseline.1MCP.Outturn	3	9641	3214	1.11	0.346
Section.Baseline.1MCP.Outturn	6	42434	7072	2.45	0.026
PreH.Baseline.Ethylene.Outturn	3	1503	501	0.17	0.914
Section.Baseline.Ethylene.Outturn	6	17644	2941	1.02	0.415
Baseline.1MCP.Ethylene.Outturn	3	2525	842	0.29	0.832
PreH.Section.Baseline.1MCP.Ethylene	2	8165	4082	1.41	0.246
PreH.Section.Baseline.1MCP.Outturn	6	17085	2847	0.98	0.437
PreH.Section.Baseline.Ethylene.Outturn	6	2145	357	0.12	0.993
PreH.Baseline.1MCP.Ethylene.Outturn	3	8099	2700	0.93	0.425
Section.Baseline.1MCP.Ethylene.Outturn	6	3506	584	0.2	0.976
PreH.Section.Baseline.1MCP.Ethylene.Outturn	6	2488	415	0.14	0.99
Residual	204	589870	2892		
Total	305	4173205			

Table J-7. ANOVA of abscisic acid glucose ester content (pmol g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	207916	207916	5.98	0.015
Section	2	2175361	1087680	31.3	<.001
Baseline	1	2444	2444	0.07	0.791
PreH.Section	2	80502	40251	1.16	0.316
PreH.Baseline	1	43696	43696	1.26	0.263
Section.Baseline	2	32943	16471	0.47	0.623
Baseline.1MCP	1	47958	47958	1.38	0.241
Baseline.Ethylene	1	65206	65206	1.88	0.172
Baseline.Outturn	3	1917217	639072	18.39	<.001
PreH.Section.Baseline	2	2657	1328	0.04	0.963
PreH.Baseline.1MCP	1	3	3	0	0.992
Section.Baseline.1MCP	2	2145	1073	0.03	0.97
PreH.Baseline.Ethylene	1	120763	120763	3.47	0.064
Section.Baseline.Ethylene	2	162696	81348	2.34	0.099
Baseline.1MCP.Ethylene	1	68059	68059	1.96	0.163
PreH.Baseline.Outturn	3	273664	91221	2.62	0.052
Section.Baseline.Outturn	6	211547	35258	1.01	0.417
Baseline.1MCP.Outturn	3	126853	42284	1.22	0.305
Baseline.Ethylene.Outturn	3	444530	148177	4.26	0.006
PreH.Section.Baseline.1MCP	2	51805	25903	0.75	0.476
PreH.Section.Baseline.Ethylene	2	86841	43420	1.25	0.289
PreH.Baseline.1MCP.Ethylene	1	72767	72767	2.09	0.149
Section.Baseline.1MCP.Ethylene	2	178838	89419	2.57	0.079
PreH.Section.Baseline.Outturn	6	297511	49585	1.43	0.206
PreH.Baseline.1MCP.Outturn	3	59007	19669	0.57	0.638
Section.Baseline.1MCP.Outturn	6	373217	62203	1.79	0.103
PreH.Baseline.Ethylene.Outturn	3	76663	25554	0.74	0.532
Section.Baseline.Ethylene.Outturn	6	468439	78073	2.25	0.04
Baseline.1MCP.Ethylene.Outturn	3	269741	89914	2.59	0.054
PreH.Section.Baseline.1MCP.Ethylene	2	26724	13362	0.38	0.681
PreH.Section.Baseline.1MCP.Outturn	6	67698	11283	0.32	0.924
PreH.Section.Baseline.Ethylene.Outturn	6	33587	5598	0.16	0.987
PreH.Baseline.1MCP.Ethylene.Outturn	3	70946	23649	0.68	0.565
Section.Baseline.1MCP.Ethylene.Outturn	6	232593	38765	1.12	0.354
PreH.Section.Baseline.1MCP.Ethylene.Outturn	6	48953	8159	0.23	0.965
Residual	204	7089619	34753		
Total	305	15491110			

Table J-8. ANOVA of 7′-hydroxyabscisic acid content (pmol g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
preH	1	1663827	1663827	14.43	<.001
Section	2	4548986	2274493	19.73	<.001
BaseLine	1	505647	505647	4.39	0.037
preH.Section	2	1354807	677403	5.88	0.003
preH.BaseLine	1	97566	97566	0.85	0.359
Section.BaseLine	2	399380	199690	1.73	0.179
BaseLine.MCP	1	6295253	6295253	54.62	<.001
BaseLine.Ethyl	1	8420651	8420651	73.05	<.001
BaseLine.Outturn	3	25872837	8624279	74.82	<.001
preH.Section.BaseLine	2	90294	45147	0.39	0.676
preH.BaseLine.MCP	1	762403	762403	6.61	0.011
Section.BaseLine.MCP	2	3464567	1732283	15.03	<.001
preH.BaseLine.Ethyl	1	1726628	1726628	14.98	<.001
Section.BaseLine.Ethyl	2	5995281	2997641	26.01	<.001
BaseLine.MCP.Ethyl	1	6044771	6044771	52.44	<.001
preH.BaseLine.Outturn	3	5757689	1919230	16.65	<.001
Section.BaseLine.Outturn	6	19203644	3200607	27.77	<.001
BaseLine.MCP.Outturn	3	18435160	6145053	53.31	<.001
BaseLine.Ethyl.Outturn	3	26539900	8846633	76.75	<.001
preH.Section.BaseLine.MCP	2	2033228	1016614	8.82	<.001
preH.Section.BaseLine.Ethyl	2	1420099	710050	6.16	0.003
preH.BaseLine.MCP.Ethyl	1	842821	842821	7.31	0.007
Section.BaseLine.MCP.Ethyl	2	3611767	1805884	15.67	<.001
preH.Section.BaseLine.Outturn	6	3992082	665347	5.77	<.001
preH.BaseLine.MCP.Outturn	3	2233134	744378	6.46	<.001
Section.BaseLine.MCP.Outturn	6	10849618	1808270	15.69	<.001
preH.BaseLine.Ethyl.Outturn	3	5367286	1789095	15.52	<.001
Section.BaseLine.Ethyl.Outturn	6	17161552	2860259	24.81	<.001
BaseLine.MCP.Ethyl.Outturn	3	17932937	5977646	51.86	<.001
preH.Section.BaseLine.MCP.Ethyl	2	2035966	1017983	8.83	<.001
preH.Section.BaseLine.MCP.Outturn	6	5907679	984613	8.54	<.001
preH.Section.BaseLine.Ethyl.Outturn	6	4148947	691491	6	<.001
preH.BaseLine.MCP.Ethyl.Outturn	3	2341761	780587	6.77	<.001
Section.BaseLine.MCP.Ethyl.Outturn	6	10708234	1784706	15.48	<.001
preH.Section.BaseLine.MCP.Ethyl.Outturn	6	6280751	1046792	9.08	<.001
Residual	204	23514063	115265		
Total	305	2.58E+08			

Table J-9. ANOVA of phaseic acid content (pmol g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	139452	139452	4.49	0.035
Section	2	2344551	1172275	37.74	<.001
Baseline	1	215535	215535	6.94	0.009
PreH.Section	2	48651	24325	0.78	0.458
PreH.Baseline	1	138146	138146	4.45	0.036
Section.Baseline	2	172366	86183	2.77	0.065
Baseline.1MCP	1	118433	118433	3.81	0.052
Baseline.Ethylene	1	29238	29238	0.94	0.333
Baseline.Outturn	3	185697	61899	1.99	0.116
PreH.Section.Baseline	2	78298	39149	1.26	0.286
PreH.Baseline.1MCP	1	3305	3305	0.11	0.745
Section.Baseline.1MCP	2	86377	43188	1.39	0.251
PreH.Baseline.Ethylene	1	10381	10381	0.33	0.564
Section.Baseline.Ethylene	2	171432	85716	2.76	0.066
Baseline.1MCP.Ethylene	1	73921	73921	2.38	0.124
PreH.Baseline.Outturn	3	63692	21231	0.68	0.563
Section.Baseline.Outturn	6	592912	98819	3.18	0.005
Baseline.1MCP.Outturn	3	102545	34182	1.1	0.35
Baseline.Ethylene.Outturn	3	301620	100540	3.24	0.023
PreH.Section.Baseline.1MCP	2	4924	2462	0.08	0.924
PreH.Section.Baseline.Ethylene	2	5263	2631	0.08	0.919
PreH.Baseline.1MCP.Ethylene	1	241284	241284	7.77	0.006
Section.Baseline.1MCP.Ethylene	2	13999	6999	0.23	0.798
PreH.Section.Baseline.Outturn	6	16144	2691	0.09	0.998
PreH.Baseline.1MCP.Outturn	3	63428	21143	0.68	0.565
Section.Baseline.1MCP.Outturn	6	64064	10677	0.34	0.913
PreH.Baseline.Ethylene.Outturn	3	17308	5769	0.19	0.906
Section.Baseline.Ethylene.Outturn	6	274275	45712	1.47	0.189
Baseline.1MCP.Ethylene.Outturn	3	40580	13527	0.44	0.728
PreH.Section.Baseline.1MCP.Ethylene	2	82027	41013	1.32	0.269
PreH.Section.Baseline.1MCP.Outturn	6	80775	13462	0.43	0.856
PreH.Section.Baseline.Ethylene.Outturn	6	27880	4647	0.15	0.989
PreH.Baseline.1MCP.Ethylene.Outturn	3	57253	19084	0.61	0.606
Section.Baseline.1MCP.Ethylene.Outturn	6	49508	8251	0.27	0.952
PreH.Section.Baseline.1MCP.Ethylene.Outturn	6	55846	9308	0.3	0.937
Residual	204	6337178	31065		
Total	305	12308285			

Table J-10. ANOVA of dihydrophaseic acid content (pmol g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	40268	40268	2.67	0.104
Section	2	3199678	1599839	106.2	<.001
Baseline	1	2072209	2072209	137.56	<.001
PreH.Section	2	37787	18894	1.25	0.287
PreH.Baseline	1	239258	239258	15.88	<.001
Section.Baseline	2	264598	132299	8.78	<.001
Baseline.1MCP	1	345784	345784	22.95	<.001
Baseline.Ethylene	1	60940	60940	4.05	0.046
Baseline.Outturn	3	1022589	340863	22.63	<.001
PreH.Section.Baseline	2	155416	77708	5.16	0.007
PreH.Baseline.1MCP	1	336	336	0.02	0.881
Section.Baseline.1MCP	2	81095	40548	2.69	0.07
PreH.Baseline.Ethylene	1	104656	104656	6.95	0.009
Section.Baseline.Ethylene	2	3048	1524	0.1	0.904
Baseline.1MCP.Ethylene	1	156952	156952	10.42	0.001
PreH.Baseline.Outturn	3	29278	9759	0.65	0.585
Section.Baseline.Outturn	6	532095	88683	5.89	<.001
Baseline.1MCP.Outturn	3	17071	5690	0.38	0.769
Baseline.Ethylene.Outturn	3	42875	14292	0.95	0.418
PreH.Section.Baseline.1MCP					
PreH.Section.Baseline.Ethylene	2	10992	5496	0.36	0.695
PreH.Baseline.1MCP.Ethylene					
Section.Baseline.1MCP.Ethylene	2	10300	5150	0.34	0.711
PreH.Section.Baseline.Outturn	6	102148	17025	1.13	0.346
PreH.Baseline.1MCP.Outturn	3	72150	24050	1.6	0.191
Section.Baseline.1MCP.Outturn	6	58357	9726	0.65	0.694
PreH.Baseline.Ethylene.Outturn	3	41593	13864	0.92	0.432
Section.Baseline.Ethylene.Outturn	6	91927	15321	1.02	0.415
Baseline.1MCP.Ethylene.Outturn	3	197437	65812	4.37	0.005
PreH.Section.Baseline.1MCP.Ethylene	2	26079	13040	0.87	0.422
PreH.Section.Baseline.1MCP.Outturn	6	60040	10007	0.66	0.679
PreH.Section.Baseline.Ethylene.Outturn	6	75383	12564	0.83	0.545
PreH.Baseline.1MCP.Ethylene.Outturn	3	142545	47515	3.15	0.026
Section.Baseline.1MCP.Ethylene.Outturn	6	6855	1143	0.08	0.998
PreH.Section.Baseline.1MCP.Ethylene.Outturn	6	101076	16846	1.12	0.353
Residual	204	3073049	15064		
Total	305	12531997			

Appendix K

K.1.1 ANOVA for figures used in Chapter Four

Table K-1. ANOVA of fructose (mg g^{-1} DW) for 'Red Baron' and 'Sherpa' during 2015/16 storage (*n* = 9 $cultivar^{-1}$ pre-/ $postharvest\ treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	1	11679.9	11679.9	84.3	<.001
Pre-harvest treatments	1	188.5	188.5	1.36	0.245
Section	2	570337	285168.5	2058.29	<.001
Baseline	1	72524.2	72524.2	523.47	<.001
Cultivar.PreH	1	1301.1	1301.1	9.39	0.003
Cultivar.Section	2	6378.3	3189.2	23.02	<.001
PreH.Section	2	278.6	139.3	1.01	0.368
Cultivar.Baseline	1	637	637	4.6	0.033
PreH.Baseline	1	189.8	189.8	1.37	0.244
Section.Baseline	2	51368.5	25684.3	185.38	<.001
Baseline.PostH)	1	6521.6	6521.6	47.07	<.001
Baseline.Outturn	2	44408.7	22204.4	160.27	<.001
Cultivar.PreH.Section	2	692.9	346.4	2.5	0.085
Cultivar.PreH.Baseline	1	2.5	2.5	0.02	0.894
Cultivar.Section.Baseline	2	374.6	187.3	1.35	0.262
PreH.Section.Baseline	2	8	4	0.03	0.972
Cultivar.Baseline.PostH	1	37.1	37.1	0.27	0.605
PreH.Baseline.PostH	1	59.7	59.7	0.43	0.512
Section.Baseline.PostH	2	1814.9	907.5	6.55	0.002
Cultivar.Baseline.Outturn	2	1820.3	910.1	6.57	0.002
PreH.Baseline.Outturn	2	376.3	188.1	1.36	0.26
Section.Baseline.Outturn	4	2006.1	501.5	3.62	0.007
Baseline.PostH.Outturn	2	5287.1	2643.5	19.08	<.001
Cultivar.PreH.Section.Baseline	2	111.1	55.6	0.4	0.67
Cultivar.PreH.Baseline.PostH	1	111	111	0.8	0.372
Cultivar.Section.Baseline.PostH	2	81.2	40.6	0.29	0.746
PreH.Section.Baseline.PostH	2	41.6	20.8	0.15	0.861
Cultivar.PreH.Baseline.Outturn	2	534.7	267.4	1.93	0.148
Cultivar.Section.Baseline.Outturn	4	339	84.7	0.61	0.655
PreH.Section.Baseline.Outturn	4	600.5	150.1	1.08	0.366
Cultivar.Baseline.PostH.Outturn	2	1063.9	532	3.84	0.023
PreH.Baseline.PostH.Outturn	2	750	375	2.71	0.07
Section.Baseline.PostH.Outturn	4	1302.7	325.7	2.35	0.056
Cultivar.PreH.Section.Baseline.PostH	2	69.7	34.9	0.25	0.778
Cultivar.PreH.Section.Baseline.Outturn	4	658.8	164.7	1.19	0.318
Cultivar.PreH.Baseline.PostH.Outturn	2	386.7	193.4	1.4	0.251
Cultivar.Section.Baseline.PostH.Outturn	4	642.6	160.6	1.16	0.331
PreH.Section.Baseline.PostH.Outturn	4	244	61	0.44	0.779
Cultivar.PreH.Section.Baseline.PostH.Outturn	4	135.8	34	0.25	0.912
Residual	168	23275.8	138.5		

Table K-2. ANOVA of DP5 fructans (mg g^{-1} DW) for 'Red Baron' and 'Sherpa' during 2015/16 storage (n = 9 $cultivar^{-1}$ pre-/ $postharvest\ treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	1	225.75	225.75	4.67	0.032
PreH	1	294.87	294.87	6.1	0.015
Section	2	283.75	141.88	2.94	0.056
Baseline	1	63015.31	63015.31	1304.03	<.001
Cultivar.PreH	1	51.38	51.38	1.06	0.304
Cultivar.Section	2	936.83	468.42	9.69	<.001
PreH.Section	2	76.54	38.27	0.79	0.455
Cultivar.Baseline	1	654.71	654.71	13.55	<.001
PreH.Baseline	1	230.61	230.61	4.77	0.03
Section.Baseline	2	17788.08	8894.04	184.05	<.001
Baseline.PostH	1	379.18	379.18	7.85	0.006
Baseline.Outturn	2	34339.13	17169.56	355.3	<.001
Cultivar.PreH.Section	2	5.41	2.7	0.06	0.946
Cultivar.PreH.Baseline	1	31.43	31.43	0.65	0.421
Cultivar.Section.Baseline	2	25.01	12.5	0.26	0.772
PreH.Section.Baseline	2	46.54	23.27	0.48	0.619
Cultivar.Baseline.PostH	1	54.9	54.9	1.14	0.288
PreH.Baseline.PostH	1	5.38	5.38	0.11	0.739
Section.Baseline.PostH	2	27.13	13.56	0.28	0.756
Cultivar.Baseline.Outturn	2	138.06	69.03	1.43	0.243
PreH.Baseline.Outturn	2	33.93	16.96	0.35	0.704
Section.Baseline.Outturn	4	202.58	50.65	1.05	0.384
Baseline.PostH.Outturn	2	53.08	26.54	0.55	0.578
Cultivar.PreH.Section.Baseline	2	0.26	0.13	0	0.997
Cultivar.PreH.Baseline.PostH	1	556.76	556.76	11.52	<.001
Cultivar.Section.Baseline.PostH	2	2.21	1.1	0.02	0.977
PreH.Section.Baseline.PostH	2	69.49	34.74	0.72	0.489
Cultivar.PreH.Baseline.Outturn	2	62.13	31.07	0.64	0.527
Cultivar.Section.Baseline.Outturn	4	68.08	17.02	0.35	0.842
PreH.Section.Baseline.Outturn	4	18.58	4.65	0.1	0.984
Cultivar.Baseline.PostH.Outturn	2	266.9	133.45	2.76	0.066
PreH.Baseline.PostH.Outturn	2	233.15	116.57	2.41	0.093
Section.Baseline.PostH.Outturn	4	10.99	2.75	0.06	0.994
Cultivar.PreH.Section.Baseline.PostH	2	39.3	19.65	0.41	0.667
Cultivar.PreH.Section.Baseline.Outturn	4	60.67	15.17	0.31	0.868
Cultivar.PreH.Baseline.PostH.Outturn	2	1647.01	823.51	17.04	<.001
Cultivar.Section.Baseline.PostH.Outturn	4	21.07	5.27	0.11	0.979
PreH.Section.Baseline.PostH.Outturn	4	26.18	6.55	0.14	0.969
Cultivar.PreH.Section.Baseline.PostH.Outturn	4	162.95	40.74	0.84	0.5
Residual	168	8118.38	48.32		
Total	251	130263.69			

Table K-3. ANOVA of DP6 fructans (mg g^{-1} DW) for 'Red Baron' and 'Sherpa' during 2015/16 storage (n = 9 $cultivar^{-1}$ pre-/ $postharvest\ treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	1	38.28	38.28	0.89	0.347
PreH	1	81.77	81.77	1.9	0.17
Section	2	4950.12	2475.06	57.41	<.001
Baseline	1	25899.64	25899.64	600.79	<.001
Cultivar.PreH	1	125.43	125.43	2.91	0.09
Cultivar.Section	2	77.22	38.61	0.9	0.41
PreH.Section	2	26.87	13.44	0.31	0.733
Cultivar.Baseline	1	177.2	177.2	4.11	0.044
PreH.Baseline	1	74.68	74.68	1.73	0.19
Section.Baseline	2	12249.91	6124.95	142.08	<.001
Baseline.PostH	1	1068.01	1068.01	24.77	<.001
Baseline.Outturn	2	4396.11	2198.05	50.99	<.001
Cultivar.PreH.Section	2	99.29	49.64	1.15	0.319
Cultivar.PreH.Baseline	1	1.34	1.34	0.03	0.86
Cultivar.Section.Baseline	2	43.09	21.54	0.5	0.608
PreH.Section.Baseline	2	122.4	61.2	1.42	0.245
Cultivar.Baseline.PostH	1	80.43	80.43	1.87	0.174
PreH.Baseline.PostH	1	49.18	49.18	1.14	0.287
Section.Baseline.PostH	2	409.38	204.69	4.75	0.01
Cultivar.Baseline.Outturn	2	0.17	0.08	0	0.998
PreH.Baseline.Outturn	2	17.04	8.52	0.2	0.821
Section.Baseline.Outturn	4	4068	1017	23.59	<.001
Baseline.PostH.Outturn	2	501.96	250.98	5.82	0.004
Cultivar.PreH.Section.Baseline	2	12.22	6.11	0.14	0.868
Cultivar.PreH.Baseline.PostH	1	27.33	27.33	0.63	0.427
Cultivar.Section.Baseline.PostH	2	48.58	24.29	0.56	0.57
PreH.Section.Baseline.PostH	2	161.35	80.68	1.87	0.157
Cultivar.PreH.Baseline.Outturn	2	26.62	13.31	0.31	0.735
Cultivar.Section.Baseline.Outturn	4	130.23	32.56	0.76	0.556
PreH.Section.Baseline.Outturn	4	136.2	34.05	0.79	0.533
Cultivar.Baseline.PostH.Outturn	2	147.49	73.75	1.71	0.184
PreH.Baseline.PostH.Outturn	2	122.96	61.48	1.43	0.243
Section.Baseline.PostH.Outturn	4	154.13	38.53	0.89	0.469
Cultivar.PreH.Section.Baseline.PostH	2	155.24	77.62	1.8	0.168
Cultivar.PreH.Section.Baseline.Outturn	4	180.53	45.13	1.05	0.385
Cultivar.PreH.Baseline.PostH.Outturn	2	179.45	89.72	2.08	0.128
Cultivar.Section.Baseline.PostH.Outturn	4	49.95	12.49	0.29	0.884
PreH.Section.Baseline.PostH.Outturn	4	185.84	46.46	1.08	0.369
Cultivar.PreH.Section.Baseline.PostH.Outturn	4	320.81	80.2	1.86	0.12
Residual	168	7242.37	43.11		
Total	251	63838.82			

Table K-4. ANOVA of DP7 fructans (mg g^{-1} DW) for 'Red Baron' and 'Sherpa' during 2015/16 storage (n = 9 $cultivar^{-1}$ pre-/ $postharvest\ treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	1	559.73	559.73	18.04	<.001
PreH	1	17.04	17.04	0.55	0.46
Section	2	3029.31	1514.65	48.83	<.001
Baseline	1	3337.68	3337.68	107.6	<.001
Cultivar.PreH	1	182.67	182.67	5.89	0.016
Cultivar.Section	2	92.18	46.09	1.49	0.229
PreH.Section	2	1.23	0.62	0.02	0.98
Cultivar.Baseline	1	1229.84	1229.84	39.65	<.001
PreH.Baseline	1	88.95	88.95	2.87	0.092
Section.Baseline	2	4017.05	2008.52	64.75	<.001
Baseline.PostH	1	15.46	15.46	0.5	0.481
Baseline.Outturn	2	963.56	481.78	15.53	<.001
Cultivar.PreH.Section	2	232.64	116.32	3.75	0.026
Cultivar.PreH.Baseline	1	2.67	2.67	0.09	0.77
Cultivar.Section.Baseline	2	157	78.5	2.53	0.083
PreH.Section.Baseline	2	1.89	0.94	0.03	0.97
Cultivar.Baseline.PostH	1	65.87	65.87	2.12	0.147
PreH.Baseline.PostH	1	54.67	54.67	1.76	0.186
Section.Baseline.PostH	2	48.25	24.13	0.78	0.461
Cultivar.Baseline.Outturn	2	1.52	0.76	0.02	0.976
PreH.Baseline.Outturn	2	1.06	0.53	0.02	0.983
Section.Baseline.Outturn	4	1878.58	469.64	15.14	<.001
Baseline.PostH.Outturn	2	2.63	1.32	0.04	0.958
Cultivar.PreH.Section.Baseline	2	12.13	6.07	0.2	0.823
Cultivar.PreH.Baseline.PostH	1	30.96	30.96	1	0.319
Cultivar.Section.Baseline.PostH	2	165.48	82.74	2.67	0.072
PreH.Section.Baseline.PostH	2	140.25	70.12	2.26	0.107
Cultivar.PreH.Baseline.Outturn	2	168.55	84.27	2.72	0.069
Cultivar.Section.Baseline.Outturn	4	0.05	0.01	0	1
PreH.Section.Baseline.Outturn	4	4.06	1.02	0.03	0.998
Cultivar.Baseline.PostH.Outturn	2	168.78	84.39	2.72	0.069
PreH.Baseline.PostH.Outturn	2	102.76	51.38	1.66	0.194
Section.Baseline.PostH.Outturn	4	17.81	4.45	0.14	0.966
Cultivar.PreH.Section.Baseline.PostH	2	85.65	42.83	1.38	0.254
Cultivar.PreH.Section.Baseline.Outturn	4	375.79	93.95	3.03	0.019
Cultivar.PreH.Baseline.PostH.Outturn	2	130.08	65.04	2.1	0.126
Cultivar.Section.Baseline.PostH.Outturn	4	300.66	75.17	2.42	0.05
PreH.Section.Baseline.PostH.Outturn	4	173.71	43.43	1.4	0.236
Cultivar.PreH.Section.Baseline.PostH.Outturn	4	234.36	58.59	1.89	0.115
Residual	168	5211.15	31.02		
Total	251	23303.75			

Table K-5. ANOVA of DP8 fructans (mg g^{-1} DW) for 'Red Baron' and 'Sherpa' during 2015/16 storage (n = 9 $cultivar^{-1}$ pre-/ $postharvest\ treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	1	12.217	12.217	11.79	<.001
PreH	1	0.119	0.119	0.11	0.735
Section	2	148.583	74.291	71.69	<.001
Baseline	1	12.382	12.382	11.95	<.001
Cultivar.PreH	1	0.426	0.426	0.41	0.522
Cultivar.Section	2	24.434	12.217	11.79	<.001
PreH.Section	2	0.238	0.119	0.11	0.892
Cultivar.Baseline	1	2.036	2.036	1.96	0.163
PreH.Baseline	1	0.02	0.02	0.02	0.89
Section.Baseline	2	24.764	12.382	11.95	<.001
Baseline.PostH	1	5.25	5.25	5.07	0.026
Baseline.Outturn	2	43.738	21.869	21.1	<.001
Cultivar.PreH.Section	2	0.853	0.426	0.41	0.663
Cultivar.PreH.Baseline	1	0.071	0.071	0.07	0.794
Cultivar.Section.Baseline	2	4.072	2.036	1.96	0.143
PreH.Section.Baseline	2	0.04	0.02	0.02	0.981
Cultivar.Baseline.PostH	1	0.17	0.17	0.16	0.686
PreH.Baseline.PostH	1	0.02	0.02	0.02	0.89
Section.Baseline.PostH	2	10.501	5.25	5.07	0.007
Cultivar.Baseline.Outturn	2	11.005	5.502	5.31	0.006
PreH.Baseline.Outturn	2	0.259	0.13	0.13	0.883
Section.Baseline.Outturn	4	87.477	21.869	21.1	<.001
Baseline.PostH.Outturn	2	3.593	1.796	1.73	0.18
Cultivar.PreH.Section.Baseline	2	0.142	0.071	0.07	0.934
Cultivar.PreH.Baseline.PostH	1	0.039	0.039	0.04	0.846
Cultivar.Section.Baseline.PostH	2	0.339	0.17	0.16	0.849
PreH.Section.Baseline.PostH	2	0.04	0.02	0.02	0.981
Cultivar.PreH.Baseline.Outturn	2	0.302	0.151	0.15	0.865
Cultivar.Section.Baseline.Outturn	4	22.01	5.502	5.31	<.001
PreH.Section.Baseline.Outturn	4	0.518	0.13	0.13	0.973
Cultivar.Baseline.PostH.Outturn	2	0.114	0.057	0.05	0.947
PreH.Baseline.PostH.Outturn	2	0.476	0.238	0.23	0.795
Section.Baseline.PostH.Outturn	4	7.186	1.796	1.73	0.145
Cultivar.PreH.Section.Baseline.PostH	2	0.078	0.039	0.04	0.963
Cultivar.PreH.Section.Baseline.Outturn	4	0.603	0.151	0.15	0.965
Cultivar.PreH.Baseline.PostH.Outturn	2	0.137	0.069	0.07	0.936
Cultivar.Section.Baseline.PostH.Outturn	4	0.228	0.057	0.05	0.994
PreH.Section.Baseline.PostH.Outturn	4	0.953	0.238	0.23	0.921
Cultivar.PreH.Section.Baseline.PostH.Outturn	4	0.274	0.069	0.07	0.992
Residual	168	174.086	1.036		
Total	251	599.791			

Table K-6. ANOVA of the pre-harvest fructose content (mg g^{-1} DW) for 'Sherpa' 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	15.5	15.5	0.14	0.715
Section	2	28609.5	14304.7	124.46	<.001
Baseline	1	1109.5	1109.5	9.65	0.003
PreH.Section	2	334.1	167	1.45	0.244
PreH.Baseline	1	237.7	237.7	2.07	0.157
Section.Baseline	2	2204.5	1102.2	9.59	<.001
Baseline.Outturn	2	1464.3	732.2	6.37	0.004
PreH.Section.Baseline	2	37.4	18.7	0.16	0.85
PreH.Baseline.Outturn	2	2115.9	1057.9	9.2	<.001
Section.Baseline.Outturn	4	912.7	228.2	1.99	0.112
PreH.Section.Baseline.Outturn	4	1105.6	276.4	2.4	0.062
Residual	48	5516.8	114.9		
Total	71	43663.4			

Table K-7. ANOVA of the pre-harvest glucose content (mg g^{-1} DW) for 'Sherpa' 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	427	427	1.88	0.177
Section	2	226359	113179.5	497.34	<.001
Baseline	1	4536.9	4536.9	19.94	<.001
PreH.Section	2	67.4	33.7	0.15	0.863
PreH.Baseline	1	97.4	97.4	0.43	0.516
Section.Baseline	2	785.6	392.8	1.73	0.189
Baseline.Outturn	2	4252.3	2126.2	9.34	<.001
PreH.Section.Baseline	2	7.8	3.9	0.02	0.983
PreH.Baseline.Outturn	2	1640.3	820.1	3.6	0.035
Section.Baseline.Outturn	4	930.4	232.6	1.02	0.405
PreH.Section.Baseline.Outturn	4	359.9	90	0.4	0.811
Residual	48	10923.4	227.6		
Total	71	250387.5			

Table K-8. ANOVA of the pre-harvest sucrose content (mg g^{-1} DW) for 'Sherpa' 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	528.6	528.6	3.26	0.077
Section	2	20054.3	10027.1	61.87	<.001
Baseline	1	5012.1	5012.1	30.92	<.001
PreH.Section	2	105	52.5	0.32	0.725
PreH.Baseline	1	61.3	61.3	0.38	0.542
Section.Baseline	2	2713	1356.5	8.37	<.001
Baseline.Outturn	2	1353.1	676.6	4.17	0.021
PreH.Section.Baseline	2	92	46	0.28	0.754
PreH.Baseline.Outturn	2	536.3	268.1	1.65	0.202
Section.Baseline.Outturn	4	1720.1	430	2.65	0.044
PreH.Section.Baseline.Outturn	4	158.3	39.6	0.24	0.912
Residual	48	7779.9	162.1		
Total	71	40113.8			

Table K-9. ANOVA of the pre-harvest kestose content (mg g^{-1} DW) for 'Sherpa' 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	0.4	0.4	0	0.955
Section	2	72507.3	36253.7	316.58	<.001
Baseline	1	2142	2142	18.7	<.001
PreH.Section	2	42.2	21.1	0.18	0.832
PreH.Baseline	1	44.8	44.8	0.39	0.534
Section.Baseline	2	370.7	185.4	1.62	0.209
Baseline.Outturn	2	36.1	18.1	0.16	0.855
PreH.Section.Baseline	2	1	0.5	0	0.995
PreH.Baseline.Outturn	2	406.1	203	1.77	0.181
Section.Baseline.Outturn	4	2072.1	518	4.52	0.004
PreH.Section.Baseline.Outturn	4	213	53.3	0.47	0.761
Residual	48	5496.9	114.5		
Total	71	83332.7			

Table K-10. ANOVA of the pre-harvest nystose content (mg g^{-1} DW) for 'Sherpa' 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	169.46	169.46	2.68	0.108
Section	2	27124.13	13562.06	214.75	<.001
Baseline	1	13.11	13.11	0.21	0.651
PreH.Section	2	108.49	54.25	0.86	0.43
PreH.Baseline	1	76.25	76.25	1.21	0.277
Section.Baseline	2	111.7	55.85	0.88	0.42
Baseline.Outturn	2	535.85	267.92	4.24	0.02
PreH.Section.Baseline	2	111.69	55.85	0.88	0.42
PreH.Baseline.Outturn	2	318.92	159.46	2.53	0.091
Section.Baseline.Outturn	4	657.54	164.39	2.6	0.047
PreH.Section.Baseline.Outturn	4	185.89	46.47	0.74	0.572
Residual	48	3031.34	63.15		
Total	71	32444.39			

Table K-11. ANOVA of the pre-harvest DP5 fructans content (mg g^{-1} DW) for 'Sherpa' 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	110.19	110.19	3.64	0.062
Section	2	8481.13	4240.57	140	<.001
Baseline	1	257.59	257.59	8.5	0.005
PreH.Section	2	29.23	14.62	0.48	0.62
PreH.Baseline	1	0.02	0.02	0	0.981
Section.Baseline	2	82.04	41.02	1.35	0.268
Baseline.Outturn	2	346.28	173.14	5.72	0.006
PreH.Section.Baseline	2	30.17	15.09	0.5	0.611
PreH.Baseline.Outturn	2	121.31	60.65	2	0.146
Section.Baseline.Outturn	4	601.57	150.39	4.97	0.002
PreH.Section.Baseline.Outturn	4	35.74	8.93	0.29	0.88
Residual	48	1453.92	30.29		
Total	71	11549.2			

Table K-12. ANOVA of the pre-harvest DP6 fructans content (mg g^{-1} DW) for 'Sherpa' (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	43.67	43.67	2.16	0.148
Section	2	2885.26	1442.63	71.35	<.001
Baseline	1	11.34	11.34	0.56	0.458
PreH.Section	2	18.75	9.37	0.46	0.632
PreH.Baseline	1	1.18	1.18	0.06	0.81
Section.Baseline	2	63.81	31.91	1.58	0.217
Baseline.Outturn	2	17.54	8.77	0.43	0.651
PreH.Section.Baseline	2	41.69	20.85	1.03	0.364
PreH.Baseline.Outturn	2	39.52	19.76	0.98	0.384
Section.Baseline.Outturn	4	261.84	65.46	3.24	0.02
PreH.Section.Baseline.Outturn	4	26.65	6.66	0.33	0.857
Residual	48	970.46	20.22		
Total	71	4381.71			

Table K-13. ANOVA of the pre-harvest DP7 fructans content (mg g^{-1} DW) for 'Sherpa' 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	59.67	59.67	2.55	0.117
Section	2	1210.11	605.06	25.84	<.001
Baseline	1	5.61	5.61	0.24	0.627
PreH.Section	2	14.3	7.15	0.31	0.738
PreH.Baseline	1	2.73	2.73	0.12	0.734
Section.Baseline	2	32.22	16.11	0.69	0.507
Baseline.Outturn	2	243.76	121.88	5.21	0.009
PreH.Section.Baseline	2	38.24	19.12	0.82	0.448
PreH.Baseline.Outturn	2	28.46	14.23	0.61	0.549
Section.Baseline.Outturn	4	44.66	11.17	0.48	0.752
PreH.Section.Baseline.Outturn	4	30.64	7.66	0.33	0.858
Residual	48	1123.75	23.41		
Total	71	2834.15			

Table K-14. ANOVA of the pre-harvest DP8 fructans content (mg g^{-1} DW) 'Sherpa' 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	1.5	1.5	0.1	0.755
Section	2	199.99	100	6.59	0.003
Baseline	1	2.5	2.5	0.16	0.687
PreH.Section	2	14.8	7.4	0.49	0.617
PreH.Baseline	1	0.03	0.03	0	0.965
Section.Baseline	2	18.33	9.17	0.6	0.55
Baseline.Outturn	2	32.58	16.29	1.07	0.35
PreH.Section.Baseline	2	4.74	2.37	0.16	0.856
PreH.Baseline.Outturn	2	13.29	6.65	0.44	0.648
Section.Baseline.Outturn	4	81.04	20.26	1.34	0.27
PreH.Section.Baseline.Outturn	4	67.02	16.75	1.1	0.365
Residual	48	727.91	15.16		
Total	71	1163.72			

Table K-15. ANOVA of the pre-harvest total sugar content (mg g^{-1} DW) for 'Sherpa' 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	39.3	39.3	0.07	0.798
Section	2	597393.7	298696.8	503.47	<.001
Baseline	1	1350.5	1350.5	2.28	0.138
PreH.Section	2	837.8	418.9	0.71	0.499
PreH.Baseline	1	304.8	304.8	0.51	0.477
Section.Baseline	2	14689.7	7344.9	12.38	<.001
Baseline.Outturn	2	3720.5	1860.2	3.14	0.052
PreH.Section.Baseline	2	110.7	55.3	0.09	0.911
PreH.Baseline.Outturn	2	4838.5	2419.3	4.08	0.023
Section.Baseline.Outturn	4	6914.8	1728.7	2.91	0.031
PreH.Section.Baseline.Outturn	4	1652.3	413.1	0.7	0.598
Residual	48	28477.3	593.3		
Total	71	660329.8			

Table K-16. ANOVA of the pre-harvest total fructans content (mg g^{-1} DW) for 'Sherpa' 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	1574.8	1574.8	2.43	0.126
Section	2	389783.1	194891.5	300.2	<.001
Baseline	1	739.4	739.4	1.14	0.291
PreH.Section	2	732.8	366.4	0.56	0.572
PreH.Baseline	1	219.8	219.8	0.34	0.563
Section.Baseline	2	1064.6	532.3	0.82	0.447
Baseline.Outturn	2	1004.8	502.4	0.77	0.467
PreH.Section.Baseline	2	759.6	379.8	0.59	0.561
PreH.Baseline.Outturn	2	3008.6	1504.3	2.32	0.109
Section.Baseline.Outturn	4	13211	3302.7	5.09	0.002
PreH.Section.Baseline.Outturn	4	1298	324.5	0.5	0.736
Residual	48	31162	649.2		
Total	71	444558.4			

Table K-17. ANOVA of the pre-harvest total non-structural carbohydrates content (mg g^{-1} DW) in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks 2016/17 (*n* = 9 pre-harvest $treatment^{-1}$).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	1116.7	1116.7	1.15	0.289
Section	2	1905494.3	952747.1	982.69	<.001
Baseline	1	4088.4	4088.4	4.22	0.045
PreH.Section	2	35.2	17.6	0.02	0.982
PreH.Baseline	1	6.9	6.9	0.01	0.933
Section.Baseline	2	10336.2	5168.1	5.33	0.008
Baseline.Outturn	2	1066.5	533.2	0.55	0.581
PreH.Section.Baseline	2	794.9	397.4	0.41	0.666
PreH.Baseline.Outturn	2	1328.8	664.4	0.69	0.509
Section.Baseline.Outturn	4	12218.9	3054.7	3.15	0.022
PreH.Section.Baseline.Outturn	4	2472.8	618.2	0.64	0.638
Residual	48	46537.2	969.5		
Total	71	1985496.8			

Table K-18. ANOVA of the fructose content (mg g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 cultivar^{-1} pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	160.7	160.7	0.78	0.379
Section	2	718150.8	359075.4	1736.11	<.001
Baseline	1	11854.1	11854.1	57.31	<.001
PreH.Section	2	329.4	164.7	0.8	0.452
PreH.Baseline	1	1156.9	1156.9	5.59	0.019
Section.Baseline	2	4115.2	2057.6	9.95	<.001
Baseline.1MCP	1	12291.9	12291.9	59.43	<.001
Baseline.Ethylene	1	41.6	41.6	0.2	0.654
Baseline.Outturn	5	43818.6	8763.7	42.37	<.001
PreH.Section.Baseline	2	455.7	227.9	1.1	0.334
PreH.Baseline.1MCP	1	74.7	74.7	0.36	0.548
Section.Baseline.1MCP	2	4181.5	2090.8	10.11	<.001
PreH.Baseline.Ethylene	1	0.6	0.6	0	0.957
Section.Baseline.Ethylene	2	24436.5	12218.2	59.07	<.001
Baseline.1MCP.Ethylene	1	17.4	17.4	0.08	0.772
PreH.Baseline.Outturn	5	447.7	89.5	0.43	0.825
Section.Baseline.Outturn	10	32612.5	3261.3	15.77	<.001
Baseline.1MCP.Outturn	5	2357.3	471.5	2.28	0.047
Baseline.Ethylene.Outturn	5	580.6	116.1	0.56	0.73
PreH.Section.Baseline.1MCP	2	88.6	44.3	0.21	0.807
PreH.Section.Baseline.Ethylene	2	84.9	42.5	0.21	0.815
PreH.Baseline.1MCP.Ethylene	1	205.4	205.4	0.99	0.32
Section.Baseline.1MCP.Ethylene	2	2671.5	1335.8	6.46	0.002
PreH.Section.Baseline.Outturn	10	402.1	40.2	0.19	0.997
PreH.Baseline.1MCP.Outturn	5	1376	275.2	1.33	0.251
Section.Baseline.1MCP.Outturn	10	816.7	81.7	0.39	0.948
PreH.Baseline.Ethylene.Outturn	5	161.2	32.2	0.16	0.978
Section.Baseline.Ethylene.Outturn	10	17036.6	1703.7	8.24	<.001
Baseline.1MCP.Ethylene.Outturn	5	391.4	78.3	0.38	0.863
PreH.Section.Baseline.1MCP.Ethylene	2	459.3	229.6	1.11	0.331
PreH.Section.Baseline.1MCP.Outturn	10	904.3	90.4	0.44	0.928
PreH.Section.Baseline.Ethylene.Outturn	10	3260.3	326	1.58	0.113
PreH.Baseline.1MCP.Ethylene.Outturn	5	498.8	99.8	0.48	0.789
Section.Baseline.1MCP.Ethylene.Outturn	10	2454.5	245.5	1.19	0.299
PreH.Section.Baseline.1MCP.Ethylene.Outturn	10	1891.8	189.2	0.91	0.52
Residual	300	62048.3	206.8		
Total	449	951835.5			

Table K-19. ANOVA of the glucose content (mg g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	129.4	129.4	0.45	0.503
Section	2	1152806.5	576403.3	2000.36	<.001
Baseline	1	30601.7	30601.7	106.2	<.001
PreH.Section	2	146.3	73.2	0.25	0.776
PreH.Baseline	1	1905.8	1905.8	6.61	0.011
Section.Baseline	2	3005.4	1502.7	5.22	0.006
Baseline.1MCP	1	1672.1	1672.1	5.8	0.017
Baseline.Ethylene	1	5.1	5.1	0.02	0.894
Baseline.Outturn	5	132892.2	26578.4	92.24	<.001
PreH.Section.Baseline	2	192.4	96.2	0.33	0.716
PreH.Baseline.1MCP	1	290	290	1.01	0.317
Section.Baseline.1MCP	2	861.8	430.9	1.5	0.226
PreH.Baseline.Ethylene	1	6.9	6.9	0.02	0.877
Section.Baseline.Ethylene	2	5472.2	2736.1	9.5	<.001
Baseline.1MCP.Ethylene	1	7.1	7.1	0.02	0.876
PreH.Baseline.Outturn	5	2120.6	424.1	1.47	0.199
Section.Baseline.Outturn	10	29408.4	2940.8	10.21	<.001
Baseline.1MCP.Outturn	5	1292.4	258.5	0.9	0.483
Baseline.Ethylene.Outturn	5	787.3	157.5	0.55	0.741
PreH.Section.Baseline.1MCP	2	71	35.5	0.12	0.884
PreH.Section.Baseline.Ethylene	2	322.6	161.3	0.56	0.572
PreH.Baseline.1MCP.Ethylene	1	183	183	0.64	0.426
Section.Baseline.1MCP.Ethylene	2	21.7	10.9	0.04	0.963
PreH.Section.Baseline.Outturn	10	1340.7	134.1	0.47	0.912
PreH.Baseline.1MCP.Outturn	5	906.3	181.3	0.63	0.678
Section.Baseline.1MCP.Outturn	10	1088.3	108.8	0.38	0.956
PreH.Baseline.Ethylene.Outturn	5	264.4	52.9	0.18	0.969
Section.Baseline.Ethylene.Outturn	10	4885.8	488.6	1.7	0.081
Baseline.1MCP.Ethylene.Outturn	5	578.6	115.7	0.4	0.848
PreH.Section.Baseline.1MCP.Ethylene	2	2555.3	1277.6	4.43	0.013
PreH.Section.Baseline.1MCP.Outturn	10	635.2	63.5	0.22	0.994
PreH.Section.Baseline.Ethylene.Outturn	10	3743.1	374.3	1.3	0.23
PreH.Baseline.1MCP.Ethylene.Outturn	5	375.5	75.1	0.26	0.934
Section.Baseline.1MCP.Ethylene.Outturn	10	4252.3	425.2	1.48	0.147
PreH.Section.Baseline.1MCP.Ethylene.Outturn	10	1434.4	143.4	0.5	0.891
Residual	300	86445.1	288.2		
Total	449	1472706.6			

Table K-20. ANOVA of the sucrose content (mg g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	259.6	259.6	1.26	0.262
Section	2	93840	46920	228.59	<.001
Baseline	1	3360.3	3360.3	16.37	<.001
PreH.Section	2	421.7	210.9	1.03	0.359
PreH.Baseline	1	472.2	472.2	2.3	0.13
Section.Baseline	2	371.1	185.5	0.9	0.406
Baseline.1MCP	1	1530.9	1530.9	7.46	0.007
Baseline.Ethylene	1	243.7	243.7	1.19	0.277
Baseline.Outturn	5	192126.3	38425.3	187.2	<.001
PreH.Section.Baseline	2	27.2	13.6	0.07	0.936
PreH.Baseline.1MCP	1	60.2	60.2	0.29	0.588
Section.Baseline.1MCP	2	464.7	232.4	1.13	0.324
PreH.Baseline.Ethylene	1	0.2	0.2	0	0.975
Section.Baseline.Ethylene	2	7406.9	3703.4	18.04	<.001
Baseline.1MCP.Ethylene	1	66.6	66.6	0.32	0.569
PreH.Baseline.Outturn	5	1530.2	306	1.49	0.193
Section.Baseline.Outturn	10	5251	525.1	2.56	0.006
Baseline.1MCP.Outturn	5	7175.4	1435.1	6.99	<.001
Baseline.Ethylene.Outturn	5	941.8	188.4	0.92	0.47
PreH.Section.Baseline.1MCP	2	211.8	105.9	0.52	0.597
PreH.Section.Baseline.Ethylene	2	772.4	386.2	1.88	0.154
PreH.Baseline.1MCP.Ethylene	1	1.8	1.8	0.01	0.926
Section.Baseline.1MCP.Ethylene	2	674.5	337.3	1.64	0.195
PreH.Section.Baseline.Outturn	10	848.6	84.9	0.41	0.94
PreH.Baseline.1MCP.Outturn	5	351.7	70.3	0.34	0.887
Section.Baseline.1MCP.Outturn	10	2024.7	202.5	0.99	0.455
PreH.Baseline.Ethylene.Outturn	5	115.5	23.1	0.11	0.99
Section.Baseline.Ethylene.Outturn	10	23229.5	2323	11.32	<.001
Baseline.1MCP.Ethylene.Outturn	5	1112.1	222.4	1.08	0.369
PreH.Section.Baseline.1MCP.Ethylene	2	3.8	1.9	0.01	0.991
PreH.Section.Baseline.1MCP.Outturn	10	615.5	61.5	0.3	0.981
PreH.Section.Baseline.Ethylene.Outturn	10	1574.2	157.4	0.77	0.661
PreH.Baseline.1MCP.Ethylene.Outturn	5	666.7	133.3	0.65	0.662
Section.Baseline.1MCP.Ethylene.Outturn	10	6481.3	648.1	3.16	<.001
PreH.Section.Baseline.1MCP.Ethylene.Outturn	10	2407.5	240.7	1.17	0.309
Residual	300	61578.7	205.3		
Total	449	418220.3			

Table K-21. ANOVA of the kestose content (mg g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated with or without 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	4.16	4.16	0.15	0.702
Section	2	80205.4	40102.7	1409.13	<.001
Baseline	1	22743.97	22743.97	799.18	<.001
PreH.Section	2	36.17	18.09	0.64	0.53
PreH.Baseline	1	188.3	188.3	6.62	0.011
Section.Baseline	2	2539.61	1269.8	44.62	<.001
Baseline.1MCP	1	0.05	0.05	0	0.968
Baseline.Ethylene	1	68.39	68.39	2.4	0.122
Baseline.Outturn	5	267708.82	53541.76	1881.35	<.001
PreH.Section.Baseline	2	51.5	25.75	0.9	0.406
PreH.Baseline.1MCP	1	321.84	321.84	11.31	<.001
Section.Baseline.1MCP	2	212.75	106.38	3.74	0.025
PreH.Baseline.Ethylene	1	22.9	22.9	0.8	0.37
Section.Baseline.Ethylene	2	61.8	30.9	1.09	0.339
Baseline.1MCP.Ethylene	1	59.25	59.25	2.08	0.15
PreH.Baseline.Outturn	5	184.92	36.98	1.3	0.264
Section.Baseline.Outturn	10	15864.51	1586.45	55.74	<.001
Baseline.1MCP.Outturn	5	1385.6	277.12	9.74	<.001
Baseline.Ethylene.Outturn	5	135.9	27.18	0.96	0.446
PreH.Section.Baseline.1MCP	2	41.05	20.53	0.72	0.487
PreH.Section.Baseline.Ethylene	2	118	59	2.07	0.128
PreH.Baseline.1MCP.Ethylene	1	1.26	1.26	0.04	0.834
Section.Baseline.1MCP.Ethylene	2	35.02	17.51	0.62	0.541
PreH.Section.Baseline.Outturn	10	80.32	8.03	0.28	0.985
PreH.Baseline.1MCP.Outturn	5	231.45	46.29	1.63	0.153
Section.Baseline.1MCP.Outturn	10	951.29	95.13	3.34	<.001
PreH.Baseline.Ethylene.Outturn	5	65	13	0.46	0.808
Section.Baseline.Ethylene.Outturn	10	3235.35	323.53	11.37	<.001
Baseline.1MCP.Ethylene.Outturn	5	158.96	31.79	1.12	0.351
PreH.Section.Baseline.1MCP.Ethylene	2	110.21	55.11	1.94	0.146
PreH.Section.Baseline.1MCP.Outturn	10	179.29	17.93	0.63	0.788
PreH.Section.Baseline.Ethylene.Outturn	10	861.44	86.14	3.03	0.001
PreH.Baseline.1MCP.Ethylene.Outturn	5	139.55	27.91	0.98	0.43
Section.Baseline.1MCP.Ethylene.Outturn	10	1283.86	128.39	4.51	<.001
PreH.Section.Baseline.1MCP.Ethylene.Outturn	10	454.62	45.46	1.6	0.106
Residual	300	8537.75	28.46		
Total	449	408280.27			

Table K-22. ANOVA of the nystose content (mg g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	91.88	91.88	1.37	0.242
Section	2	4302.34	2151.17	32.19	<.001
Baseline	1	22020.56	22020.56	329.52	<.001
PreH.Section	2	309.29	154.64	2.31	0.101
PreH.Baseline	1	0.61	0.61	0.01	0.924
Section.Baseline	2	4843.36	2421.68	36.24	<.001
Baseline.1MCP	1	88.22	88.22	1.32	0.251
Baseline.Ethylene	1	547.41	547.41	8.19	0.005
Baseline.Outturn	5	145966.95	29193.39	436.85	<.001
PreH.Section.Baseline	2	36.15	18.08	0.27	0.763
PreH.Baseline.1MCP	1	67.37	67.37	1.01	0.316
Section.Baseline.1MCP	2	358.43	179.22	2.68	0.07
PreH.Baseline.Ethylene	1	8.74	8.74	0.13	0.718
Section.Baseline.Ethylene	2	9048.11	4524.06	67.7	<.001
Baseline.1MCP.Ethylene	1	13.56	13.56	0.2	0.653
PreH.Baseline.Outturn	5	781.42	156.28	2.34	0.042
Section.Baseline.Outturn	10	5276.67	527.67	7.9	<.001
Baseline.1MCP.Outturn	5	3203.11	640.62	9.59	<.001
Baseline.Ethylene.Outturn	5	1237.49	247.5	3.7	0.003
PreH.Section.Baseline.1MCP	2	2.38	1.19	0.02	0.982
PreH.Section.Baseline.Ethylene	2	102.23	51.12	0.76	0.466
PreH.Baseline.1MCP.Ethylene	1	13.6	13.6	0.2	0.652
Section.Baseline.1MCP.Ethylene	2	208.17	104.09	1.56	0.212
PreH.Section.Baseline.Outturn	10	229.54	22.95	0.34	0.968
PreH.Baseline.1MCP.Outturn	5	318.25	63.65	0.95	0.447
Section.Baseline.1MCP.Outturn	10	466.9	46.69	0.7	0.726
PreH.Baseline.Ethylene.Outturn	5	68.67	13.73	0.21	0.96
Section.Baseline.Ethylene.Outturn	10	18071.9	1807.19	27.04	<.001
Baseline.1MCP.Ethylene.Outturn	5	303.6	60.72	0.91	0.476
PreH.Section.Baseline.1MCP.Ethylene	2	195.27	97.64	1.46	0.234
PreH.Section.Baseline.1MCP.Outturn	10	699.09	69.91	1.05	0.405
PreH.Section.Baseline.Ethylene.Outturn	10	466.97	46.7	0.7	0.726
PreH.Baseline.1MCP.Ethylene.Outturn	5	36.31	7.26	0.11	0.99
Section.Baseline.1MCP.Ethylene.Outturn	10	4437.54	443.75	6.64	<.001
PreH.Section.Baseline.1MCP.Ethylene.Outturn	10	546.41	54.64	0.82	0.612
Residual	300	20048.1	66.83		
Total	449	244416.59			

Table K-23. ANOVA of the DP5 fructans content (mg g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	5.89	5.89	0.23	0.633
Section	2	6076.89	3038.45	118.07	<.001
Baseline	1	5283.38	5283.38	205.3	<.001
PreH.Section	2	121.06	60.53	2.35	0.097
PreH.Baseline	1	0.07	0.07	0	0.958
Section.Baseline	2	1465.68	732.84	28.48	<.001
Baseline.1MCP	1	627.31	627.31	24.38	<.001
Baseline.Ethylene	1	51.08	51.08	1.98	0.16
Baseline.Outturn	5	48817.2	9763.44	379.38	<.001
PreH.Section.Baseline	2	16	8	0.31	0.733
PreH.Baseline.1MCP	1	122.7	122.7	4.77	0.03
Section.Baseline.1MCP	2	445.69	222.84	8.66	<.001
PreH.Baseline.Ethylene	1	0.13	0.13	0	0.944
Section.Baseline.Ethylene	2	129.66	64.83	2.52	0.082
Baseline.1MCP.Ethylene	1	9.49	9.49	0.37	0.544
PreH.Baseline.Outturn	5	30.53	6.11	0.24	0.946
Section.Baseline.Outturn	10	1925.14	192.51	7.48	<.001
Baseline.1MCP.Outturn	5	532.64	106.53	4.14	0.001
Baseline.Ethylene.Outturn	5	362.89	72.58	2.82	0.017
PreH.Section.Baseline.1MCP	2	33.07	16.53	0.64	0.527
PreH.Section.Baseline.Ethylene	2	45.64	22.82	0.89	0.413
PreH.Baseline.1MCP.Ethylene	1	50.21	50.21	1.95	0.164
Section.Baseline.1MCP.Ethylene	2	90.14	45.07	1.75	0.175
PreH.Section.Baseline.Outturn	10	241.24	24.12	0.94	0.499
PreH.Baseline.1MCP.Outturn	5	428.23	85.65	3.33	0.006
Section.Baseline.1MCP.Outturn	10	230.87	23.09	0.9	0.536
PreH.Baseline.Ethylene.Outturn	5	21.5	4.3	0.17	0.975
Section.Baseline.Ethylene.Outturn	10	1863.82	186.38	7.24	<.001
Baseline.1MCP.Ethylene.Outturn	5	31.16	6.23	0.24	0.944
PreH.Section.Baseline.1MCP.Ethylene	2	71.14	35.57	1.38	0.253
PreH.Section.Baseline.1MCP.Outturn	10	212.83	21.28	0.83	0.603
PreH.Section.Baseline.Ethylene.Outturn	10	338.65	33.86	1.32	0.221
PreH.Baseline.1MCP.Ethylene.Outturn	5	257.4	51.48	2	0.078
Section.Baseline.1MCP.Ethylene.Outturn	10	232.43	23.24	0.9	0.531
PreH.Section.Baseline.1MCP.Ethylene.Outturn	10	559.42	55.94	2.17	0.019
Residual	300	7720.54	25.74		
Total	449	78451.71			

Table K-24. ANOVA of the DP6 fructans content (mg g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	0.03	0.03	0	0.973
Section	2	11033.46	5516.73	225.79	<.001
Baseline	1	2605.98	2605.98	106.66	<.001
PreH.Section	2	18.45	9.22	0.38	0.686
PreH.Baseline	1	2.05	2.05	0.08	0.772
Section.Baseline	2	1096.64	548.32	22.44	<.001
Baseline.1MCP	1	972.68	972.68	39.81	<.001
Baseline.Ethylene	1	13.26	13.26	0.54	0.462
Baseline.Outturn	5	18682.05	3736.41	152.93	<.001
PreH.Section.Baseline	2	7.3	3.65	0.15	0.861
PreH.Baseline.1MCP	1	48.11	48.11	1.97	0.162
Section.Baseline.1MCP	2	427.38	213.69	8.75	<.001
PreH.Baseline.Ethylene	1	18.38	18.38	0.75	0.386
Section.Baseline.Ethylene	2	60.62	30.31	1.24	0.291
Baseline.1MCP.Ethylene	1	8.44	8.44	0.35	0.557
PreH.Baseline.Outturn	5	26.86	5.37	0.22	0.954
Section.Baseline.Outturn	10	3096.86	309.69	12.68	<.001
Baseline.1MCP.Outturn	5	1733.67	346.73	14.19	<.001
Baseline.Ethylene.Outturn	5	304.96	60.99	2.5	0.031
PreH.Section.Baseline.1MCP	2	94.35	47.18	1.93	0.147
PreH.Section.Baseline.Ethylene	2	0.02	0.01	0	1
PreH.Baseline.1MCP.Ethylene	1	18.86	18.86	0.77	0.38
Section.Baseline.1MCP.Ethylene	2	303.64	151.82	6.21	0.002
PreH.Section.Baseline.Outturn	10	70.44	7.04	0.29	0.984
PreH.Baseline.1MCP.Outturn	5	130.07	26.01	1.06	0.38
Section.Baseline.1MCP.Outturn	10	569.38	56.94	2.33	0.012
PreH.Baseline.Ethylene.Outturn	5	6.13	1.23	0.05	0.998
Section.Baseline.Ethylene.Outturn	10	1696.52	169.65	6.94	<.001
Baseline.1MCP.Ethylene.Outturn	5	38.26	7.65	0.31	0.905
PreH.Section.Baseline.1MCP.Ethylene	2	13.86	6.93	0.28	0.753
PreH.Section.Baseline.1MCP.Outturn	10	115.42	11.54	0.47	0.907
PreH.Section.Baseline.Ethylene.Outturn	10	104.47	10.45	0.43	0.933
PreH.Baseline.1MCP.Ethylene.Outturn	5	65.78	13.16	0.54	0.747
Section.Baseline.1MCP.Ethylene.Outturn	10	349.18	34.92	1.43	0.166
PreH.Section.Baseline.1MCP.Ethylene.Outturn	10	50.95	5.09	0.21	0.995
Residual	300	7329.77	24.43		
Total	449	51114.26			

Table K-25. ANOVA of the DP7 fructans content (mg g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	2.13	2.13	0.14	0.71
Section	2	16282.87	8141.44	532.14	<.001
Baseline	1	1245.48	1245.48	81.41	<.001
PreH.Section	2	50.84	25.42	1.66	0.192
PreH.Baseline	1	30.1	30.1	1.97	0.162
Section.Baseline	2	1455.39	727.7	47.56	<.001
Baseline.1MCP	1	243.42	243.42	15.91	<.001
Baseline.Ethylene	1	21.65	21.65	1.41	0.235
Baseline.Outturn	5	4446.96	889.39	58.13	<.001
PreH.Section.Baseline	2	36.03	18.01	1.18	0.309
PreH.Baseline.1MCP	1	5.7	5.7	0.37	0.542
Section.Baseline.1MCP	2	37.85	18.93	1.24	0.292
PreH.Baseline.Ethylene	1	1.18	1.18	0.08	0.782
Section.Baseline.Ethylene	2	29.93	14.97	0.98	0.377
Baseline.1MCP.Ethylene	1	0.3	0.3	0.02	0.889
PreH.Baseline.Outturn	5	14.22	2.84	0.19	0.968
Section.Baseline.Outturn	10	4538.63	453.86	29.67	<.001
Baseline.1MCP.Outturn	5	375.49	75.1	4.91	<.001
Baseline.Ethylene.Outturn	5	36.93	7.39	0.48	0.789
PreH.Section.Baseline.1MCP	2	26.26	13.13	0.86	0.425
PreH.Section.Baseline.Ethylene	2	18.56	9.28	0.61	0.546
PreH.Baseline.1MCP.Ethylene	1	16.68	16.68	1.09	0.297
Section.Baseline.1MCP.Ethylene	2	21.63	10.82	0.71	0.494
PreH.Section.Baseline.Outturn	10	168.35	16.84	1.1	0.361
PreH.Baseline.1MCP.Outturn	5	19.69	3.94	0.26	0.936
Section.Baseline.1MCP.Outturn	10	264	26.4	1.73	0.074
PreH.Baseline.Ethylene.Outturn	5	15.41	3.08	0.2	0.962
Section.Baseline.Ethylene.Outturn	10	413.21	41.32	2.7	0.003
Baseline.1MCP.Ethylene.Outturn	5	29.95	5.99	0.39	0.855
PreH.Section.Baseline.1MCP.Ethylene	2	22.34	11.17	0.73	0.483
PreH.Section.Baseline.1MCP.Outturn	10	54.56	5.46	0.36	0.964
PreH.Section.Baseline.Ethylene.Outturn	10	208.88	20.89	1.37	0.196
PreH.Baseline.1MCP.Ethylene.Outturn	5	129.19	25.84	1.69	0.137
Section.Baseline.1MCP.Ethylene.Outturn	10	78.86	7.89	0.52	0.879
PreH.Section.Baseline.1MCP.Ethylene.Outturn	10	149.83	14.98	0.98	0.461
Residual	300	4589.83	15.3		
Total	449	35082.34			

Table K-26. ANOVA of the DP8 fructans content (mg g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	11.46	11.46	0.77	0.381
Section	2	9050.96	4525.48	303.56	<.001
Baseline	1	1.22	1.22	0.08	0.775
PreH.Section	2	14.75	7.37	0.49	0.61
PreH.Baseline	1	3.64	3.64	0.24	0.621
Section.Baseline	2	668.56	334.28	22.42	<.001
Baseline.1MCP	1	22.5	22.5	1.51	0.22
Baseline.Ethylene	1	5.85	5.85	0.39	0.531
Baseline.Outturn	5	1378.86	275.77	18.5	<.001
PreH.Section.Baseline	2	75.08	37.54	2.52	0.082
PreH.Baseline.1MCP	1	0	0	0	0.997
Section.Baseline.1MCP	2	14.38	7.19	0.48	0.618
PreH.Baseline.Ethylene	1	0.1	0.1	0.01	0.934
Section.Baseline.Ethylene	2	3.63	1.82	0.12	0.885
Baseline.1MCP.Ethylene	1	36.32	36.32	2.44	0.12
PreH.Baseline.Outturn	5	43.9	8.78	0.59	0.708
Section.Baseline.Outturn	10	3610.75	361.07	24.22	<.001
Baseline.1MCP.Outturn	5	23.19	4.64	0.31	0.906
Baseline.Ethylene.Outturn	5	40.95	8.19	0.55	0.739
PreH.Section.Baseline.1MCP	2	0.2	0.1	0.01	0.993
PreH.Section.Baseline.Ethylene	2	1.26	0.63	0.04	0.959
PreH.Baseline.1MCP.Ethylene	1	0.18	0.18	0.01	0.913
Section.Baseline.1MCP.Ethylene	2	73.1	36.55	2.45	0.088
PreH.Section.Baseline.Outturn	10	36.17	3.62	0.24	0.992
PreH.Baseline.1MCP.Outturn	5	7.65	1.53	0.1	0.992
Section.Baseline.1MCP.Outturn	10	74.18	7.42	0.5	0.891
PreH.Baseline.Ethylene.Outturn	5	15.24	3.05	0.2	0.96
Section.Baseline.Ethylene.Outturn	10	95.7	9.57	0.64	0.777
Baseline.1MCP.Ethylene.Outturn	5	58.45	11.69	0.78	0.562
PreH.Section.Baseline.1MCP.Ethylene	2	25.04	12.52	0.84	0.433
PreH.Section.Baseline.1MCP.Outturn	10	14.31	1.43	0.1	1
PreH.Section.Baseline.Ethylene.Outturn	10	30.05	3.01	0.2	0.996
PreH.Baseline.1MCP.Ethylene.Outturn	5	32.11	6.42	0.43	0.827
Section.Baseline.1MCP.Ethylene.Outturn	10	117.16	11.72	0.79	0.642
PreH.Section.Baseline.1MCP.Ethylene.Outturn	10	124.33	12.43	0.83	0.596
Residual	300	4472.41	14.91		
Total	449	20183.66			

Table K-27. ANOVA of the total sugar content (mg g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	1613	1613	1.42	0.235
Section	2	4903742	2451871	2152.64	<.001
Baseline	1	15382	15382	13.5	<.001
PreH.Section	2	2063	1031	0.91	0.405
PreH.Baseline	1	3129	3129	2.75	0.098
Section.Baseline	2	66	33	0.03	0.971
Baseline.1MCP	1	36438	36438	31.99	<.001
Baseline.Ethylene	1	392	392	0.34	0.558
Baseline.Outturn	5	644976	128995	113.25	<.001
PreH.Section.Baseline	2	789	394	0.35	0.708
PreH.Baseline.1MCP	1	321	321	0.28	0.596
Section.Baseline.1MCP	2	13324	6662	5.85	0.003
PreH.Baseline.Ethylene	1	5	5	0	0.946
Section.Baseline.Ethylene	2	98397	49199	43.19	<.001
Baseline.1MCP.Ethylene	1	225	225	0.2	0.657
PreH.Baseline.Outturn	5	3256	651	0.57	0.722
Section.Baseline.Outturn	10	124922	12492	10.97	<.001
Baseline.1MCP.Outturn	5	16472	3294	2.89	0.014
Baseline.Ethylene.Outturn	5	3693	739	0.65	0.663
PreH.Section.Baseline.1MCP	2	663	331	0.29	0.748
PreH.Section.Baseline.Ethylene	2	1520	760	0.67	0.514
PreH.Baseline.1MCP.Ethylene	1	704	704	0.62	0.432
Section.Baseline.1MCP.Ethylene	2	5178	2589	2.27	0.105
PreH.Section.Baseline.Outturn	10	4378	438	0.38	0.953
PreH.Baseline.1MCP.Outturn	5	5228	1046	0.92	0.47
Section.Baseline.1MCP.Outturn	10	8220	822	0.72	0.704
PreH.Baseline.Ethylene.Outturn	5	1236	247	0.22	0.955
Section.Baseline.Ethylene.Outturn	10	84382	8438	7.41	<.001
Baseline.1MCP.Ethylene.Outturn	5	5029	1006	0.88	0.493
PreH.Section.Baseline.1MCP.Ethylene	2	4889	2444	2.15	0.119
PreH.Section.Baseline.1MCP.Outturn	10	3733	373	0.33	0.973
PreH.Section.Baseline.Ethylene.Outturn	10	14919	1492	1.31	0.224
PreH.Baseline.1MCP.Ethylene.Outturn	5	3708	742	0.65	0.661
Section.Baseline.1MCP.Ethylene.Outturn	10	19536	1954	1.72	0.077
PreH.Section.Baseline.1MCP.Ethylene.Outturn	10	11058	1106	0.97	0.469
Residual	300	341702	1139		
Total	449	6385287			

Table K-28. ANOVA of the total fructans content (mg g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	269.1	269.1	0.82	0.365
Section	2	44624.5	22312.2	68.36	<.001
Baseline	1	207511.2	207511.2	635.81	<.001
PreH.Section	2	2127.3	1063.7	3.26	0.04
PreH.Baseline	1	14.4	14.4	0.04	0.834
Section.Baseline	2	63969.3	31984.6	98	<.001
Baseline.1MCP	1	8505.8	8505.8	26.06	<.001
Baseline.Ethylene	1	2326.2	2326.2	7.13	0.008
Baseline.Outturn	5	1716591.4	343318.3	1051.93	<.001
PreH.Section.Baseline	2	351.9	176	0.54	0.584
PreH.Baseline.1MCP	1	77.3	77.3	0.24	0.627
Section.Baseline.1MCP	2	6504.6	3252.3	9.97	<.001
PreH.Baseline.Ethylene	1	227.8	227.8	0.7	0.404
Section.Baseline.Ethylene	2	11739.5	5869.7	17.98	<.001
Baseline.1MCP.Ethylene	1	277.7	277.7	0.85	0.357
PreH.Baseline.Outturn	5	1048.1	209.6	0.64	0.668
Section.Baseline.Outturn	10	104548.8	10454.9	32.03	<.001
Baseline.1MCP.Outturn	5	3108.1	621.6	1.9	0.093
Baseline.Ethylene.Outturn	5	1591.8	318.4	0.98	0.433
PreH.Section.Baseline.1MCP	2	327.3	163.7	0.5	0.606
PreH.Section.Baseline.Ethylene	2	79.9	40	0.12	0.885
PreH.Baseline.1MCP.Ethylene	1	364.5	364.5	1.12	0.291
Section.Baseline.1MCP.Ethylene	2	559.4	279.7	0.86	0.425
PreH.Section.Baseline.Outturn	10	1194.7	119.5	0.37	0.96
PreH.Baseline.1MCP.Outturn	5	1323.3	264.7	0.81	0.543
Section.Baseline.1MCP.Outturn	10	2571.6	257.2	0.79	0.64
PreH.Baseline.Ethylene.Outturn	5	240.1	48	0.15	0.981
Section.Baseline.Ethylene.Outturn	10	7596.8	759.7	2.33	0.012
Baseline.1MCP.Ethylene.Outturn	5	447.6	89.5	0.27	0.927
PreH.Section.Baseline.1MCP.Ethylene	2	1224.9	612.4	1.88	0.155
PreH.Section.Baseline.1MCP.Outturn	10	3402.5	340.3	1.04	0.407
PreH.Section.Baseline.Ethylene.Outturn	10	3466.4	346.6	1.06	0.392
PreH.Baseline.1MCP.Ethylene.Outturn	5	1124.8	225	0.69	0.632
Section.Baseline.1MCP.Ethylene.Outturn	10	2018.7	201.9	0.62	0.798
PreH.Section.Baseline.1MCP.Ethylene.Outturn	10	2334	233.4	0.72	0.71
Residual	300	97911.3	326.4		
Total	449	2301602.5			

Table K-29. ANOVA of the total non-structural carbohydrates content (mg g^{-1} DW) during storage in different sections (top, bottom and baseplate) on onion 'Sherpa' bulbs grown under full or deficit irrigation, harvested at full maturity, cured under glass for six weeks, treated or untreated with 1-methylcyclopropene (at 1 µL L^{-1}) 24 h and stored at 1 °C under ethylene (10 µL L^{-1}) or air 2016/17 (*n* = 9 pre-/ postharvest treatment^{-1}).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
PreH	1	3171	3171	2.61	0.107
Section	2	4158251	2079125	1711.63	<.001
Baseline	1	337735	337735	278.04	<.001
PreH.Section	2	4	2	0	0.998
PreH.Baseline	1	2713	2713	2.23	0.136
Section.Baseline	2	68532	34266	28.21	<.001
Baseline.1MCP	1	11052	11052	9.1	0.003
Baseline.Ethylene	1	883	883	0.73	0.394
Baseline.Outturn	5	3434813	686963	565.54	<.001
PreH.Section.Baseline	2	1864	932	0.77	0.465
PreH.Baseline.1MCP	1	54	54	0.04	0.833
Section.Baseline.1MCP	2	1747	874	0.72	0.488
PreH.Baseline.Ethylene	1	237	237	0.19	0.659
Section.Baseline.Ethylene	2	41753	20877	17.19	<.001
Baseline.1MCP.Ethylene	1	3	3	0	0.964
PreH.Baseline.Outturn	5	1511	302	0.25	0.94
Section.Baseline.Outturn	10	277487	27749	22.84	<.001
Baseline.1MCP.Outturn	5	11125	2225	1.83	0.106
Baseline.Ethylene.Outturn	5	5293	1059	0.87	0.501
PreH.Section.Baseline.1MCP	2	1538	769	0.63	0.532
PreH.Section.Baseline.Ethylene	2	1852	926	0.76	0.467
PreH.Baseline.1MCP.Ethylene	1	2156	2156	1.78	0.184
Section.Baseline.1MCP.Ethylene	2	2688	1344	1.11	0.332
PreH.Section.Baseline.Outturn	10	5032	503	0.41	0.939
PreH.Baseline.1MCP.Outturn	5	5877	1175	0.97	0.438
Section.Baseline.1MCP.Outturn	10	13760	1376	1.13	0.337
PreH.Baseline.Ethylene.Outturn	5	2110	422	0.35	0.884
Section.Baseline.Ethylene.Outturn	10	79766	7977	6.57	<.001
Baseline.1MCP.Ethylene.Outturn	5	6531	1306	1.08	0.374
PreH.Section.Baseline.1MCP.Ethylene	2	1684	842	0.69	0.501
PreH.Section.Baseline.1MCP.Outturn	10	6240	624	0.51	0.88
PreH.Section.Baseline.Ethylene.Outturn	10	23851	2385	1.96	0.037
PreH.Baseline.1MCP.Ethylene.Outturn	5	5926	1185	0.98	0.433
Section.Baseline.1MCP.Ethylene.Outturn	10	15647	1565	1.29	0.236
PreH.Section.Baseline.1MCP.Ethylene.Outturn	10	18353	1835	1.51	0.134
Residual	300	364412	1215		
Total	449	8915652			

Appendix L

Results from all the chapters have been published or presented as follows:

Chapter Two

- Oral presentation - Ohanenye, I. C., Alamar, M. C., Thompson, A. J., Terry, L. A. Physiological regulation of onion dormancy as influenced by pre-harvest irrigation and postharvest ethylene supplementation. SCI Young Researchers in Agri-Food 2016: Food Quality and Sustainability from Plough to Plate, 12th May 2016, University of Reading, UK.

- Poster presentation - Ohanenye, I. C., Alamar, M. C., Thompson, A. J., Terry, L. A. Physiological Regulation of Onion Dormancy as Influenced by Pre-harvest Irrigation and Postharvest Ethylene Supplementation. International Symposium on Sensing Plant Water Status - Methods and Applications in Horticultural Science, October 5th to 7th, 2016, Potsdam, Berlin, Germany.

- Flash presentation - Ohanenye, I. C., Alamar, M. C., Thompson, A. J., Terry, L. A. Physiological and biochemical regulation of onion dormancy as influenced by pre-harvest irrigation and postharvest storage regimes. Horticulture and Potato Initiative (HaPI) Event, March 8th and 9th 2017, Manchester, UK.

- Oral Presentation - Ohanenye, I. C., Alamar, M. C., Thompson, A. J., Terry, L. A. Effect of deficit irrigation and continuous ethylene supplementation on the quality of cold stored onion bulbs. The 11th International FRUTIC Symposium, February 6th to 9th, 2018 Berlin, Germany.

- Flash presentation - Ohanenye, I. C., Alamar, M. C., Thompson, A. J., Terry, L. A. Understanding the Molecular and Metabolic Regulation of Onion Dormancy as Influenced by Pre-harvest Irrigation and Post-harvest Storage Regimes. BBSRC Horticulture and Potato Initiative (HaPI) Workshop March 13th to 14th 2018, Nottingham, UK.

Chapter Three

- e-Posters with short oral presentation – Ohanenye, I. C., Alamar, M. C., Thompson, A. J., Terry, L. A. Physiological and metabolic regulation of onion dormancy as influenced by preharvest irrigation regime during post-harvest storage. VIII International Postharvest Symposium, June 21st to 24th, 2016 Cartagena, Spain.

- Poster presentation - Ohanenye, I. C., Alamar, M. C., Thompson, A. J., Terry, L. A. Biochemical regulation of onion dormancy as influenced by pre-harvest deficit irrigation and postharvest storage regimes. 2016 AHDB Crops PhD Studentship Conference, November 16th to 17th 2016, Warwickshire, UK.

Chapter Four

- Ohanenye, I. C., Alamar, M. C., Thompson, A. J., Terry, L. A., 2019. Fructans redistribution prior to sprouting in stored onion bulbs is a potential marker for dormancy break. Postharvest Biology and Technology, 149, 221-234.

- Oral presentation - Ohanenye, I. C., Alamar, M. C., Thompson, A. J., Terry, L. A. Understanding the Molecular and Metabolic Regulation of Onion Dormancy as Influenced by Pre-harvest Irrigation and Post-harvest Storage Regimes. HaPI meeting, March 14th, 2017, Ardrie, Scotland, UK.

Physiological Regulation of Onion Dormancy as Influenced by Pre-harvest Irrigation and Post-harvest Regimes

Background

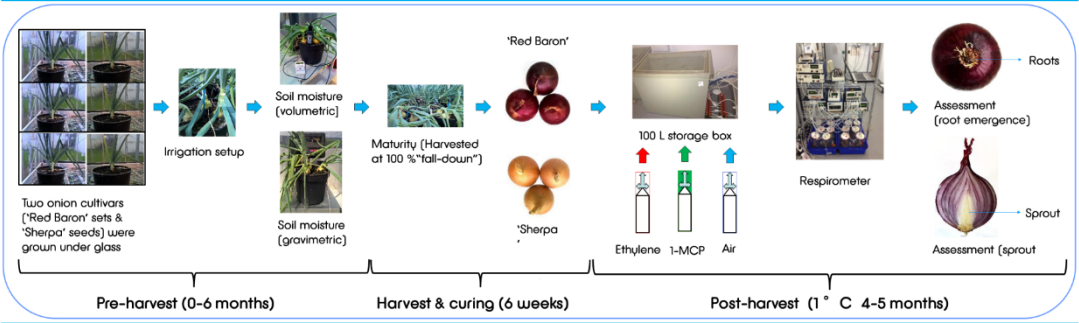
The UK onion industry loses up to 10 % of the 786.7 kilo tonnes produced annually, due to diseases and sprouting.[1,2] Currently, maleic hydrazide is the industrial standard sprout suppressant, a chemical whose continued future use is uncertain.[3] Moreover, pre-harvest practices (e.g. irrigation)[4] and post-harvest regimes (e.g. continuous ethylene supplementation) were revealed to influence onion bulb dormancy and sprouting.[5] However, previous studies often treated pre- and post-harvest factors separately. This research investigated the influence of deficit irrigation and post-harvest regimes on the storage qualities of onion bulbs.

Aim and Objectives

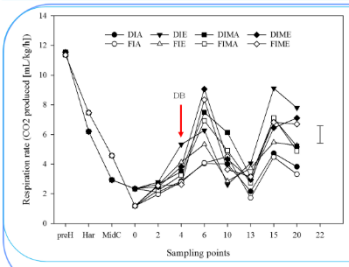
Aim: To investigate how pre-harvest irrigation influences the physiological changes occurring in onion bulb during curing and storage.
Objectives: To determine the combined effect of differential irrigation, continuous ethylene supplementation and 1-MCP treatment on

- the respiration rates of onion bulbs during storage
- dormancy-break and sprout growth in stored onion bulbs.

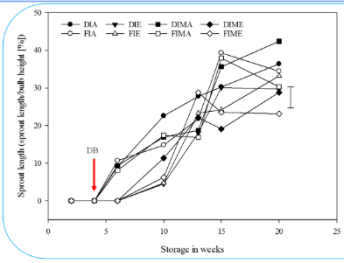
Plant Material & Experimental Design



Results



Respiration rate (RR) increased 2-fold at dormancy-break, and average RR during storage was significantly higher in bulbs stored under ethylene when compared to bulbs stored in air. However, there was no significant difference in RR between DI and FI.



Ethylene suppressed sprout length by a quarter and extended dormancy-break by four weeks when compared to bulbs stored in air ($p < 0.001$). In addition, 1-MCP and ethylene (combined treatment) suppressed sprout length by 1.3 and 1.4-fold compared to individual treatments with 1-MCP and ethylene.

Key: RB = 'Red Baron', SH = 'Sherpa', DI = deficit irrigated, FI = fully irrigated, A = air, E = ethylene, and M = 1-MCP, DB = dormancy-break, preH = pre-harvest, Har = harvest, MidC = mid-curing

Conclusions

- ✓ Deficit irrigation (DI), as an independent factor, did not affect the respiration rate of stored onion bulbs, irrespective of cultivar.
- ✓ Ethylene extended dormancy-break and suppressed sprout growth, irrespective of pre-harvest treatment or cultivar.
- ✓ 1-MCP and ethylene, synergistically suppressed sprout growth in stored onion bulbs.

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2. Terry, L. A., Mena, C., Williams, A., Jenney, N. and Whitehead, P. (2011) Fruit and vegetable resource maps. WRAP (RSC008).
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Biochemical and physiological regulation of onion dormancy as influenced by pre-harvest irrigation and post-harvest regimes

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Background

The UK, produces 388 kilo tonnes (kT) of onion annually and imports 405 kT (1). However, 3-10 % of these are lost to disease and sprouting (2). Current industrial approaches to sprout suppression involves the use of maleic hydrazide (MH); a chemical whose continued future use is uncertain (3). Nonetheless, post-harvest studies on onion have revealed that continuous ethylene supplementation suppressed sprout growth (4). In addition, phytohormones (e.g. abscisic acid (ABA)), and pre-harvest practices (e.g. irrigation) were also shown to influence dormancy-break and sprouting (5, 6). However, previous studies often treated pre-harvest and post-harvest factors separately. This research is investigating the influence of pre-harvest irrigation on post-harvest storage.

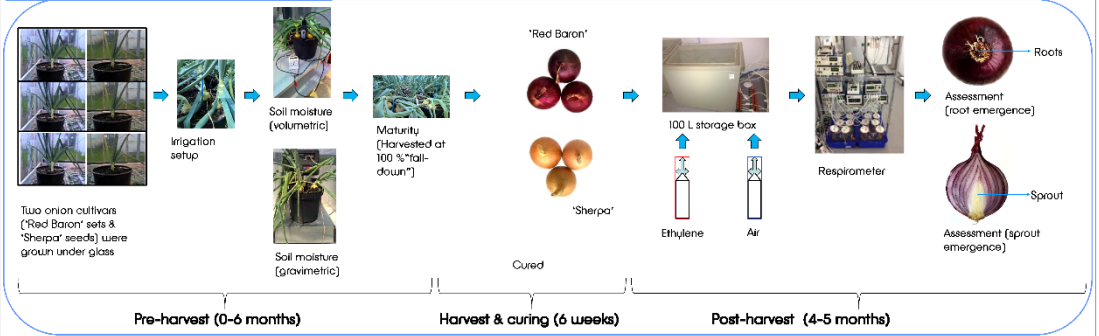
Aim and Objectives

Aim: To investigate how irrigation and post-harvest storage regimes influence the metabolic and molecular changes occurring in onion during storage.

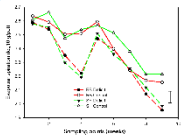
Objectives: To determine

- the combined effect of differential irrigation and post-harvest treatment on the regulation of onion dormancy during storage
- the molecular response to pre-harvest and post-harvest storage treatments in relation to dormancy-break.

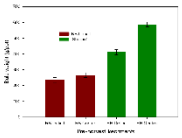
Plant Material & Experimental Design



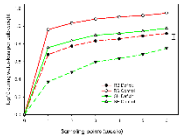
Results



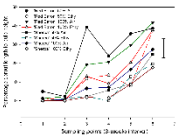
Evapotranspiration (ET) was ca. 14 % and 34 % higher in the fully irrigated 'Red Baron' and 'Sherpa, respectively ($p < 0.05$)



Bulb weight was 14% and 43 % higher in the fully irrigated 'Red Baron' and 'Sherpa', respectively ($p < 0.01$)



Water-loss during curing was two fold higher in the fully irrigated crops for both cultivars ($p < 0.01$)



Ethylene suppressed sprout elongation by 26 % and 21 % when compared to air treatment in 'Red Baron' and 'Sherpa', respectively ($p < 0.001$)

Cultivar	Pre-harvest irrigation	IUUE fresh weight ($Kg.ha^{-1}.mm^{-1}$)	IUUE dry weight ($Kg.ha^{-1}.mm^{-1}$)
'Red Baron'	Full irrigation	189	158
	Deficit irrigation	262	236
Difference in IUUE between treatments		79	88
'Sherpa'	Full irrigation	401	362
	Deficit irrigation	456	423
Difference in IUUE between treatments		55	61

Irrigation water-use efficiency (IUUE) was 17 % and 54 % higher in deficit irrigated 'Sherpa' and 'Red Baron' when compared to fully irrigated.

Conclusions

- ✓ Deficit irrigation (DI) decreased evapotranspiration, bulb weight and water-loss during curing.
- ✓ Ethylene supplementation reduced sprout elongation.
- ✓ Further biochemical and molecular analysis will be concluded to further understand the effect of DI on onion quality during storage.

Next steps

- ✓ Investigate, quantify and analyse the impact of DI and continuous ethylene supplementation on the concentration, spatial distribution and metabolism of phytohormones and non-structural carbohydrates linked to dormancy-break during onion bulb storage.
- ✓ Investigate the influence of DI and continuous ethylene supplementation on differential gene expression and its interaction with post-harvest continuous ethylene supplementation.

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Physiological and Metabolic Regulation of Onion Dormancy as Influenced by Pre-harvest Irrigation and Post-harvest Regimes

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Introduction

Onion is cultivated as a seasonal crop, but the demand for onion bulbs is all-year-round. To ensure continuous supply, the onion industry relies on storing onion bulbs for up to 9 months. As a result, major losses due to sprouting are recorded during storage. Post-harvest continuous ethylene supplementation is known to suppress sprout growth. Pre-harvest irrigation is also known to influence sprouting. However, the interaction between pre-harvest irrigation and continuous ethylene supplementation on sprouting is not known.

Objectives

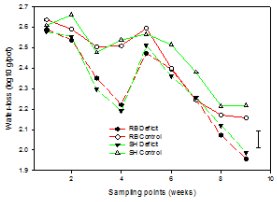
To determine the combined effect of differential irrigation and post-harvest continuous ethylene supplementation on the regulation of onion dormancy and sprouting during storage.

Materials and Methods

Two onion cultivars 'Sherpa' seeds and 'Red Baron' sets were grown under glass. Growth media was John Innes Compost (No 3). Plants were subjected to two irrigation treatments at bulb initiation stage until harvest: replenishing of evapotranspiration (ET) at 100 or 50% for the control or test plants, respectively. Bulbs were harvested at 100% 'fall-down', weighed and cured under glass for six weeks. Bulbs were stored at 1°C and subjected to two postharvest treatments: air and continuous ethylene supplementation at 10 µL/L. Bulbs were assessed for internal sprout emergence and sprout length; measured as ratio of sprout length to bulb height.

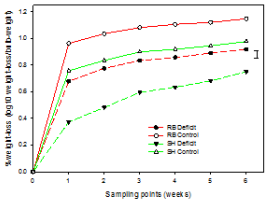
Results

Evapotranspiration (ET)



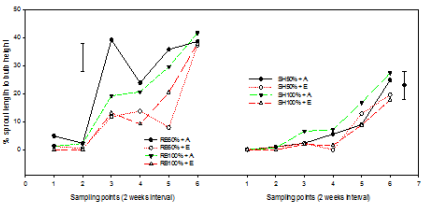
ET was significantly higher in the control by one-third when compared to the test plants in both cultivars.

Bulb curing water-loss



Bulb water-loss during curing was two-fold higher in the fully irrigated crops for 'Red Baron' and 'Sherpa'.

Sprout length in ratio to bulb height



Ethylene suppressed sprout elongation by one-quarter when compared to air.

RB = 'Red Baron'; SH = 'Sherpa'; E = ethylene; A = Air; 100% and 50% = replenished ET.

Conclusions

1. Water-loss during curing was influenced by the pre-harvest irrigation regimes; the fully irrigated bulbs lost twice amount of water when compared to the deficit irrigated bulbs.
2. Continuous ethylene supplementation significantly suppressed sprout elongation when compared to onion bulbs grown under full irrigation and stored under air.
3. Finally, there is a potential to use continuous ethylene supplementation in combination with pre-harvest irrigation to manipulate onion dormancy.

Physiological Regulation of Onion Dormancy as Influenced by Pre-harvest Irrigation and Post-harvest Regimes

Background

In 2015, the UK produced 388 kilo tonnes of onion and imported 405 kT [1]. However, 3-10 % of these are annually lost to disease and sprouting [2]. Current industrial approaches to sprout suppression involves the use of maleic hydrazide; a chemical with an uncertain continued future [3]. Nonetheless, pre-harvest practices [e.g. irrigation] may also influence dormancy-break and sprouting [4]. Previous studies often treat pre-harvest and post-harvest factors in isolation.

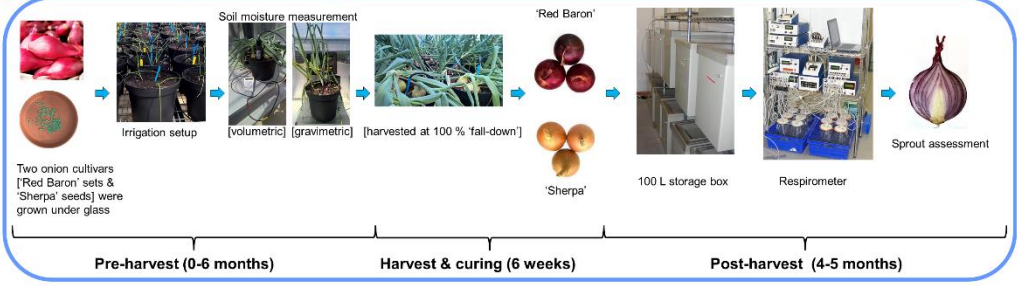
Aim and Objectives

Aim: To investigate how pre-harvest irrigation influences physiological changes during onion storage.

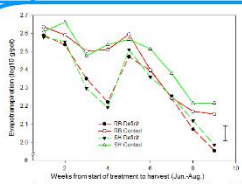
Objectives: To determine

- the combined effect of differential irrigation and post-harvest weight-loss during onion storage
- sprout response to pre-harvest irrigation and post-harvest storage in relation to onion dormancy-break.

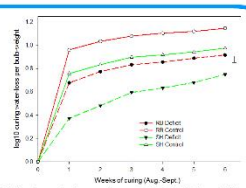
Plant Material & Experimental Design



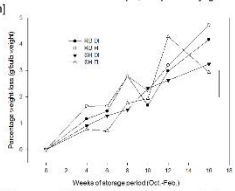
Results



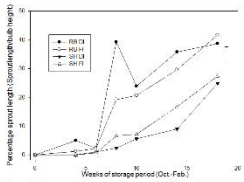
Evapotranspiration was ca. 14 % and 34 % higher in the fully irrigated 'Red Baron' and 'Sherpa, respectively [$p<0.05$; LSD shown]



Water-loss during curing was 2-fold higher in the fully irrigated crops for both cultivars [$p<0.01$; LSD shown]



Weight-loss was 1.8-fold higher in 'Red Baron' when compared to 'Sherpa' [$p<0.001$; LSD shown]



At week 8, sprout growth was 4-fold and 2-fold higher in 'Red Baron' DI and FI when compared to 'Sherpa' DI and FI, respectively [$p<0.01$; LSD shown]

RB = 'Red Baron'; SH = 'Sherpa'; DB = Dormancy-break; FI = Full irrigation; DI = Deficit irrigation

Conclusions

- ✓ Deficit irrigation reduced evapotranspiration, bulb weight and water-loss during curing.
- ✓ Water-loss was continuous during onion storage, however, water-loss was higher in 'Red Baron.
- ✓ Deficit irrigation caused an increase in sprout elongation during post-harvest storage.
- ✓ Biochemical and molecular analyses will be required to further understand the influence of water-loss on sprouting.

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Fructans redistribution prior to sprouting in stored onion bulbs is a potential marker for dormancy break

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ABSTRACT

Continuous supply of high quality onion bulbs to meet year-round demand is dependent on maintaining dormancy and bulb quality during storage. Sprouting impacts negatively on the storage quality of onion bulbs. Ethylene supplementation has previously been revealed to inhibit sprout growth in stored onion bulbs. Fructans content, especially those at higher degree of polymerisation (DP), are reported to positively correlate with delayed sprouting. However, little is known about the impact of pre-harvest irrigation regimes on fructans accumulation and redistribution in relation to onion bulb dormancy and quality in store. Across two seasons, onion plants of cultivars 'Red Baron' and 'Sherpa' were subjected to full irrigation (FI) (100% replenishment of crop evapotranspiration) or deficit irrigation (DI) (50% of FI treatment) from bulb initiation to harvest. Bulbs were harvested at full maturity and stored at 1 °C for five months. Bulbs were treated with or without 1-MCP (1 μL L^{-1}) for 24 h before storage under continuous ethylene supplementation (10 μL L^{-1}) or air. DI had no effect on dormancy-break, sprout emergence, total fructans content and total sugar content. In contrast, ethylene delayed sprout emergence and suppressed sprout growth; added 1-MCP enhanced this effect. The concentration of DP3-8 fructans were higher in top and bottom sections compared to the baseplate. Before sprout emergence, fructans of DPs 7–8 were no longer present in the top and bottom wedges, while they accumulated in the baseplate; irrespective of pre- or postharvest treatments. This redistribution of fructans within the bulb suggested a transition in dormancy state and could be used as a predictive marker for sprouting in stored onion bulbs.

1. Introduction

Despite being a seasonal crop, the demand for onion bulbs is all-year-round, therefore, the onion industry relies on innate dormancy and storage treatments to extend availability. Dormancy break elicits various changes including increased weight-loss, sprouting and breakdown of sugars and fructans. Currently, the industrial standard sprout suppressant regime involves the use of maleic hydrazide (MH) - applied as a foliar spray - in conjunction with low temperature storage and, occasionally controlled atmosphere (CA).

Ethylene supplementation has previously been shown to delay and suppress sprouting in stored onion bulbs. Onion bulbs are low endogenous ethylene producers (Cools et al., 2011; Chope et al., 2012); nonetheless, continuous exogenous ethylene supplementation at 10 μL L^{-1} during storage suppresses sprout growth (Briddon and Sbeu, 2006; Bufler, 2009; Cools et al., 2011; Chope et al., 2012). Further evidence of ethylene sprout suppression in other crops was revealed by Foukaraki et al. (2014) (potatoes) and Amoah et al. (2016) (sweet potatoes). Perhaps unexpectedly, the treatment of onion bulbs with 1-methylcyclopropene (1-MCP) - a known inhibitor of ethylene activities – also suppressed sprout growth and caused the accumulation of sugars (Chope et al., 2006a); and this sprout suppression was further enhanced when applied in combination with ethylene supplementation (Cools et al., 2011).

Fructans are polymers of fructose and the major reserve carbohydrates in onion bulbs, which vary in their degree of polymerisation (DP) (Valluru and Van Den Ende, 2008). Fructans and simple sugars are known to decrease in bulbs during dormancy transition (Chope et al., 2012). The concentration of fructans at harvest was reported to positively correlate with long storage and delayed sprouting in onion bulbs (Suzuki and Cutcliffe, 1989; Jaime et al., 2001; Benkeblia et al., 2006). Furthermore, Jaime et al. (2001), reported a positive correlation between long storing onion bulbs and higher DP fructans content compared to poorer storing onion bulbs, yet, the mechanism of fructans mobilisation and metabolism in relation to onion dormancy, is still unclear.

Previous studies on the role of deficit irrigation (DI) on the postharvest qualities of onion bulbs are contradictory. Rattin et al. (2011)

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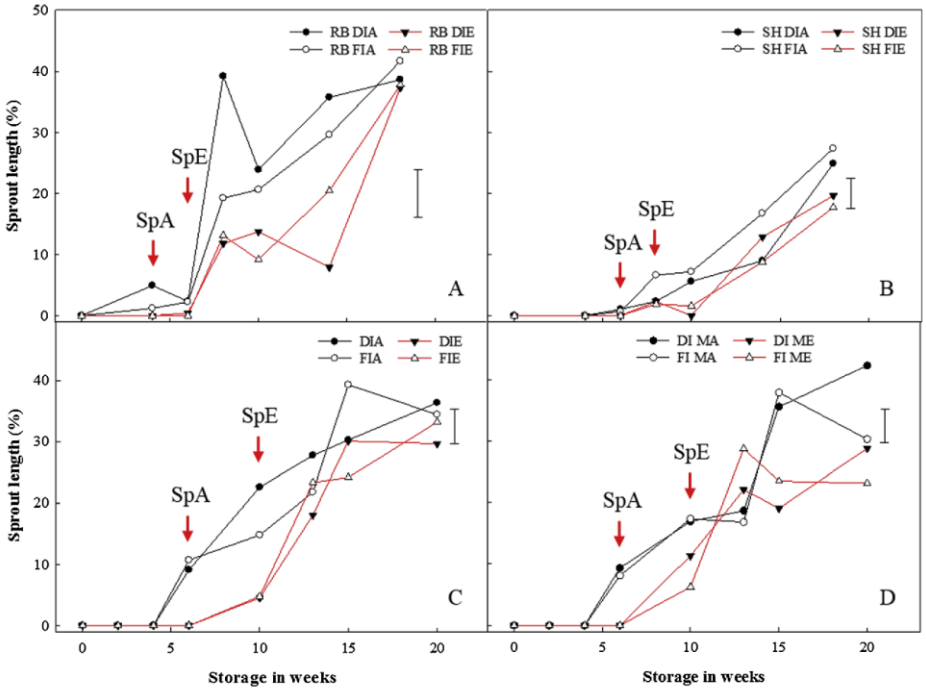

Fig. 1. Sprout length in stored onion bulbs. Sprout length was measured as sprout length in proportion to bulb height represented as a percentage for onion bulbs of cultivar 'Red Baron' (RB) and 'Sherpa' (SH) grown in 2015 (A and B) and 2016 (C and D) under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. After curing, bulbs were either treated with 1-MCP at 1 μL L^{-1} for 24 h or untreated before storage; and then, stored at 1 °C under continuous ethylene supplementation at 10 μL L^{-1} or air. DIA and FIA are DI and FI bulbs in air, DIE and FIE are DI and FI bulbs stored under ethylene, DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95% confidence shown.

and Vickers et al. (2015) reported that DI caused early sprouting and greater postharvest losses; Leskovar et al. (2012) reported an increase in postharvest storage life; while Martín de Santa Olalla et al. (2004) and Enciso et al. (2009) found no such differences. Notably, none of these earlier studies combined DI with any postharvest sprout mitigation treatments. Moreover, DI had previously been shown to cause the accumulation of non-structural carbohydrates such as sugars in strawberry (Bordonaba and Terry, 2010) and tomato fruits (Kirda et al., 2004). In *Aloe vera* leaves, DI treatments caused up to 60% increase in the fructans content when compared to the fully irrigated plants (Salinas et al., 2016). In the leaves of transgenic tobacco, Pilon-Smits et al. (1995a, 1995b) reported that polyethylene glycol-mediated drought stress caused a 7-fold increase in fructans concentration and overall biomass, while starch level was not affected. Similarly, drought stress caused the accumulation of fructans in the shoots and roots of transgenic sugar beets (Pilon-Smits et al., 1999) and in the rhizophores (the storage organ) of *Vernonia herbacea* (Garcia et al., 2011). Salinas et al. (2016) further reported that DI caused the accumulation of fructans with higher DP when compared to fully irrigated leaves of *Aloe vera* plants. Despite these reports, and the implication that fructans may regulate onion dormancy (Suzuki and Cutcliffe, 1989; Jaime et al., 2001; Benkeblia et al., 2006; Chope et al., 2012), it remains unknown if fructans content can be manipulated through DI.

The aim of this study was to investigate the influences of pre-harvest DI and postharvest ethylene supplementation on the accumulation and distribution of fructans in relation to dormancy-break and sprouting in stored onion bulbs.

2. Materials and methods

2.1. Plant material

In this study, two different onion cultivars ('Red Baron' and 'Sherpa') were used in 2015 and only 'Sherpa' in 2016. In 2015, 'Red Baron' sets and 'Sherpa' seeds were sourced from Elsom Seeds (Lincs., UK), and Steve Howe Seeds (Lincs., UK), respectively; while in 2016, 'Sherpa' seeds were sourced from Limagrain (Lincs., UK). For both years, 'Sherpa' seeds were planted into trays with John Innes No 1 seed media and seedlings were transplanted into pots six weeks after germination, while 'Red Baron' were planted directly into pots. Final growth media was John Innes Compost No 3. Equal weights of the growth media (7.3 kg) were measured into 96 pots of 8 L capacity per cultivar for 2015 and 264 pots for 2016 experiments. Plants were transplanted at the rate of three plants per pot (used as pseudo-reps) for both years. Plants were split into three completely randomised blocks (replicates) formed across two benches in the glasshouse. The first and

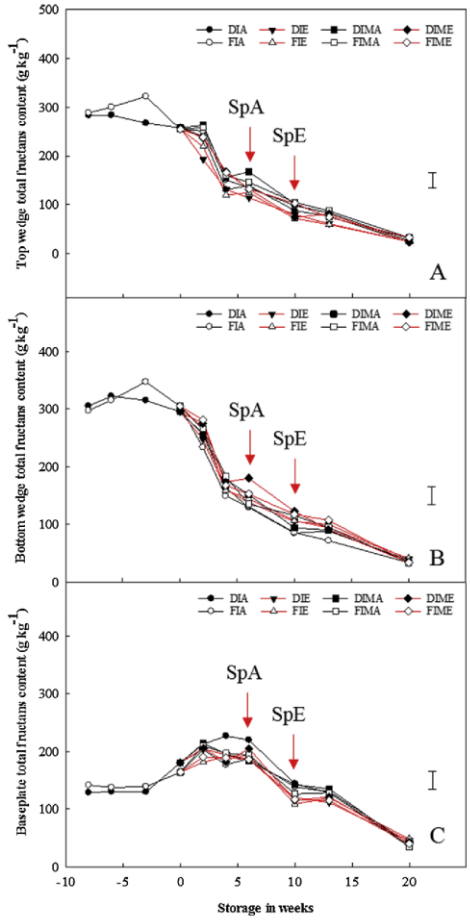

Fig. 2. Pre- and postharvest total fructans content in the top wedge, bottom wedge and baseplate of stored onion bulbs treated or untreated with 1-MCP. Total fructans contents (sum of fructans of DP3-8 content) per dry weight for the top wedge (A), bottom wedge (B) and baseplate (C) of onion bulbs of cultivar 'Sherpa' grown in 2016 under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. Post-curing, bulbs were treated with 1-MCP at 1 μL L^{-1} for 24 h before storage or untreated with 1-MCP, and were stored at 1 °C under continuous ethylene supplementation at 10 μL L^{-1} or air. DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. Where pre-harvest = –8; harvest = –6; mid-curing = –3; and end of curing = 0 in weeks. LSD bar at 95% confidence shown.

last rows of plants in blocks 1 and 3, respectively, served as guard plants (not included in the analysis) for both years. Plants were fertilised with 800 mL of Hoagland's solution as two single 400 mL applications per treatment. For both years, bulbs were harvested manually at full maturity when all plant foliage had lodged (100% fall-down) and were cured under glass for six weeks (August - September).

2.2. Experimental design

Plants were subjected to two pre-harvest treatments: full irrigation (FI) and deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c), and DI corresponded to 50% of the FI treatment. Irrigation was uniformly applied using an automated irrigation system (AC4, Hozelock, Warwickshire, UK). Polyvinyl tubing (20 mm diameter) were fitted with pressure-compensating emitters of 1.2 L min^{-1} flow rate, which were connected to polyvinyl tubes (5 mm diameter) to the pots (one emitter per pot). Soil moisture content was monitored weekly both gravimetrically (based on individual pot weight) and volumetrically using soil moisture probes (HH2 and ML2x, Delta-T, Cambs., UK); three pots per treatment per block were assessed. The onion plants were subjected to differential pre-harvest irrigation treatments for seven weeks - from bulb initiation stage until two weeks before harvest, when no more irrigation was applied thereafter.

At harvest, all three bulbs per pot were collected, tagged and weighed together. Afterwards, the bulbs were spread out in a single layer on the benches in the glasshouse for curing (18–35 °C and 40–90 % relative humidity). Bulbs were weighed weekly throughout the six weeks' curing period. After curing, bulbs were transferred to 100 L storage boxes and stored at 1 °C for 18 and 20 weeks for 2015 and 2016, respectively. For 2015, bulbs were subjected to two treatments: continuous ethylene supplementation at 10 μL L^{-1} or continuous air, as described elsewhere (Chope et al., 2006a; Amoah et al., 2017). For 2016, bulbs were treated with or without 1-MCP at 1 μL L^{-1} for 24 h before storage; and then stored in air or under continuous ethylene supplementation at 10 μL L^{-1} (Cools et al., 2011). Thus, the postharvest treatments were: air, air + ethylene, 1-MCP + air, and 1-MCP + ethylene. At each sampling point, three bulbs were collected in triplicate, per treatment and throughout the storage duration (3 bulbs x 3 replicates x treatments). All experiments (pre- and postharvest) were arranged in a completely randomised design.

Bulbs were stored for 18 weeks in 2015 with six postharvest sampling points, while in 2016, bulbs were stored for 20 weeks with seven postharvest sampling points with two pre-storage sampling points (pre-harvest and mid-curing) added. The pre-harvest samples for 2016 were collected at the termination of irrigation at 100% fall-down (two weeks before harvest), while the mid-curing samples were collected three weeks into curing. Time 0 (week 0) samples for both years were collected at the end of curing, prior to bulbs being transferred to the cold room. Afterwards, sampling was conducted bi-weekly until sprout length was 40% in proportion to bulb height (Chope, 2006). At each sampling point, bulb weight, real-time respiration rate and sprout assessment (sprout length as a percentage of total bulb height) were recorded. Bulbs were sliced in two equal parts from top to bottom with a sharp stainless-steel knife; then, the individual baseplates were excised, after which the remaining section (including the skin) was divided equally into top and bottom (Supplement 6). All samples were immediately snap-frozen in liquid nitrogen and stored at − 80 °C before lyophilisation at -55 °C in a freeze-drier (Scanvac, Lynge, Denmark) in the dark for 7 days. Freeze-dried samples were stored at − 40 °C prior to biochemical analyses.

2.3. Estimation of crop evapotranspiration (ET_c) and soil moisture measurements

Three pots per block, per treatment, per cultivar were randomly selected, weighed and reweighed after 24 h. The ET_c was then calculated as the difference between the recorded initial and final weights (in grams). Soil moisture content were measured using Delta-T soil moisture probes (type: HH2 and ML2x, Delta-T, Cambs., UK). The

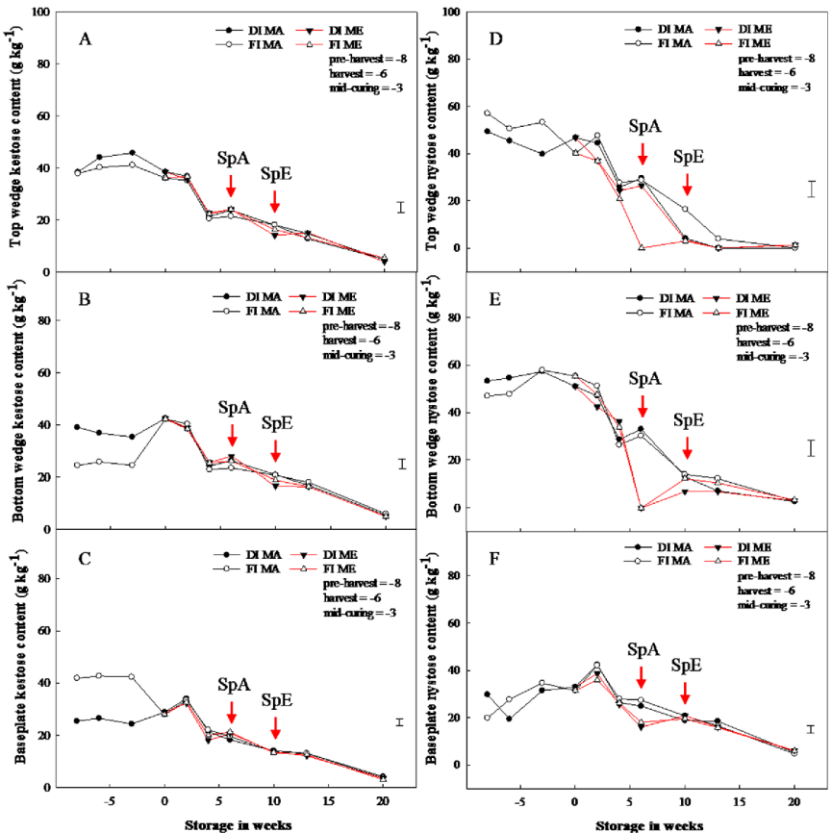

Fig. 3. Pre- and postharvest kestose and nystose contents in the top section, bottom section and baseplate of stored onion bulbs treated with and without 1-MCP and ethylene. Kestose and nystose contents per dry weight for the top wedge (A and D), bottom wedge (B and E) and baseplate (C and F) of onion bulbs of cultivar 'Sherpa' grown in 2016 under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. Post-curing, bulbs were treated with 1-MCP at 1 μL L^{-1} for 24 h before storage, and were stored at 1 °C under continuous ethylene supplementation at 10 μL L^{-1} or air. DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95% confidence shown.

probes were inserted fully into the soil and the percentage soil moisture content reading was recorded. Measurements were conducted weekly, from bulb initiation to harvest (June – August) for both years. Where there was a need, irrigation was readjusted according to the treatment.

2.4. Curing and storage bulb weight-loss

Onion bulb weight-loss was measured weekly during curing and at each sampling point during storage.

2.5. Real-time respiration rate (RR)

Real-time respiration rate measurements were taken at each sampling point using the Sable Respirometry System (Model 1.3.8 Pro, Sable Systems International, NV, USA), as described previously by Collings et al. (2013). Each replicate (3 replicates x 3 onion bulbs per replicate) were taken out from the storage boxes and placed on the laboratory bench for a minimum of one hour to be acclimatised to room temperature. Each replicate was then placed in a 3 L sealed gas jar with gas inlet for air supply and outlet for respiration rate measurement as CO_2 production. The measured CO_2 produced (in mL h^{-1}) was adjusted to get the final respiration rate values as CO_2 produced in milligrams per kilogram of bulb weight per hour (mg kg^{-1} h^{-1}) as previously described by Collings et al. (2018).

2.6. Sprout assessment

Sprout incidence were assessed as previously described elsewhere (Chope et al., 2006a, 2006b). Onion bulbs were cut in half (vertically from top to bottom) and internal sprout length was presented as a percentage of the total bulb height (Appendix A).

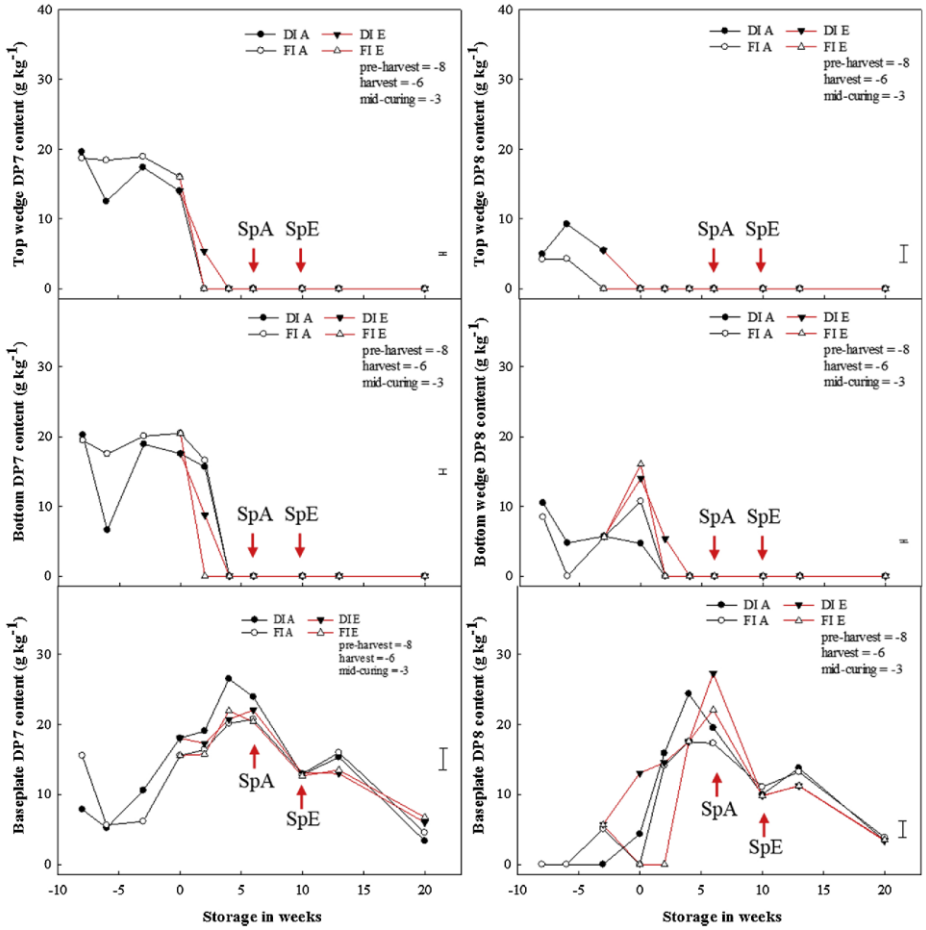

Fig. 4. Pre- and postharvest fructans of DP7 and 8 contents for the top section, bottom section and baseplate of stored onion bulbs. Fructans of DP7 and 8 contents per dry weight for the top wedge, bottom wedge and baseplate of onion bulbs of cultivar 'Sherpa' grown in 2016 under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. Post-curing, bulbs were stored at 1 °C under continuous ethylene supplementation at 10 μL L^{-1} or air. DIA and FIA are DI and FI bulbs stored in air and DIE and FIE are DI and FI bulbs stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95% confidence shown.

2.7. Non-structural carbohydrates extraction and quantification

Freeze-dried samples (top wedge, bottom wedge and baseplate [Appendix B]) were ground into fine powder using metallic ball bearings (size: 5 mm) placed in each tube with freeze-dried samples and placed into a Star-beater (VWR International bvba, Leuven, Belgium). Non-structural carbohydrates (NSCs) from the powdered samples were extracted and quantified as described elsewhere, with modifications. Briefly, 150 mg of pulverised samples were thoroughly vortexed in 3 ml of 62.5% (v/v) aqueous HPLC grade methanol, incubated in a shaking bath for 15 min. at 55 °C and filtered through a 13 mm diameter × 0.2 μm PTFE filter unit (Jaytee Biosciences Ltd, Kent, UK). Extracts (10 μL) were injected into a Prevail Carbohydrate ES (250 × 4.6 mm, 5 μm particle size; Grace Discovery Sciences, IL, USA) with a guard column (ZORBAX Eclipse Plus-C18, 2.1 × 12.5 mm, 5 μm particle size) in Agilent 1260 Infinity HPLC system coupled to an Evaporative Light-Scattering Detector (Agilent Technologies LDA UK Limited, Cheshire, UK). The mobile phase was a linear increase/decrease amount of water in acetonitrile (% water): 0–15 minutes, 20–50 %; 15–20 minutes, 50-20%; 20–23 minutes, 20-15%; 23–30 minutes, 15-10%. The flow rate and injection volumes were 0.8 mL min^{-1} and 5 μL, respectively, and the oven temperature was set at 30 °C. The presence and concentration of fructose, glucose, sucrose, kestose and nystose were calculated by comparing the peak areas in each sample to

Table 1
Correlation between sugars, fructans, total sugars, total fructans and total non-structural carbohydrates for onion bulbs grown under deficit irrigation, treated with 1-methylcyclopropene (at 1 μL L^{-1}) 24 h prior to storage and stored under continuous ethylene (at 10 μL L^{-1}) or air.

Compounds	Fructose	Glucose	Sucrose	DP3	DP4	DP5	DP6	DP7	DP8	Total Sugars	Total fructans	Total NSc
Fructose	1.00	0.81	0.66	0.44	0.07	−0.05	−0.19	−0.33	−0.33	0.90	0.12	0.68
Glucose	0.81	1.00	0.77	0.77	0.51	0.37	0.17	−0.08	−0.28	0.96	0.53	0.91
Sucrose	0.66	0.77	1.00	0.83	0.60	0.54	0.39	0.20	0.05	0.87	0.68	0.92
DP3	0.44	0.77	0.83	1.00	0.83	0.77	0.58	0.31	0.02	0.75	0.89	0.93
DP4	0.07	0.51	0.60	0.83	1.00	0.88	0.73	0.58	0.25	0.43	0.95	0.73
DP5	−0.05	0.37	0.54	0.77	0.88	1.00	0.86	0.70	0.41	0.31	0.95	0.64
DP6	−0.19	0.17	0.39	0.58	0.73	0.86	1.00	0.81	0.57	0.13	0.84	0.47
DP7	−0.33	−0.08	0.20	0.31	0.58	0.70	0.81	1.00	0.71	−0.09	0.66	0.23
DP8	−0.33	−0.28	0.05	0.02	0.25	0.41	0.57	0.71	1.00	−0.22	0.35	0.00
Total Sugars	0.90	0.96	0.87	0.75	0.43	0.31	0.13	−0.09	−0.22	1.00	0.48	0.92
Total fructans	0.12	0.53	0.68	0.89	0.95	0.95	0.84	0.66	0.35	0.48	1.00	0.79
Total NSC	0.68	0.91	0.92	0.93	0.73	0.64	0.47	0.23	0.00	0.92	0.79	1.00

standards (0.05–5 mg mL^{-1}) using Agilent ChemStation software version 4 (Agilent Technologies, CA, USA). Fructose, glucose, sucrose, kestose and nystose standards were obtained from Sigma-Aldrich Co. (Dorset, UK). Fructans with degree of polymerisation higher than four were quantified using the standard curve for nystose as previously described by Downes (2010). Total sugar content was calculated as the sum of the concentrations of fructose glucose and sucrose while total fructans was calculated as the sum of the concentrations of fructans of DP3-8 on a per section and per sample bases.

2.8. Statistical analyses and plots

All statistical analyses were conducted using Genstat for Windows 10th Edition (VSN International Ltd, Herts., UK). Analysis of variance (ANOVA) was performed to identify factors that significantly affected variance in the physiological and biochemical data collected. ANOVA was performed on the data specifying a nested treatment structure of a common baseline (observation before postharvest treatments). Least significant difference (LSD) values were calculated from each analysis, for comparison of appropriate treatment means, using general analysis of variance. A significance threshold of $p < 0.05$ was adopted, for all analyses. SigmaPlot for Windows SPW13 (Systat Software, Inc., London, UK) was used for all plots.

3. Results

3.1. Crop evapotranspiration, soil moisture content and curing weight-loss

Mean crop evapotranspiration (ET_c), soil moisture content and curing weight-loss (Appendix C– Fig. B1[2015] and B [2016]) varied between pre-harvest treatments. A comparison between cultivars showed that curing weight-loss was 15.0 and 8.6% for 'Red Baron' FI and DI bulbs, respectively, compared to 9.8 and 6.2% for 'Sherpa' FI and DI bulbs, respectively, in 2015. While a comparison between years showed that curing weight-loss for 'Sherpa' in 2015 was 9.8 and 6.2% for FI and DI bulbs, respectively, compared to 6.8 and 3.6% for FI and DI bulbs, respectively, in 2016 (Appendix C – Fig. B1). Interestingly, weight-loss during curing was twice as high in FI compared to DI, irrespective of cultivar or year. Bulb storage weight-loss was continuous throughout the storage period, however, there were no significant differences between pre- or postharvest treatments (Appendix C – Fig. B1 [2015] and D [2016]). Overall, bulb weight was reduced in DI bulbs for all years; such that in 2015, bulb weights were significantly (12.2 and 41.8%) higher for fully irrigated 'Red Baron' and 'Sherpa' onion bulbs compared to the DI bulbs, respectively. For 2016, 'Sherpa' bulb weights were significantly (14%) higher for FI compared to DI bulbs.

3.2. Sprout length

There was no significant difference in sprout emergence between pre-harvest treatments, irrespective of cultivar or year (Fig. 1). However, sprout emergence and sprout length varied according to the postharvest regime. Ethylene delayed sprout emergence by two and four weeks (2015 and 2016, respectively) and suppressed sprout growth compared to bulbs stored in air. Overall, 1-MCP-treated bulbs stored under ethylene produced the shortest sprouts followed by untreated bulbs under ethylene supplementation, untreated bulbs stored in air and 1-MCP treated bulbs stored in air.

3.3. Fructans content

Neither pre- nor postharvest treatments significantly influenced the total fructans content within the bulbs (Fig. 2). The highest total fructans content (sum of DP3-8 fructans concentration) in the top (283–288.6 g kg^{-1}) and bottom (297–305.3 g kg^{-1}) sections were measured at pre-harvest and this declined continuously throughout the storage period; irrespective of pre- or postharvest treatments. In contrast, the total fructans content for the baseplate (128–141.7 g kg^{-1}) increased continuously, irrespective of ethylene treatment, from pre-harvest until six weeks of storage (at sprout emergence for bulbs stored in air), before gradually declining continuously thereafter. Nevertheless, there were no significant differences between pre-harvest treatments as independent factors ($p = 0.365$), or with ethylene ($p = 0.404$) and 1-MCP ($p = 0.627$).

The kestose (DP3) content between harvest and mid-curing in the bottom wedge was significantly higher in DI compared to FI, while for the baseplate, DP3 was significantly higher in FI compared to DI bulbs. However, at the end of curing, these differences had disappeared. No similar differences were found for nystose (Fig. 3) or other fructans of higher DP; irrespective of pre-harvest treatments or year. Moreover, by the end of curing and during storage, the differences in the accumulation of kestose between pre-harvest treatments had ceased to exist. Besides a significant decline in the nystose content for the bottom section and baseplates of 1-MCP-treated bulbs stored under ethylene at week 6, there was no consistent effect of ethylene or 1-MCP on kestose or nystose contents during storage.

For 2015 onion bulbs, fructans of DP3-6 were present across all sections in the cured bulbs (DP6 shown in Appendix D); while fructans of DP7 and 8 were present only in the baseplate. For 2016 onion bulbs, fructans of DP3-7 were present across all sections at pre-harvest, harvest, and mid-curing stages, with DP8 fructans being totally absent in the baseplate. However, at the end of curing, irrespective of pre-harvest treatments, DP8 fructans were no longer present in the top wedge (Fig. 4).

Prior to sprout emergence, there was a significant decline in fructans content, especially for fructans of DP6 and above. For 2015 bulbs, fructans of DP6 in the top and bottom wedges declined from *ca.* 47.7 to

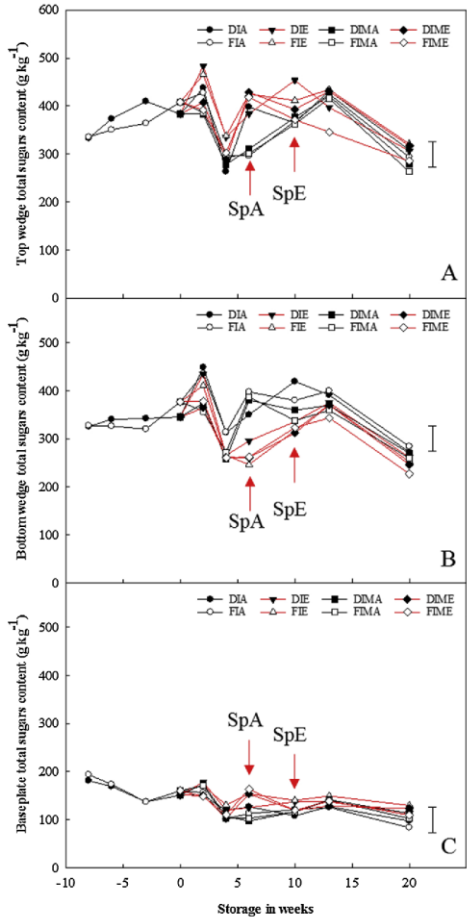

Fig. 5. Pre- and postharvest total sugar content in the top section, bottom section and baseplate of stored onion bulbs treated with and without 1-MCP and ethylene. Total fructans contents (sum of fructose glucose and sucrose content) per dry weight for the top wedge (A), bottom wedge (B) and baseplate (C) of onion bulbs of cultivar 'Sherpa' grown in 2016 under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. Post-curing, bulbs were treated with 1-MCP at 1 μL L^{-1} for 24 h before storage or untreated with 1-MCP, and were stored at 1 °C under continuous ethylene supplementation at 10 μL L^{-1} or air. DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. Where pre-harvest = –8; harvest = –6; mid-curing = –3; and end of curing = 0 in weeks. LSD bar at 95% confidence shown.

0 g kg^{-1} (Appendix D). A similar decline was found in 2016 bulbs. Fructans of DPs 7 and 8 (Fig. 4) in the top and bottom wedges declined from 20.5 to 0 and 16 to 0 g kg^{-1} DW, for DPs 7 and 8, respectively. In contrast to the decline of higher DP fructans in the top and bottom wedges, they increased in the baseplate; irrespective of cultivar, pre- or post-harvest treatments or year.

Correlations were found to be stronger between fructans of closer DPs (e.g. DP3 and DP4) when compared to fructans with DPs wider apart (e.g. DP3 and DP8) as shown in Table 1.

3.4. Total sugar (fructose, glucose, and sucrose) content

There were no significant differences in the total sugar content (the sum of fructose, glucose, and sucrose concentrations) between deficit and fully irrigated bulbs. Moreover, the total sugar content declined across all sections after four weeks of storage, with no significant differences between pre- and postharvest treatments (Fig. 5); a similar trend was found in 2015.

Overall, fructose content increased continuously throughout the storage period. There were no significant differences in the fructose and sucrose contents for 1-MCP untreated bulbs stored under ethylene. In contrast, fructose and sucrose contents increased by 1.5-fold for 1-MCP treated bulbs stored under ethylene (Fig. 6). Glucose and sucrose contents increased slightly in the first two weeks of storage before they declined by a half across all sections after four weeks of storage (prior to sprout emergence); nevertheless, there were no differences between pre-harvest treatments.

The strongest correlation between sugars were found between fructose and glucose (0.81) when compared to that between glucose and sucrose (0.77) and between fructose and sucrose (0.66) (Table 1).

3.5. Real-time respiration rate

In 2015, real-time respiration rates (RR) measured after curing prior to storage were 9.58 and 6.73 mg kg^{-1} h^{-1} of CO_2 for 'Red Baron and 9.64 and 7.03 mg kg^{-1} h^{-1} of CO_2 for 'Sherpa'; for DI and FI bulbs, respectively (Fig. 7 A and B). At the end of curing, RR was not significantly different between pre-harvest treatments within cultivars. After four weeks of storage, mean RR increased by at least 50 and 25% for 'Red Baron' and 'Sherpa' bulbs, respectively. Overall, the mean RR during storage was significantly higher in cv. 'Red Baron' (by 25%) when compared to 'Sherpa' for the 2015 experiment.

To understand the impact of curing on RR, during the 2016 experiment, RR was measured for onion bulbs two weeks prior to harvest (pre-harvest), at harvest, at mid-curing, and after curing. RR declined continuously from 22.8 mg kg^{-1} h^{-1} of CO_2 at pre-harvest to 4.6 mg kg^{-1} h^{-1} of CO_2 at the end of curing (i.e. pre-harvest > harvest > mid-curing > post-curing). Although RR declined 6-fold from pre-harvest to end of curing, there was no significant differences in RR between DI and FI bulbs (Fig. 7 C and D). During cold storage, RR increased steadily until dormancy-break at week 6 (7.9–18.0 mg kg^{-1} h^{-1} of CO_2), when the RR for 1-MCP-treated bulbs were twice as high when compared to the untreated bulbs stored under ethylene or air. The RR at dormancy-break were: 1-MCP treated bulbs under ethylene > 1-MCP treated bulbs stored in air > bulbs stored under ethylene > bulbs stored in air. Thereafter, RR declined until week 13, after which the RR for bulbs stored under ethylene became significantly higher in DI compared to FI bulbs.

3.6. Dry matter content

Mean dry matter content (DMC) for DI and FI onion bulbs across all postharvest treatments and time points was 127.3 and 129.1 g kg^{-1} FW, respectively. There were no significant differences in DMC between DI and FI at pre-harvest, harvest, and throughout the curing period. DMC fluctuated during storage, and after 20 weeks DW was 17% higher in the FI compared to DI bulbs stored in air, while there was no significant difference between DI and FI bulbs stored under ethylene.

4. Discussion

Evapotranspiration, soil moisture content and curing weight-loss

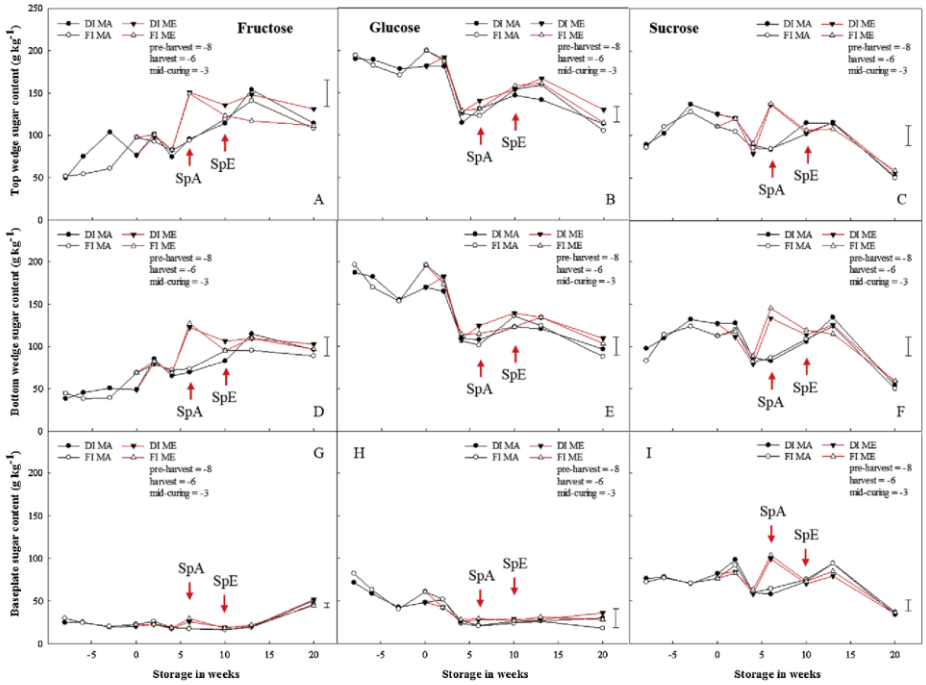

Fig. 6. Pre- and postharvest sugar content in stored onion bulbs treated with and without 1-MCP and ethylene. Fructose, glucose and sucrose contents per dry weight for the top wedge (A, B and C), bottom wedge (D, E and F) and baseplate (G, H and I) of onion bulbs of cultivar 'Sherpa' grown in 2016 under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. After curing, bulbs were treated with 1-MCP at 1 μL L^{-1} for 24 h before storage and were stored at 1 °C under continuous ethylene supplementation at 10 μL L^{-1} or air. DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95% confidence shown.

were significantly higher in the fully irrigated compared to deficit irrigated plants

Onion plants grown under deficit irrigation (DI) recorded a lower ET_c when compared to plants under full irrigation (FI) for both years. This showed that the pre-harvest treatment in this study successfully created differential transpiration between FI and DI plants, as was previously reported by Begum et al. (1993); thus validating the use of resulting bulbs in the postharvest studies reported herein.

4.1. Fructans redistribution prior to sprouting may predict dormancy-break

Fructans of higher DP initially present across all bulb sections at pre-storage could only be detected in the baseplate prior to sprouting. Fructans are polymers of fructose and the major reserve carbohydrates of onion. The concentration of fructans was reported to be highest at harvest, but decreased continuously to a minimum at dormancy-break and sprouting during storage (Suzuki and Cutcliffe, 1989; Jaime et al., 2001). The data herein showed that whilst this is true for the fructans content in the top and bottom sections, the opposite was true for the fructan content in the baseplate region; where the fructans content increased continuously until sprout emergence after which it declined (Fig. 2). However, previous studies on onion fructans sampled either the whole bulb (Suzuki and Cutcliffe, 1989), equatorial sections (Darbyshire and Henry, 1978) or the inner scales (Jaime et al., 2001) of onion bulbs. This study here represents the first investigation of fructans content in baseplate of onion bulbs. The reasons behind this accumulation of fructans in the baseplate prior to sprouting is unclear; though they quickly declined after sprout emergence. Notably, neither ethylene nor 1-MCP affected this decline. Notwithstanding this, specific accumulation of fructans in the baseplate prior to sprouting may have been to ensure adequate reserve energy was available for the successful initiation of sprouting whilst also offering osmo-protection to the meristematic tissues.

Fructans vary in their degree of polymerisation (DP) and are known to possess some osmo-regulation activities (Asega and Machado De Carvalho, 2004; Garcia et al., 2011). The higher accumulation of kestose (a DP3 fructan) content in the bottom section of DI and in the baseplates of FI bulbs between pre-harvest and mid-curing shown here (Fig. 3) was thought to be in response to water-stress. More so, these differences occurred in the sections closer to the roots where water availability differed between DI and FI plants. Interestingly, the differences in kestose content had disappeared at the end of curing, which suggested that this accumulation of kestose was not related to dormancy. In addition, the higher DP fructans content did not vary according to the imposed irrigation regime. Since, kestose is the first and last product of fructan biosynthesis from sugars and fructan catabolism to sugars, respectively, their biosynthesis and catabolism may be central to osmoregulation in onion plants under water-stress. This is supported by the equal and strong positive correlations of 0.83 shown by kestose to sucrose and nystose

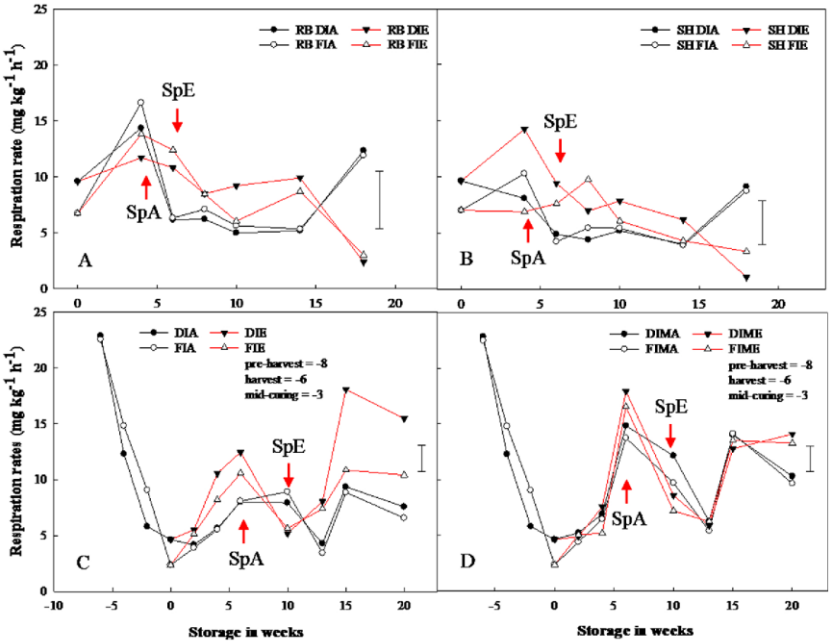

Fig. 7. Real-time respiration rates as CO_2 production (mg kg^{-1} h^{-1}) of stored onion bulbs. Onion bulbs of cultivar 'Red Baron' (RB) and 'Sherpa' (SH) were grown in 2015 (A and B) and 'Sherpa' in 2016 (C and D) under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. Post-curing, bulbs were treated with or without 1-MCP at 1 µL L^{-1} for 24 h before storage; and then, stored at 1 °C under continuous ethylene supplementation at 10 µL L^{-1} or air. DIA and FIA are DI and FI bulbs in air, DIE and FIE are DI and FI bulbs stored under ethylene, DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95% confidence shown.

(Table 1). Nevertheless, the differences in kestose content between pre-harvest treatments were no longer significant in cured bulbs prior to storage. Overall, correlations between fructans diminished with increasing differences in DP as shown in Table 1. Fructans accumulation had previously been reported in transgenic tobacco leaves and roots (Pilon-Smits et al., 1995a; Li et al., 2007), rice leaves (Kawakami et al., 2008) and sugar beet roots and shoots (Pilon-Smits et al., 1999), grown under drought-stress. While these allude to a role for kestose content prior to end of curing, it contrasts with data on total fructan content. This then suggests that fructans may be recycled in relation to the plants' responses to certain stimuli (in this case drought stress) without affecting total fructan content. Therefore, the accumulation of fructans in response to drought-stress may be typical of fructan type, plant tissues and species.

A positive relationship between onion bulb higher DP fructans content and delayed sprouting has been previously reported; such that long storing onion bulbs are known to typically have higher accumulation of higher DP fructans while poorer storing onion bulbs tend to accumulate low DP fructans (Jaime et al., 2001). While this suggests that the accumulation of higher DP fructans may extend dormancy, the data presented herein is at odds with this understanding. 'Red Baron' had a higher fructans content, including those of higher DP, when compared to 'Sherpa', however, 'Red Baron' sprouted two weeks earlier. This suggests that the accumulation of higher DP fructans in the whole bulb alone is not sufficient to predict dormancy-break or length of storage of onion bulbs.

Fructans of DP3-7 were present across all sections of the onion bulb before harvest, while fructans of DP8 also present in the top and bottom wedges, was conspicuously absent from the baseplate. Previous authors (Suzuki and Cutcliffe, 1989; Jaime et al., 2001; Benkeblia et al., 2006; Chope et al., 2012) reported that the concentration of fructans declined to a minimum, which coincided with dormancy-break and sprout emergence. Understandably, dormancy-break and sprout emergence had not been clearly differentiated in previous onion studies; thus, they are used interchangeably. Dormancy-break is the release of meristematic tissues for regrowth – at which point there might not be any physical signs of sprouting; while sprout emergence is when there is a physical appearance of sprout. Furthermore, considering the bulb sections sampled, it becomes apparent why previous studies reported a decline in fructans content at dormancy-break and sprout emergence. The data herein has shown for the first time that: (1) fructans of DP7 and 8 became conspicuously absent from the top and bottom wedges two and six weeks prior to sprout emergence (for bulbs stored in air and ethylene, respectively); and (2) the decline of fructans from the top and bottom wedges coincided with the accumulation of higher DP in the baseplate. This phenomenon suggests a top-to-bottom remobilisation of higher DP fructans. Notably, even though ethylene and 1-MCP treatments delayed and suppressed sprout growth, neither treatments affected the decline of these fructans, thereby suggesting that ethylene-related sprout suppression, while effective in the regulation of eco-dormancy, may not be involved in mediating endo-dormancy. Nevertheless, it was unclear if this redistribution of fructans of DP7 and 8 resulted from or was caused by dormancy-break.

Total sugar content declined across all sections at dormancy-break irrespective of pre- or postharvest treatments. Sugars are substrates for fructans

biosynthesis where the first step in the fructans biosynthesis pathway involves the transfer of the fructosyl group from one sucrose to another sucrose creating kestose, a DP3 fructan. This first step is catalysed by sucrose:sucrose 1-fructosyltranferase (1-SST); while the elongation of the fructan chain (increase in degree of polymerisation) is catalysed by fructan:fructan 6(G) fructosyltranferase (6G-FFT) (Vijn et al., 1998; Lüscher et al., 2000). Catabolism of fructans into its individual sugar components is by hydrolysis, which releases the fructose moiety; catalysed by fructan 1-exohydrolase (1-FEH) (Van Den Ende et al., 2003). The total sugar content data herein showed an increase in the first two weeks of storage across all sections; which coincided with reduced real-time respiration rate (RR) (Fig. 7). Thereafter, the total sugar content declined prior to sprout emergence (Fig. 5) at which point there was an increase in respiration rate. Individual sugars (*viz.* fructose, glucose and sucrose) content varied during storage and more so at sprout emergence. Pre-harvest fructose content increased continuously during storage, irrespective of a steep decline in RR from pre-harvest to end of curing; and this coincided with the decline in fructans as reported herein and elsewhere (Suzuki and Cutcliffe, 1989; Jaime et al., 2001; Benkeblia et al., 2006). Furthermore, there was a spike in the concentration of fructose and sucrose across all sections for bulbs stored in ethylene, which was more obvious for 1-MCP-treated bulbs stored in ethylene. The reason behind this transient increase is unclear, since there was also a spike in the RR for these bulbs. However, this finding is also supported by Chope et al. (2006a) who previously reported an increased accumulation of sugars in 1-MCP treated onion bulbs. Dissimilar to fructose, glucose content declined by a half after four weeks of storage irrespective of pre- or postharvest treatments. Following this decline, sprouts were recorded two and six weeks later for bulbs stored in air and ethylene, respectively, and coincided with the disappearance of DP7 and 8 fructans from the top and bottom wedges and concomitant accumulation in the baseplate. The observation suggests that glucose may be the preferred source of energy at dormancy-break. Interestingly, neither pre- nor postharvest treatments influenced the decline in glucose prior to sprouting; suggesting that neither ethylene nor 1-MCP is involved in endodormancy-break but instead in eco-dormancy (sprout suppression).

Three classes of dormancy exist *viz.* endo-dormancy, para-dormancy and eco-dormancy; where endo-dormancy is time-dependent, irrespective of the environmental condition, para-dormancy is dependent on the transfer of biochemical compounds, while eco-dormancy is dependent on environmental conditions (Campbell, 2006). Endo-dormancy is thought to occur at around bulb initiation, when the growth of all meristematic tissues is arrested (Terry et al., 2015). Notably, significant changes in sugar concentration occurred only after sprout emergence.

5. Conclusions

Deficit irrigation had no effect on sprout emergence, or the accumulation or distribution of total fructans content within the onion bulbs under cold storage. Therefore, the accumulation of fructans and the degrees of polymerisation thereof in onion bulbs may be genetically driven rather than being dependent on pre-harvest irrigation regimes. Ethylene as an independent factor delayed sprout emergence and when combined with 1-MCP produced the shortest sprouts as reported by (Cools et al., 2011); however, neither significantly influenced the fructans content within the onion bulbs in relation to dormancy-break. This suggests that the mechanism by which ethylene and 1-MCP reduce sprouting may not be through fructans remobilisation. Prior to sprout emergence and regardless of pre- or postharvest treatments, fructans of DP7 and 8 were redistributed from the top and bottom sections of the bulb to the baseplate - in a para-dormancy-like remobilisation. It is unclear whether this redistribution resulted from, or was caused by dormancy-break; however, it occurred prior to sprout emergence. As such, although ethylene and 1-MCP treatments influenced eco-dormancy through delaying sprouting, neither seemed to affect endo-dormancy. Furthermore, neither the interactions between pre-harvest irrigation, postharvest ethylene nor 1-MCP treatments significantly influenced the accumulation and redistribution of fructans within the onion bulb in relation to dormancy-break and sprouting. Given these findings, the redistribution of fructans prior to sprouting could serve as a potential marker to predict the transition from endo-dormancy.

Declaration of interests

None.

Acknowledgements

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Appendix A. Sprout assessment

Fig. A1

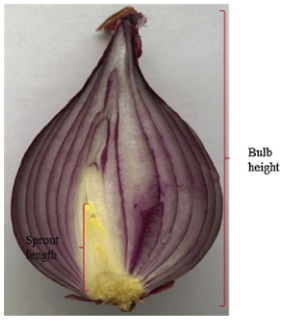

Fig. A1. Depiction of sprout assessment.

Appendix B. sampled sections (top, bottom and baseplate)

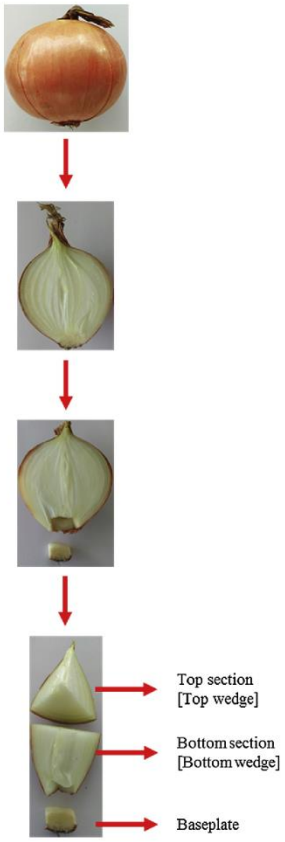

Fig. B1. Onion bulb cut into top wedge, bottom wedge and baseplate.

Appendix C. Curing weight-loss and Storage weight-loss

Fig. C1

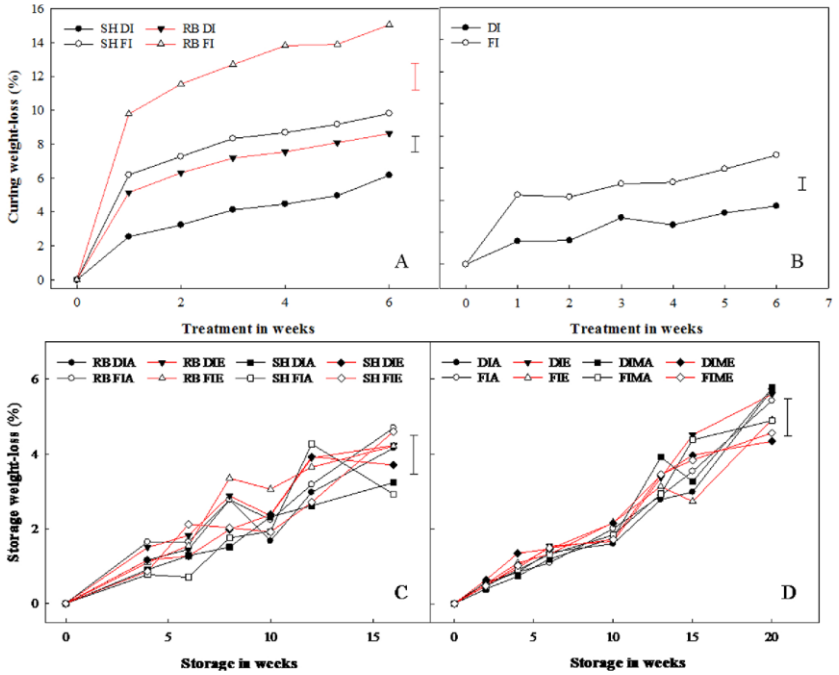

Fig. C1. Postharvest curing weight-loss (A and B) and storage weight losses (C and D) in stored onion bulbs. Onion bulbs of cultivar 'Red Baron' (RB) and 'Sherpa' (SH) were grown in 2015 (A and C) and 2016 (B and D) under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. Post-curing, bulbs were either treated with 1-MCP at 1 μL L^{-1} for 24 h or untreated before storage; and then, stored at 1 °C under continuous ethylene supplementation at 10 μL L^{-1} or air. DIA and FIA are DI and FI bulbs in air, DIE and FIE are DI and FI bulbs stored under ethylene, DIMA and FIMA are DI and FI bulbs treated with 1-MCP and stored in air and DIME and FIME are DI and FI bulbs treated with 1-MCP and stored under ethylene. LSD bar at 95% confidence shown.

Appendix D. DP6 fructans in the top, bottom and baseplate (2015)

Fig. D1

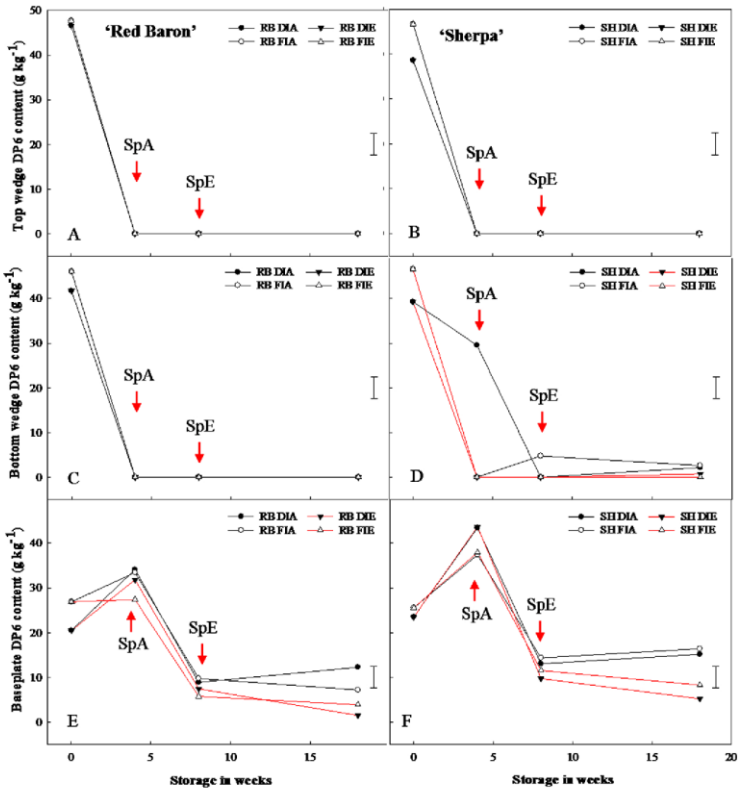

Fig. D1. Pre- and postharvest fructans DP6 content in stored onion bulbs DP6 fructans contents per dry weight for the top wedge (A and B), bottom wedge (C and D) and baseplate (E and F) of onion bulbs of cultivar 'Sherpa' grown in 2015 under full irrigation (FI) or deficit irrigation (DI); where FI amounted to 100% replenishment of crop evapotranspiration (ET_c) and DI was ET_c replenishment at 50%. Bulbs were harvested at full maturity (100% fall-down) and cured under glass for six weeks for both years. Post-curing, bulbs were stored at 1 °C under continuous ethylene supplementation at 10 μL L^{-1} or air. DIA and FIA are DI and FI bulbs stored in air and DIE and FIE are DI and FI bulbs stored under ethylene. SpA and SpE are sprout emergences for bulbs stored in air and ethylene, respectively. LSD bar at 95% confidence shown.

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