Effects of drought acclimation on drought stress resistance in three potato (*Solanum tuberosum* L.) genotypes

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ABSTRACT

Potato crops are generally sensitive to drought. Even a short period of water shortage can affect tuber production and quality. However, field potato crops undergoing mild water deficit conditions may acclimate to subsequent severe water deficits. While responses may be both acclimation and genotype-dependent, few studies have examined whole plant physiological factors leading to enhanced drought stress resistance. Identification of these key factors may increase selection efficiency in breeding programs. This study examined the effects of drought acclimation on drought stress resistance in three potato genotypes [‘Fv12246-6’ (Fv), ‘Vigor’ (V) and ‘Russet Burbank’ (RB)] in a low relative humidity greenhouse. Non-Acclimated and Non-Stressed (NA), Drought Acclimated and Drought Stressed (DAS) and Non-Acclimated and Drought Stressed (NAS) treatments were applied. Tuber yield and number were genetically determined and acclimation had no effect on increasing these components under drought stress. However, water conservation mechanisms based on leaf and stem characteristics were both genotype and treatment-dependent. When leaves were drought stressed while attached to the stem, genotype V and RB maintained a higher percentage of leaf water content (%LWC) than Fv, likely from the greater water stored in their stems that may have been delivered through continued leaf transpiration. Acclimation induced a thicker leaf cuticular layer and partially open stomata under drought stress in both RB and Fv. Nevertheless, Fv was the most drought sensitive potato genotype, displaying the highest degree of leaf wilting and lowest %LWC under drought stress. The observed drought stress-induced smaller stomatal size in Fv did not confer greater resistance. In addition, Fv displayed the lowest percentage shoot water content (%SWC) and slowest recovery time after drought stress. RB underwent the fastest recovery from drought stress, possibly due to its equivalent xylem to pith ratio which might have enhanced greater water

uptake in RB than in V and Fv. Finally, compared to application of drought stress directly (NAS), a pre-treatment of drought acclimation cycles followed by drought stress (DAS) reduced leaf wilting, induced thicker cuticular layer and more open stomata under stress. Without a DAS approach, potentially key drought stress resistance mechanisms will be missed. The role of the stem as a potential water reservoir to adapt against drought stress should be examined to further identify key elements for drought stress survival and recovery at the level of the potato whole plant.

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θ_v	Soil volumetric water content
Ψ	Soil water potential
DA	Drought Acclimation cycle
DAP	Date after planting
DAS	Drought-Acclimated and Drought-Stressed
DA-R	Recovery after watering at the end of Drought Acclimation cycle
DS	Drought Stress cycle
DS-R	Recovery after watering at the end of Drought Stress cycle
ET	Evapotranspiration
Fv	Potato genotype Fv12246-6
LWC	Leaf water content
NA	Non-Acclimated and Non-Stressed
NAS	Non-Acclimated and Drought Stressed
pET	Potential evapotranspiration
R	Recovery cycle
RB	Potato genotype ‘Russet Burbank’
RLER	Relative leaf expansion rate
RWC	Relative water content
SWC	Stem water content
V	Potato genotype ‘Vigor’
WUE	Water-use efficiency

1.0 INTRODUCTION

Numerous abiotic (temperature, water, solar radiation and salinity), biotic (disease, insect and weed) and physico-chemical properties of the air and soil create significant economic stresses on plants (Boyer 1982, Mahajan and Tuteja 2005, Shao et al. 2008). Environmental stresses represent the most limiting factors for agricultural productivity, have detrimental effects on plant growth and yield and are serious threats to agriculture (Boyer 1982, Wang et al. 2003). Global water scarcity and increased salinization cause significant crop loss worldwide, reducing average yields for most major crops by more than 50% (Riadh et al. 2010). Among the environmental stresses, drought stress is one of the most adverse factors to plant growth and productivity (Debaeke and Aboudrare 2004, Levy et al. 2006, Shao et al. 2008). Faced with scarcity of water resources, drought is the single most critical threat to world food security (Farooq et al. 2009). Approximately four-tenths of the world's land surface is within the arid and semiarid zones and in both zones water is the major limiting factor to plant productivity (Fischer and Turner 1978, Belin et al. 2010).

After wheat, rice and corn, potato is the fourth most important food crop worldwide based on yield per hectare (FAO crop statistics database: http://faostat.fao.org/). Potatoes are good source of carbohydrate (starch), vitamin C, vitamin B6 and potassium and also contain protein and fibre (USDA National Nutrient Database). Potato is generally considered to be sensitive to drought (van Loon 1981, Watkinson et al. 2008). Limited research has been undertaken on drought stress resistance in potato cultivars in western Canada. A short growing season and terminal drought are the major production constraints in western Canada (Thavarajah and Ball 2005).

Short root length in the soil profile (Iwama and Yamaguchi 2006) result in limited ability of potato roots to transport water and has been suggested as the basis of potato's drought sensitivity

(Gregory and Simmonds 1992). Drought stress affects the development and growth of shoots, roots and tubers (Lahlou et al. 2003). Even a short period of water shortage can result in reduction both of tuber production and of tuber quality (Costa et al. 1997, Deblonde and Ledent 2001). Drought stress at the beginning of the tuberisation stage induced a longer period of tuber formation but decreased tuber number (MacKerron and Jefferies 1986), growth and yield (Shock et al. 1992). Stolon formation and tuberization stages are the most sensitive stages to drought stress (MacKerron and Jefferies 1986, Haverkort et al. 1990). Potato response to drought varies widely among cultivars (Martin and Miller 1983) and also differs according to the extent and timing of the water deficits (Miller and Martin 1987). Before and during tuber initiation, soil water potential less than -25 kPa affects tuber set (Shock et al. 1992).

Different growth strategies by the potato plant enables adaptation to different drought conditions without significantly affecting tuber yield (Deblonde and Ledent 2001). Characterization of drought tolerance in potato cultivars is complicated by the fact that differential yield responses have not consistently related to specific physiological or morphological traits (Stark et al. 2013). Most used indicators of drought stress resistance are yield (Farshadfar and Elyasi 2012), leaf water content (Omae et al. 2005) and excised leaf water loss (Wang and Clarke 1993). Plant water management is a combination of increasing water uptake and reducing water loss during drought stress and is of obvious importance.

Plants have evolved a number of adaptive responses to overcome drought stress. An important aspect of abiotic stress is the ability of some plants to increase stress resistance after exposure to a lower level of the stress (acclimation). This is dependent upon both the genotype as well as the environment. Understanding the physiological basis of acclimation, their genetic

basis of tolerance, gene manipulation and mitigating the stresses agronomically, are important tools to increase resistance.

Drought acclimation also involves osmotic adjustment (Shao et al. 2008). By increasing the concentration of solutes in the symplast, turgor can be maintained at low tissue water potentials by enabling water to continue to be extracted from dry soil (Khalil and Grace 1992). The turgor allows stomatal opening and cell expansion, root growth and increase in productivity (Kozolowski and Pallardy 2002). Another response mechanism is the closure of stomata in response to a reduction in soil water content (Khalil and Grace 1992). The first response of virtually all the plants to acute water deficit is stomatal closure to prevent transpirational water loss (Yordanov et al. 2000, Mahajan and Tuteja 2005). Stomata primarily control transpiration which in turn influences water-use efficiency, WUE (Levy et al. 2013).

When stomata are closed, the leaf cuticular layer plays a particularly fundamental protective role against water loss (Boyer et al. 1997, Jenks and Ashworth 1999, Yoo et al. 2009). Leaf water content is a more important indicator of water status than other water potential parameters under drought stress conditions (Sinclair and Ludlow 1985) and was proposed as a selection criterion for drought tolerance in barley (Matin et al. 1989), wheat (Schonfeld et al. 1988) and pigeonpea (Kimani et al. 1994). Stem water reservoir is able to augment water supply to the leaves and provides a substantial degree of protection against desiccation in tropical alpine rosette plants (*Espeletia* and *Senetio* species, Holbrook 1995). The assessment of water loss from excised leaves showed promise for characterizing drought resistance in wheat genotypes (Clarke et al. 1989).

Although numerous molecular projects have been conducted on drought stress in potato, research is lacking in the area of drought stress resistance in the whole plant level which takes into account: drought acclimation and recovery and mechanisms of drought stress resistance. The overall long-term goal of this study was to determine the key elements of potato drought stress resistance. Overall hypotheses were:

i) There are genotypic differences in drought stress resistance.

ii) Pre-exposure to water deficit will induce drought acclimation in potato genotypes.

iii) Leaf and stem characteristics would distinguish genotypes and ability to acclimate to drought stress.

2.0 LITERATURE REVIEW

2.1 The potato crop

Potato crop is an important global food source. Potato originated from the highlands of Ecuador and Peru and is a species of annual vegetable plants grown for their food value in most countries around the world. Potatoes are best adapted to the cool temperature zones of the high altitudes in the Andes (2000-3500 m), at sea level in temperate regions of North America, Europe, South Chile and Argentina and at appropriate altitudes in intermediate latitudes (Hawkes 1992). It is an herbaceous C3 plant. The botanical name, *Solanum tuberosum* L., from the family Solanacea and it is related to deadly nightshade and tomatoes. Potatoes are easy to grow, easy to harvest and most varieties store well. The potato tuber is an enlarged portion of the underground stem or stolon. Tuber "eyes" are the lateral buds from which next season's growth will emerge. The number of stems per plant is mainly determined by the size of the seed tuber, its condition and by soil type and soil moisture at planting (Haverkort et al. 1990). The life cycle of the potato after being planted in the soil can be divided into the following stages: planting till emergence, emergence till tuber initiation, the period of maximum tuber production and senescence and tuber ripening (van Loon 1981). Growth and quality of potatoes are influenced by environmental factors such as temperature, moisture, light, soil type and nutrients. Among other cultivated species, *Solanum tuberosum* is widely grown worldwide. It is a tetraploid and has 48 chromosomes (Huaman 1986).

The potato root system in tuber-based seed systems is composed of branched fibrous roots. Many potato cultivars are shallow rooted and often produce most of their roots in the plow layer up to 30 cm below the hill crest (Lesczynski and Tanner 1976, Iwama and Yamaguchi 2006). Short days and moderate temperature enhance tuber formation while long photoperiods

enhance flowering (Moreno 1985, Watkinson et al. 2008). Once the tuber has initiated, it becomes a strong sink and develops rapidly due to a massive influx of sucrose, which is rapidly converted to starch (Fernie et al. 2001). The rapid conversion of sucrose to starch maintains low sucrose concentrations in the tuber and promotes continued delivery of photosynthate to this sink tissue. Thus, tuber formation and development serve as the best measure of drought resistance and reflects the continued storage of energy in the form of starch under unfavorable conditions (Watkinson et al. 2008).

2.2 Economic importance of the potato crops

Potato is one of the predominant crops in the world, constituting a large percentage of the staple diet in many developing countries (Watkinson et al. 2008). After wheat, rice and corn, potato is the fourth most important food crop, with a worldwide production of 364 million tonnes in 2012 (FAO crop statistics database 2014, http://faostat.fao.org/). Root and tuber crops will play an important role in feeding the developing world in the coming decades. The growth rates in production are particularly strong for potato with an annual average increase of 4.5 million tonnes per year, exceeding those of rice and wheat (Visser et al. 2009). Increases in Asia have been particularly striking. By 2020, more than two billion people in Asia, Africa and Latin America will depend on these crops for food, feed or income (Song et al. 1998). Potato has a high caloric value, which combined with high protein and vitamin content, make it an excellent food source (Watkinson et al. 2008). Potatoes are good source of carbohydrate (starch), vitamin C, vitamin B6 and potassium and also contain protein and fibre (USDA National Nutrient Database).

2.3 Effects of drought stress in potato plants

2.3.1 Effects of drought on tuber yield and tuber number

In many countries, potatoes are cultivated in arid and semi-arid regions where shortage or poor water quality are major factors limiting plant growth and yield (Heur and Nadler 1998). Potato is generally considered to be sensitive to drought (van Loon 1981, Jefferies and MacKerron 1987, Watkinson et al. 2008) with reduced ability to extract the available water from the soil in comparison with other crops (Watkinson et al. 2008). Small root length in the soil profile (Iwama and Yamaguchi 2006) and thus limited ability of potato roots to transport water has been suggested as the basis of potato's drought sensitivity (Gregory and Simmonds 1992). Even a short period of water shortage can result in reduction both of tuber production and of tuber quality (Miller and Martin 1987). A continuous and adequate water supply is required and soil water content needs to be maintained within a relatively narrow range (Wright and Stark 1990) from tuber initiation until near maturity for high yields and good grade and quality. To obtain maximum yields, soil moisture should not drop below 50% of available soil water, although others suggest 25% or 75% (Costa et al. 1997). Tuber set on potato cv. 'Maris Piper' plants grown in the greenhouse declined in proportion to the number of days that soil water potential was less than -25 kPa before and during tuber initiation (MacKerron and Jefferies 1986). Increasing duration of soil water potential below -60 kPa early in the season was associated with declining total yield in a study by Shock et al. (1992).

The effects of drought stress on a plant and the extent of tolerance depend on the timing, duration and the severity of the stress (Jefferies 1994), the cultivar involved and the developmental stage of the crop (Levy 1986, Miller and Martin 1987, Haverkort et al. 1990).

Responses of potatoes to soil moisture are generally assessed in terms of survival, vegetative growth, tuber size or total tuber production (van Loon 1981, MacKerron and Jefferies 1988). Yields are frequently constrained by drought in most environments, drought stress affecting the development and growth of shoots, roots and tubers (Lahlou et al. 2003). Water stress before tuber initiation was reported to reduce tuber set for certain varieties (van Loon 1981). Drought stress at the beginning of the tuberisation stage induced a longer period of tuber formation but decreased tuber number, growth and yield (Cavagnaro et al. 1971). This effect on tuber number was also confirmed by MacKerron and Jefferies (1986) and Haverkort et al. (1991). Costa et al. (1997) found that withholding water during tuberisation severely penalized tuber yield. Stark and McCann (1992) observed the greatest tuber yield reduction with water deficit during the mid-bulking period. Conversely, Miller and Martin (1987) found that the damaging effect of drought during tuber initiation and tuber bulking was almost the same. But no clear common reaction of early versus late varieties to drought was found in the study by Lahlou et al. (2003). Drought stress occurring during the last period of tuber growth leads to the extraction of water from tubers (Costa et al. 1987, Moorby 1978).

The number of tubers per plant is the product of the number of stems per plant and the number of tubers per stem (Heuer and Nadler 1995). The number of tubers per stem is reduced when water stress is imposed very early in the growing season, at the time of tuber initiation, but not when it was imposed later (Heuer and Nadler 1995, Shock et al. 1992, MacKerron and Jefferies 1986). Tubers per plant decreased with duration and intensity of early soil moisture stress (MacKerron and Jefferies 1986, Shock et al. 1992). Lahlou et al. (2003) found that tuber number was reduced only in early cultivars. In a study by Haverkort et al. (1990), during an early stage drought treatment with cv. ‘Radosa’, the number of stolons per stem was greatly reduced

but the number of tubers (+ initials) per stolon remained unchanged. The authors' observations were: the mechanism by which tuber numbers were reduced occurred through the reduction of the number of stolons per stem and not through a reduction of the number of tubers per stolon; once stolons were initiated, they would yield tubers regardless of a subsequent drought period. The development of soil moisture stress after tuber initiation had no effect on tuber number per stem in the study by MacKerron and Jefferies (1986). Also the later dry period did not affect numbers of stolons and tubers (+ initials) as found by Haverkort et al. (1990). Therefore, the most sensitive stage influencing final potato tuber yield is considered to be stolon initiation. In order to produce the maximum number of tubers, irrigation may be necessary at a very early stage when the stolon tips are just swelling (MacKerron and Jefferies 1986).

Number of tubers is influenced by environmental conditions and also by seed tuber characteristics (Lahlou et al. 2003). Moreover, the effects of spacing between plants and of the period between emergence and tuber initiation may all exert their influence on tuber number through their effects on assimilate supply (MacKerron and Jefferies 1986). Delaying initial irrigation resulted in a higher percent of large-sized tubers in both the US No. 1 and No. 2 grade classes in a study by Shock et al. (1992). Larger tuber size is probably related to the lower number of tubers per plant in plots receiving early-season moisture stress. It is known that average tuber size for healthy plants is inversely related to the number of tubers per plant. With the imposition of drought stress, accumulation of tuber dry matter and final tuber yield have been shown to decline (Levy 1985) although the effect may depend on the stage of potato development during the stress period (Schafleitner 2009). A lower tuber number may be compensated for by a higher assimilate partitioning to tubers (Jefferies and MacKerron 1989) and therefore a higher tuber size (Deblonde and Ledent 2001). In the study by Deblonde and

Ledent (2001), reduction in tuber number (17%) in the drought treatment was not associated to lower tuber yields due to compensation by average tuber dry weight (6%). So, the analysis of the response to drought stress of the cultivars should not be restricted to differences in yield (Lahlou et al. 2003). Yield depends on accumulation of dry matter and on its partitioning into tubers as well as on the tuber dry matter content (Heuer and Nadler 1995). Water stress resulted in a preferential supply of assimilates to the tubers (Munns and Pearson 1974). Dry matter production and its accumulation in the tubers are important quality criteria and important parameters for assessment of adaptation to stress conditions.

2.3.2 Cultivar responses under drought stress

The ability to minimize potato yield and quality losses due to drought can be greatly improved by understanding the relative responses of different cultivars to seasonal variations in water supply (Stark et al. 2013). Potatoes are an irrigated crop and can use water effectively as reflected by a high harvest index (Vos and Haverkort 2007). But many potato cultivars are sensitive to drought stress (Iwama and Yamaguchi 2006). Potato response to drought varies widely among cultivars (Table 2.1). Cultivars within a similar maturity class can be affected differently by water stress. Gandar and Tanner (1976) found that 'Russet Burbank (RB)' was very sensitive to water stress during the major period of tuber expansion. Martin and Miller (1983) showed that RB was more sensitive than other cultivars to soil water deficits with consistently low specific gravity and poor tuber quality. Stark et al. (2013) found RB produced comparatively high total yields across the range of drought treatments, but U.S. No. 1 yields (tubers with diameters greater than 48 mm and less than 5% internal and external defects) were substantially reduced but was less sensitive to changes in drought severity than other cultivars. Stark et al. (2013) also concluded that late maturing and stress susceptible cultivars like RB is

susceptible to large losses of marketable tubers under either moderate season-long stress, or sudden severe water stress caused by termination of irrigation. Shock et al. (1992) suggested that to obtain optimum yield and processing quality of RB, the first irrigation should be at or after full plant emergence. Shock et al. (1992) found that early-season water stress did not affect specific gravity of RB potatoes. Deblonde and Ledent (2000) took the difference in tuber dry weight between the irrigated and the drought treatments as a measure of sensitivity to water shortage. They found the potato cv. ‘Nicola’ as a drought tolerant and ‘Krostar’ as a sensitive type.

Table 2.1. Potato cultivar responses to drought stress

Cultivar	Drought tolerant	Drought sensitive	Author	Stress Application	Response Measurement
'Alturas'		X	Stark et al. (2013)	Field. Irrigation: 100% to 50% ET from tuber initiation stage and terminating at late bulking period.	Yield.
'GemStar Russet'		X	Stark et al. (2013)	Field. Irrigation: 100% to 50% ET from tuber initiation stage and terminating at late bulking period.	Yield.
'Horizon'	X		Puértolas et al. (2014)	Greenhouse and field. Irrigation: $\theta_v < 0.20$ cm^3cm^{-3} from 2/3 weeks after emergence.	Gas exchange, leaf water potential, leaf xylem ABA concentration, shoot biomass and root growth.
'Kennebec'	X		Wolfe et al. (1983)	Field. Differential irrigation treatment starting from tuber initiation stage.	Seasonal pattern of soil water depletion, WUE, leaf area development and dry matter partitioning.
'Konyu-2'	X		Deguchi et al. (2010)	Field. Irrigation: $\Psi < -60$kPa at 48 DAP.	Yield, Harvest index, leaf area index.
'Maris piper'		X	Puértolas et al. (2014)	Greenhouse and field. Irrigation: $\theta_v < 0.20$ cm^3cm^{-3} from 2/3 weeks after emergence.	Gas exchange, leaf water potential, leaf xylem ABA concentration, shoot biomass and root growth.
'Monona'	X		Stark et al. (1991)	Field. Irrigation ≈ 40 to 50% of pET during tuber bulking stage.	Canopy temperature.
'Nooksack'	X		Martin and Miller (1983)	Field. Variable irrigation from 0 to 100% ET at 70 DAP.	Yield.
	X		Stark et al. (1991)	Field. Irrigation ≈ 40 to 50% of pET during tuber bulking stage.	Canopy temperature.
'Red Pontiac'		X	Stark et al. (1991)	Field. Irrigation ≈ 40 to 50% of pET during tuber bulking stage.	Canopy temperature.

Table 2.1 continued…

Cultivar	Drought tolerant	Drought sensitive	Author	Stress Application	Response Measurement
'Russet Burbank'	X		Stark et al. (2013)	Field. Irrigation: 100% to 50% ET from tuber initiation stage and terminating at late bulking period.	Yield.
		X	Bélanger et al. (2001)	Soil moisture reserve was reduced to 65% of the soil water holding capacity during early tuber bulking stage.	Tuber bulking, biomass partitioning to tubers and large roots.
		X	Stark and Mccann (1992)	Field. Irrigation: 60 or 80% ET during tuber bulking period.	Yield.
		X	Stark et al. 1991	Field. Irrigation ≈ 40 to 50% of pET during tuber bulking stage.	Canopy temperature.
		X	Martin and Miller (1983)	Field. Variable irrigation from 0 to 100% ET at 70 DAP.	Yield.
'Russet Norkotah'		X	Stark et al. (2013)	Field. Irrigation: 100% to 50% ET from tuber initiation stage and terminating at late bulking period.	Yield.
'Shepody'	X		Bélanger et al. (2001)	Soil moisture reserve was reduced to 65% of the soil water holding capacity during early tuber bulking stage.	Tuber bulking, biomass partitioning to tubers and large roots.
		X	Coleman 1986	Greenhouse. When plants were 6-8 weeks old; irrigation: twice @200 ml in 5 litre pots over 16 days.	Leaf water retention, epicuticular wax levels, desiccation tolerance, root growth, transpiration rate and stomatal resistance.
'White Rose'		X	Wolfe et al. (1983)	Field. Differential irrigation treatment starting from tuber initiation stage.	Seasonal pattern of soil water depletion, WUE, leaf area development and dry matter partitioning.

2.3.3 Effects of drought on physical growth parameters

Characterization of drought tolerance in potato cultivars is complicated by the fact that differential yield responses have not been consistently related to specific physiological or morphological traits (Stark et al. 2013). Different growth strategies can be followed by the potato plant to adapt to different drought conditions without tuber yield being significantly affected (Lahlou et al. 2003, Deblonde and Ledent 2001). The first morphological manifestation of drought effects in the potato is a reduction in leaf size (Jefferies and MacKerron 1987). It results in a reduction in the amount of intercepted radiation and leads to a decrease in tuber dry mass accumulation (Jefferies 1993). Drought affects potato canopy architecture by decreasing leaf size and leaf expansion rate while limiting formation of new leaves and increasing the rate of senescence (Fleisher et al. 2008). Jefferies (1993) showed that the final size of individual leaves was reduced by drought.

Deblonde and Ledent (2001) found that potato stem height was sensitive to drought and the effect was stronger on later cultivars especially when water shortage started early. They also found in case of drought tolerant potato cultivar Désirée that drought affected more strongly stem height than tuber yield. Severe water stress (40% of pot capacity) reduced plant height by 46%, stem diameter by 51%, total dry weight by 43% and relative leaf expansion rate (RLER) by up to 75% (Kirnak et al. 2001). Drought stress reduced the total stem number by 28% in the field but had no influence on this component in the greenhouse in a study by Lahlou et al. (2003) who also found that drought stress reduced total aerial biomass, dry mass of leaves, leaf area index and leaf area duration. Kirnak et al. (2001) found that the root to shoot ratio was 2.1 times higher in water stressed eggplants than in controls and concluded that water stress in eggplants altered the pattern of dry matter distribution favoring the roots. The decrease in fruit yield, quality and

plant growth induced by water deficit was a consequence of a reduction in both RLER and transpiration (Kirnak et al. 2001).

Large root mass, high harvest index coupled with high leaf/stem ratio with low number of branches may contribute to high and stable yields in drought prone environments (Deguchi et al. 2010). A small shoot dry weight is considered to be associated with poor drought tolerance in potato (Deguchi et al. 2010). Schittenhelm et al. (2006) reported that a cultivar with a compact canopy easily showed a reduced radiation interception even by a small reduction of shoot dry weight. Jefferies and MacKerron (1987) also reported that yield reduction by drought was mainly due to a reduction in radiation interception caused by reduced canopy expansion.

Deblonde and Ledent (2000) found that moderate drought conditions did not influence the harvest index but Jeffries and MacKerron (1993) showed that the harvest index under severe drought was lower than in control. Shock et al. (1992) found that plant top growth was reduced by stress but stems per plant, stolons per plant and stolons per stem were unaffected by water stress. Various mechanisms for coping with drought-prone environments have been described (Levitt 1980). Large root mass, high harvest index coupled with high leaf/stem ratio with low number of branches may contribute to achieve high and stable yields in drought prone environments (Deguchi et al. 2010). Reduced leaf growth and accelerated leaf senescence are common responses to water deficits and could be an adaptation of plants to water deficit (Lahlou et al. 2003). Early maturation and shortening the period that plants are exposed to severe stress (stress evasion or escape) diminished yield loss. However, early maturity is often associated with lower yield potential under non-stress or mild stress conditions, compared with later-maturing varieties, due to a shorter growth period (Levy et al. 2006).

2.4 Effects of drought in water relation parameters

Plant water management is a combination of increasing water uptake and reducing water loss during drought stress. While this thesis did not examine water uptake mechanisms, parameters related to reducing water loss were studied. Most used indicators of drought stress resistance are yield (Farshadfar and Elyasi 2012), leaf water content (Omae et al. 2005) and excised leaf water loss (Wang and Clarke 1993). If water relation parameters are to be selected as drought resistance selection criteria, these must be associated with improved yield under drought stress, have a high heritability (Blum 1989) and can be measured simply and accurately in large populations (Dhanda and Sethi 1998).

2.4.1 Leaf water content

Numerous studies (Schonfeld et al. 1988, Martin et al. 1989, Omae et al. 2005) expressed leaf water content at full turgor which is termed as relative water content (RWC) which helps to normalize the variation of sampling particularly at noon when leaf water loss is highest and more variability between samples can occur (Turner 1981). Leaf water content is a more important indicator of water status than other water potential parameters under drought stress conditions (Sinclair and Ludlow 1985) and was proposed as a selection criterion for drought tolerance in barley (Matin et al. 1989), wheat (Schonfeld et al. 1988) and pigeonpea (Kimani et al. 1994).

2.4.2 Stomatal characteristics

Stomata play a major role in controlling gas exchange, especially in carbon dioxide uptake and in transpiration in response to changes in the surrounding environment (Tanaka et al. 2005). The number, distribution, and morphology of stomata on leaf surfaces are an important trait in the adaptation of plants to changing environmental conditions because stomata partly

control CO_2 uptake and transpiration (Malone et al. 1993). Plants regulate the exchange of CO_2 and water vapor with the atmosphere by adjusting their photosynthetic capacity and changing stomatal aperture (Kamakura et al. 2011). When plants suddenly encounter drought, the most important quick response is stomatal closure (Yordanov et al. 2000). Stomata express differential degrees of closure in response to drought to limit water loss through transpiration. Compared to many other crop plants, potatoes close their stomata at relatively low soil moisture deficits (Sadras and Milroy 1996).

Changes in stomatal density (number of stomata per unit leaf area); length, width and area of stomatal apertures contribute to changes in water use efficiency, photosynthetic rate and biomass accumulation under different environmental conditions (Malone et al. 1993). In maize, drought resistant lines showed higher stomatal frequency and smaller stomatal apparatus (Ristic and Cass 1991). To prevent water loss and facilitate CO_2 diffusion to mesophyll cells, the stomatal aperture mechanism responds variably to environmental factors including light intensity, water status, temperature and nutrient supply (Kamakura et al. 2011). Stomatal density is influenced by plant nutrition and environmental variables that influence leaf development.

Stomatal aperture changes in response to humidity (Okamoto et al. 2009). Stomatal closure during soil drying is mediated by changes in root water status through chemical signals ascending from the root to the leaves and lead to the closure of stomata in concert with the level of soil water stress (Yordanov et al. 2000). There are many signals that induce stomatal closure. The best known signal is ABA (Wright 1969) along with secondary messengers, such as Ca_2+, H_2O_2 and NO (Arve et al. 2011). Passive loss of turgor pressure also results in stomatal closure. Stomatal closure is correlated with a decline in leaf turgor as a consequence of low water potential (Kramer 1988). The turgor allows stomatal opening and cell expansion, root growth

and increase in productivity (Kozolowski and Pallardy 2002). By increasing the concentration of solutes in the symplast, turgor can be maintained at low tissue water potentials by enabling water to continue to be extracted from dry soil (Khalil and Grace 1992). When stomata are closed, leaf cuticle layer plays a fundamental protective role against water loss (Jenks and Ashworth 1999).

2.4.3 Leaf cuticle layer

Outer surfaces of the aerial parts of the plant body are covered with a thin continuous layer of predominantly lipid material known as the cuticular membrane or cuticle (Holloway 1982). The cuticle has an important role as a boundary layer between the body of the plant and its environment. It prevents the loss of plant components by leaching; is a supplement to the action of stomata in regulating the passage of water from within the plant to the atmosphere (Hajibaghery et al. 1983). Although stomata serve as the primary regulator of water loss to the environment, the cuticle also plays an important role. When stomata close during drought stress, the waxy cuticle can become the controlling factor in water loss (Conner and Conner 1984, Jenks and Ashworth 1999) and, to a lesser extent, CO_2 exchange (Boyer et al. 1997, Yoo et al. 2009).

An adaptation to plant life in dry environments is thick cuticles and wax layers as thick cuticles and wax layers reduce extra-stomatal transpiration (Arve et al. 2011). Plants adapted to dry environments have thicker cuticle and lower rates of transpiration through the cuticular membrane than plants from less dry environments (Oppenheimer 1960). Many plants possess the ability to respond to water-limited environments by increasing the deposition of epicuticular waxes which likely increases the cuticle's ability to function as a hydrophobic barrier (Mamrutha et al. 2010). When potato cultivars were exposed to mild drought stress, the drought resistant cv. Raritan doubled its epicuticular wax level whereas the drought sensitive cv. Shepody increased

its wax level by less than 20% (Coleman 1986). A study with maize by Ristic and Jenks (2002) revealed an inverse relationship between the epidermal water loss and cuticle thickness where a maize line with lower rates of shoot and detached leaf water loss had thicker cuticlular layer than a line with higher rates of shoot and detached-leaf water loss.

Structurally, the cuticle is composed of three layers: a) the outermost layer is epicuticular (cuticular) wax, b) beneath the epicuticular wax is the cuticle proper and c) below the cuticle proper is the cuticular layer, containing cell wall material primarily cellulose (Ristic and Jenks 2002). Compositionally, the cuticle is characterized by two specific groups of lipid substances: insoluble polymeric cutins and soluble waxes. The role of wax components (organic compounds, mainly alkanes, alcohols, fatty acids and *n*-alkyl esters) is to protect plants from loss of water (Halinski and Szafranek 2006). The relationship between the water permeability of a cuticle and its chemical composition was described in detail by Zhang et al. (2013).

The cuticle is a mechanically important structure and its properties are dynamically modified by the plant in response to internal and external stimuli (Bargel et al. 2006). Hajibagher et al. (1983) reported on the structure of the cuticle and on cuticular transpiration in the leaves of the halophyte *Suaeda maritima* (L.) grown either in the presence or absence of sodium chloride. Cuticular transpiration was found as inversely related to cuticular thickness and with increasing salinity.

2.4.4 Excised-leaf water loss

Optimum CO_2 fixation is essential for optimal crop production in potato (Blum 2009). Complete restriction of transpiration through stomatal closure as a drought avoidance mechanism may not be a realistic option for production systems (Levy et al. 2013). However, reduced

transpiration leading to sub-optimal yield at relatively consistent production levels may be acceptable for subsistence farming in drought prone environments (Sinclair 2011). The assessment of water loss from excised leaves showed promise for characterizing drought resistance in wheat genotypes (Clarke and McCaig 1982, Clarke et al. 1989). This trait is moderately heritable (Clarke and Townley-Smith 1986) and can be easily determined (Clarke and McCaig 1982, Dhanda and Sethi 1998). Detached rose leaf water loss was dependent on humidity and photoperiod (Mortensen and Fjeld 1998, Torre and Fjeld 2001, Arve et al. 2013). Similar results were also found in *Solanum Laciniatum* (Conner and Conner 1984) and *Arabidopsis thaliana* (Louise Elisabeth Arve p.c.).

Transpiration is a primary determinant of leaf energy balance and plant water status (Pearcy et al. 1996). It determines the WUE together with the exchange of CO_2. Stomatal closure is the main cause for transpiration decline as water stress develops. Stomates close in excised leaves and have been used to monitor rate of water loss which enters into a linear phase after 20 to 30 minutes that lasts for several hours (McCaig and Romagosa, 1991). During this latter phase, the water is lost from incompletely closed stomates (Dhanda and Sethi 1998).

Transpiration is directly proportional to the gradient of water vapor concentration from the internal evaporation surface to the bulk air outside the leaf. Transpiration is inversely proportional to the total resistance to water vapor transport of the air boundary layer and of the leaf. Since stomata control only one part of the total resistance, their closure will vary in effect with the magnitude of stomatal resistance relative to that of boundary layer resistance and cuticular resistance (Hsiao 1973). Water stress causes a decrease in transpiration, an increase in foliage temperature and closure of stomata (Tan and Buttery 1982).

2.4.5 Stem as a water reservoir during drought stress

Externally, the stem is bounded by the epidermis which contains typical epidermal cells, guard cells, idioblasts and trichomes (Fahn 1982). Internal to the epidermis is the cortex which is sometimes differentiated into three layers: the outermost layer is the hypodermis, middle is the general cortex and the innermost is the endodermis. Hypodermis is made up of three to four layers of cholenchymatous cells. It gives mechanical strength to young stems. General cortex is made up of parenchymatous cells and the function is storage. The endodermis is a single layer with (thin or thick-walled) barrel-shaped cells. Next to the endodermis is the pericycle. The pericycle is composed of several layers, made up of sclenchymatous cells and a separate cortex from vascular bundle. Vascular bundles are one of the most important structures in dicot stems and are arranged in a ring. These vascular bundles are conjoint (both phloem and xylem are arranged in the same radii), colateral (phloem lies outside the xylem), open (cambium ring is present between the phloem and xylem that produces secondary xylem and phloem) and withmetaxylem is towards the periphery and protoxylem is towards the centre of the stem (Taiz and Zeiger 2002). There is a medullary ray presents between two adjacent vascular bundles and this helps in radial passage for the conduction of water and minerals. Central tissue is the pith and it is made up of loosely arranged thin walled parenchymatous cells.

Xylem is a complex tissue and consists of four kinds of cell such as xylem fibre, xylem parenchyma, tracheids and vessels (Sinha 2004). Tracheids, vessels and fibres are dead cells (Salisbury and Ross 1992). Vessels and tracheids take part in translocation of water. Vessels are long continuous pipe-like cells formed by the dissolution of end walls of tracheids. Vessels lack protoplasm at maturity and the cell wall gets depositions of lignified tissues. Movement of water in vessels is not obstructed by living matter as these are dead cells (Nobel 2005). Tracheids are

elongated, spindle shaped cells tapering at their ends and overlap each other in longitudinal order. Vessels are elongated, tubular structures with pitted side walls and perforated end walls with blunt ends. Vessels are arranged longitudinally, one upon another, to make a continuous tube which are called conduits. Xylem fibres are long thin cells with thickly lignified cell walls, lack protoplast at maturity and hence are mechanical in nature. Xylem parenchyma are living cells and used for the storage of carbohydrate, in the lateral movement of water and solutes in and out of the conducting cells (Noble 2005).

Phloem is also a complex tissue consisting of four kinds of cells: sieve tubes, companion cells, phloem fibres and phloem parenchyma (Sinha 2004). Sieve tube elements are elongated and connected end-to-end to form a continuous system throughout the length of the plant (Nobel 2005). The end walls separating two vertical adjacent sieve tube elements are specialized as sieve plates. Sieve plates are perforated structures. Adjoining sieve tubes are connected by plasmodesmata which help in increasing the continuity of the conducting system. Plasmodesmata pass through these openings in the sieve plates. The phloem fibres lend support to the tissue. Thin-walled living parenchyma cells contribute to the process of translocation. Lateral transport of organic solutes and water occurs through parenchyma cells. These cells also store food.

The pith is a more or less cylindrical body of tissue in the centre of the axis, enclosed by the vascular tissues. The pith consists of rather uniform tissue, mainly thin-walled parenchymatous, in which the cells are arranged usually loosely. Often thick-walled, lignified parenchyma cells and sclereids are also present. In the ontogeny of the stem, the pith cells in the internodes of many species mature very early and stop growing, whereas the surrounding tissues are still meristematic and continue to enlarge longitudinally and in circumference (Fahn 1982).

Thus the pith may be torn apart and a hollow pith is formed, with the broken cell walls lining the cavity. This is common in herbaceous plants.

Stems of the herbaceous plant can function as a temporary water reservoir and the stored water in the stem can be used to prevent excessive decrease in leaf water potential when the leaf transpiration is rapidly increased (Kitano and Eguchi 1989). Because of the large volume of pith parenchyma in the stems of many herbaceous plants, they function as significant water reservoirs (Kramer 1983). Stark et al. (1991) found that the ability of drought tolerant potato genotypes to maintain adequate transpiration during drought was due in part to a greater ability to conserve soil water when conditions were optimal. In a study with intact stem of cucumber plants by Kitani and Eguchi (1989), root water absorption lagged about 10 minutes behind leaf transpiration; dynamics of water fluxes were affected by the lag of water absorption in roots; temporary water loss caused by rapid increase in leaf transpiration was buffered by about 5% of the water content in the stem. Therefore the authors suggested that the stem water storage might have preserved water balance, avoiding excessive leaf water deficit caused temporarily by the rapid increase in evaporative demand and by the lag of root water absorption. Tree trunks also function as water reservoirs (Kramer 1983). Stem water reservoir is able to augment water supply to the leaves and provides a substantial degree of protection against desiccation stress in tropical alpine rosette plants (*Espeletia* and *Senetio* species, Holbrook 1995). Stem water replaces transpirational losses in tropical forest canopy trees before soil water (Goldstein et al. 1998). Stem reservoir in white oak trees played a key role in supplying water for transpirational use especially as the soil reservoir depletes (Hinckley et al. 1974). The amount of water stored in the trunk of *Pinus pinaster* accounted for 12% of the daily transpiration when soil water was

abundant, but increased to 25% of the daily transpiration at the end of summer following a period of drought (Loustau et al. 1996).

2.5 Impact of drought acclimation on drought stress resistance

Acclimation is defined as a "non-heritable modification of structure and function as a response to a given climatic constraint which minimizes damage and improves the fitness of an individual plant" (Kacperska 1999). Gradually increasing stresses may induce physiological adjustment that protects plants from growth inhibition and injury when environmental stresses are suddenly imposed.

Four mechanisms of acclimation to soil drying have been identified. The first of these involves a shift in the allocation of assimilates from shoot to root (Khalil and Grace 1992) that reduces transpirational demand relative to water absorption (Pallardy 1981). The second mechanism of acclimation involves osmotic adjustment. By increasing the concentration of solutes in the symplast, turgor can be maintained at low tissue water potentials, as low water potential enables water to continue to be extracted from dry soil (Khalil and Grace 1992). The turgor allows stomatal opening and cell expansion, root growth and increase in productivity (Kozolowski and Pallardy 2002). The third mechanism of acclimation is the closure of stomata in response to a reduction in soil water content (Khalil and Grace 1992). Stomatal closure is correlated with a decline in leaf turgor as a consequence of low water potential (Kramer, 1988). Stomatal closure during soil drying is mediated by changes in root water status through chemical signals ascending from the root to the leaves and lead to the closure of stomata in concert with the level of soil water stress (Yordanov et al 2000). The other mechanism is a relationship between osmoregulation, turgor maintenance and growth under water stress conditions (Wright

et al. 1996). Osmotic adjustment (OA), defined as a cellular adaptation resulting in net solute accumulation during water deficit, is considered to be an adaptive response of plants to maintain turgor, growth and yield during drought (Levy et al. 2006). OA facilitates the maintenance of cell turgor during periods of water stress (Turner and Jones 1980) and contributes to leaf survival by maintaining higher relative water content at low water potentials (Flowers and Ludlow 1986) and during Recovery. The contribution of OA to recovery is especially important when plants are exposed to occasional periods of water deficit and need to cope with transient drought (Levy 1992).

Watkinson et al. (2008) identified accessions of *Solanum tuberosum* ssp. *andigena* that showed varying degrees of physiological acclimation or adaptation to repeated drought stress. Major adaptive responses to high temperature of tuber initiation and bulking, the degree of osmotic regulation and turgor maintenance, as well as the capacity to develop extensive root systems, could be used as selective criteria for adaptation to hot and dry climates (Levy 1983). Etehadnia et al. (2008) reported the ability of diploid and tetraploid potatoes to acclimate to salinity, a physiological drought. Positive impact of drought acclimation towards drought stress resistance was found in in *Acer pseudoplatanus* L. (Sycamore) (Khalil and Grace 1992) and in woody plants (Kozolowski and Pallardy 2002).

2.6 Measuring soil water content

The state of water in soils can be described in two ways: in terms of the quantity present and in terms of the energy status of the water. The quantity present is expressed either in gravimetric (mass) terms or volumetric terms. The energy status of water in soil can be expressed as total soil water potential. Six techniques for measurement of soil water were described by Rundel and Jarrell (1996). Gravimetric analysis, neutron probe measurements and time domain

reflectometry (TDR) measure soil water content. Tensiometers and resistance blocks measure soil metric potential as an analog of soil water potential. Soil psychrometers measure soil water potential.

Time domain transmission, TDT sensors (Sun and Young 2001) measures the transmissivity of the soil by sending an electromagnetic signal along a wave guide and measuring the propagation time of that wave (Miralles-Crespo and van Iersel 2011). The wave propagation time depends on the soil dielectric properties, which are mainly governed by the water content of the soil surrounding the probes (Blonquist et al. 2005). TDT sensors have potential use in drought stress research (Miralles-Crespo and van Iersel 2011) and are comparable to standard time domain reflectometry (TDR) measurements of soil with < 40% clay and < 2 Dsm^{-1}. Measuring soil water content by direct gravimetric sampling of the soil can be time-consuming and can be accomplished only under steady-state conditions (Munoz-Carpena 2004) and thus TDT was used.

3.0 MATERIALS AND METHODS

3.1 Establishment of tubers and plants

Tubers of three potato genotypes 'Vigor' (V), 'Russet Burbank' (RB) and 'Fv12246-6' (Fv) tubers were provided by Dr. Benoit Bizimungu of Agriculture and Agri-Food Canada, AAFC (Fredericton, NB). Vigor originated from a cross between 'Agria' and 'Wischip' at AAFC, Lethbridge, Alberta. 'Agria' has medium to high drought resistance. Fv is an unregistered breeding clone (F72117 X ND860-2). Clones of these germplasm using both tubers and cuttings were propagated for this study to determine drought stress resistance under a low RH greenhouse environment. Russet Burbank was originally released in 1902 as May's Netted Gem by L. L. May & Co. (St. Paul MN). The names Netted Gem and Russet Burbank were used synonymously for many decades. Isoenzyme, multiplex PCR, and SNP data confirmed Russet Burbank as a mutation of Burbank and do not support a seedling origin (Bethker et al. 2014).

During a previous study in our lab in 2010, potato genotypes Fv, V and RB were studied to determine their resistance to drought stress. The treatments were Non-Acclimated (NA) and Drought Acclimated (DA). DA plants were exposed to drought acclimation cycle for 13 times over 7 weeks period. The soil moisture content was maintained as 25-35% and let to drop down to 10% and go up to 25-35% by re-watering. Harvested tubers were kept in a cold room (4°C) for 3 months. NA tubers (50-250g) were planted in 11 L pots with SM#4 Mix (Sunshine Mix No. 4, Sungro Horticulture Canada Limited) just to cover the tubers. Pots were placed in a greenhouse (day/night temperature 25/22°C, RH 50%, 18 hours photoperiod and light intensity 250-300 µmol m^{-2} sec^{-1}). When plants had reached 5 cm above the pot rim, multiple stems were reduced to a single main stem and additional SM#4 mix was added to fill the pots up to 2.54 cm below the pot rim. Soil water content in the pots was monitored by time domain transmission, TDT

sensors (20.5 cm length, Gro Point Lite, ESI Environmental Sensors Inc., Sidney, BC, Canada) (Sun and Young 2001) and was maintained as 30-45% (Costa et al. 1997). Plants were fertilized twice a week (N:P:K 20:20:20, 1 g L^{-1} with 500 mL per pot). As the growth of the plants was not uniform, cuttings were taken from 8 week old plants and at 3-4 cm below the shoot apical meristem of each of Fv, V and RB during September - December, 2011 in the College of Agriculture and Bioresources Greenhouses (45 Innovation Blvd., Saskatoon, SK Canada).

All cuttings were dipped in rooting hormone (PlantProd StimRoot No. 1, 0.1% indole-3-butyric acid rooting powder, Spectrum Brands IP Inc., Canada). Approximately 50 cuttings from each genotypes were potted into SM#4 Mix with approximately 10 g of granular slow release fertilizer (N:P:K::14:14:14, Nutricote, Chisso-Asahi Fertilizer Co., Japan) on a polystyrene tray (59.5 cm x 35cm x 14.5cm). The trays were then placed on a heated mist bed (100% RH, mist from 5 am to 11 pm at 15 min interval and 30 s duration). Three week old cuttings were transplanted into SM#4 Mix in 11 L pots and the pots were placed in a greenhouse (day/night temperatures 25/22°C, RH 17-25%, 18 hours photoperiod and light intensity 250 µmol m^{-2} s^{-1}). Plants were watered as needed and fertilized twice a week. When plants had reached 5 cm above the pot rim, all other stems except the main stem were removed. Additional SM#4 Mix was added up to 2.5 cm below the rim. Pots were placed randomly into 60 cm x 40 cm x 21.5 cm white plastic trays. Four TDT sensors were placed vertically into NA, DAS and NAS pots (Table 3.1) and captured soil moisture through the vertical soil column. Twenty-seven plants (9 plants from each of the three genotypes Fv, V and RB) were used. The treatments and codes were assigned as shown in Table 3.1. This entire experiment was repeated between December 2011 – April 2012. In the second experiment, when plant cuttings were transferred from mist bed to 11 L pots and placed in the greenhouse, all V plants died of aphid attack. So, this second experiment

was conducted with Fv and RB genotypes. Specific responses of interest (cuticular/stomatal responses and stem cross section, IR thermal imaging, stem water content) were taken in the second and third experiments, respectively. Tubers with no previous drought stress history (NA) were used to generate the plants. In summary, the following parameters represent the mean of two separate experiments: yield, leaf wilting score, %leaf water content, leaf water loss, plant height, shoot dry weight, stem diameter and stem number. The consistency between the two experiments was not checked due to insufficient replications within each of the experiments. However, the mean response of each experiment to the treatment was similar.

Table 3.1. Codes for genotypes and treatments

Genotypes	Treatments	Codes
Fv12246-6 (Fv)	Non-Acclimated and Non-Stressed (NA) Drought Acclimated and Drought Stressed (DAS) Non-Acclimated and Drought Stressed (NAS)	Fv-NA
		Fv-DAS
		Fv-NAS
Vigor (V)		V-NA
		V-DAS
		V-NAS
Russet Burbank (RB)		RB-NA
		RB-DAS
		RB-NAS

3.2 Drought Acclimation, Drought Stress and Recovery cycles

Cycles of Drought Acclimation, Drought Stress and Recovery periods are outlined in Fig. 3.1. When plants were about six weeks old, the first Drought Acclimation cycle (1^{st} DA) was imposed by withholding water down to 10% soil moisture content (Fv-DAS, V-DAS and RB-DAS) and then re-watered to 25 – 35% soil water content. At this time, all NA and NAS

treatments were watered to maintain 30 - 40% soil water content and fertilized as usual. Another Drought Acclimation cycle (2^{nd} DA) was applied about 5 - 7 days later. The critical stages of stolon formation and tuber initiation corresponded to blooming of the first four flowers (Appendix A1) on the potato cymose inflorescence (Huaman 1986). At this stage, all DAS and NAS treatments were exposed to the first severe Drought Stress cycle (1^{st} DS). All NA plants were watered and fertilized as usual. When the soil water content in the DAS and NAS pots dropped to 0%, plants were not watered until they showed visible wilting (after 7-10 days, 75% leaves wilted (stage 3), Table 3.2). Soil water content was recorded by TDT sensors and corresponded to the volumetric method by Sun and Young (2001). A similar correlation was also found in this study (Appendix A2). All Fv-NAS plants were the first to reach stage 3-4. All genotypes and treatments were then re-watered to 25-35%. All plants were allowed to recover (1^{st} R) in the greenhouse until Stage 0 was reached (in 5-7 days, Table 3.2). Subsequently, another severe Drought Stress cycle (2^{nd} DS) was applied followed by a second Recovery cycle (2^{nd} R).

Table 3.2. Visual scoring for leaf wilting during Drought Stress treatments

Stage	% leaves wilted
0	None
1	25
2	50
3	75
4	100
5	100 + stem wilting

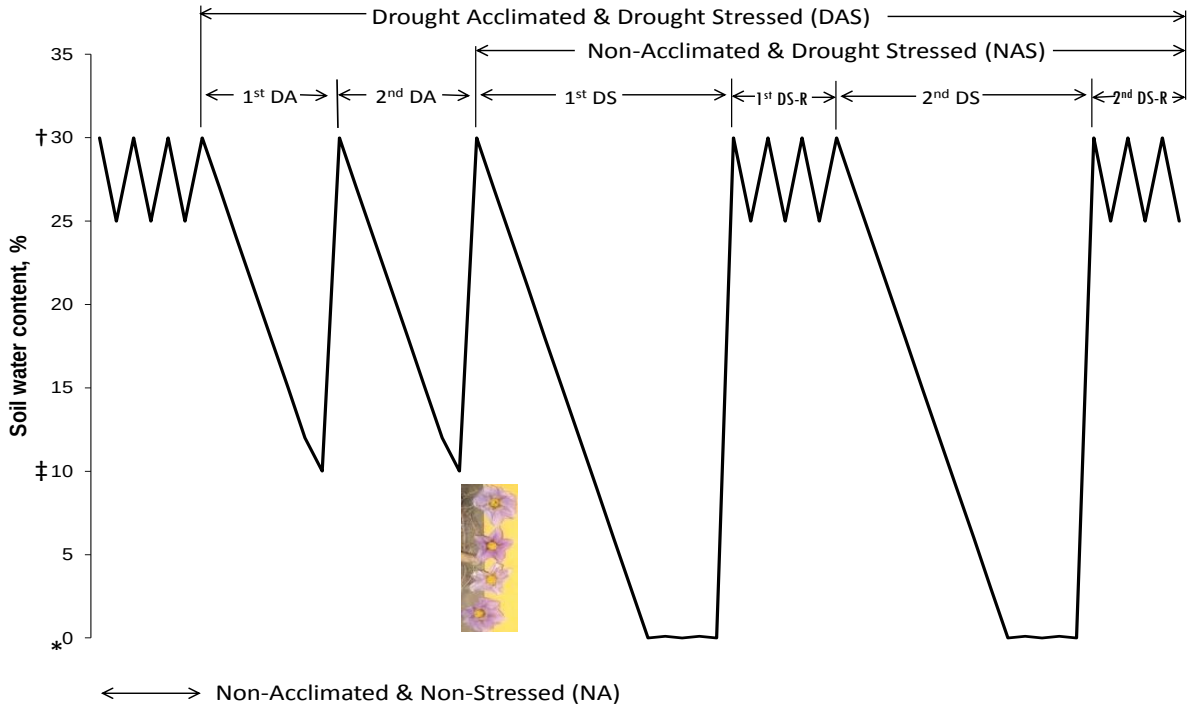


Fig. 3.1. Schematic diagram of applying Drought Acclimation and Drought Stress cycles. * 4%, ‡ 12% and † 32% of soil water content as determined by volumetric method (Fig. A2). DA- Drought Acclimation cycle, DS- Drought Stress cycle, R- Recovery cycle. NA: Non-Acclimated and Non-Stressed, DAS: Drought Acclimated and Drought Stressed and NAS: Non-Acclimated and Drought Stressed.

3.3 Shoot characteristics

Plant height was measured from the top of the soil surface to the apical meristem in all plants and treatments. Final stem diameter was measured at 2.5 cm above the soil surface. Fresh stem sections were then cut at this point in all plants and treatments. Cross sections were observed through low vacuum scanning electron microscopy with default setting (JSM 6010, JEOL Ltd., USA). Area of xylem vessels was measured by using ArcGIS software. Cross-sectional length of the entire xylem and pith regions was measured perpendicularly from the stem perimeter towards the centre of the stem using ImageTool (ImageTool version 3.0, UTHSCSA Dental Diagonostic Science, San Antonio, USA). Final stem number was recorded at

the end of the experimental period when the plants were more than three months old. Shoots (stem and leaf) of all plants were then placed in a drying room at 40 ^{0}C and data recorded when constant weight was reached.

3.4 Drought stress application to intact leaves on stems

Drought stress was applied to intact whole plants when leaves were still attached to the stems. Percentage leaf water content (%LWC) was then recorded at Stage 3 (Table 1) of Drought Stress when the first genotype (Fv-NAS) reached 75% wilting. At the end of both DA and DS cycles, the youngest fully expanded leaf of NA, DAS and NAS treatments was sampled for fresh weight (F_w) and dry weight (D_w) and %LWC expressed as:

$$\frac{F_w - D_w}{F_w} X100 \ldots\ldots\ldots (1)$$

In this study, %LWC was recorded at a standard stage (stage 3, Table 3.1) of Drought Stress which aimed to minimize variation between samples.

3.5 Leaf cuticular characteristics

At the end of DA and DS cycles (maximum soil water stress level), adaxial leaf imprints on one lateral leaflet of the youngest fully expanded leaf of each treatment (NA, DAS and NAS) and from genotypes Fv and RB were taken by using Suzuki's Universal Micro-Printing method (SUMP method, SUMP Laboratory, Tokyo) (Tanaka et al. 2005). Leaf imprints were analyzed by using Dinocapture software (AnMo Elevtronics Corporation, New Taipei City, Taiwan). Number of stomata per square mm area of the imprint was recorded. About 10-15 separate images were taken from one leaf imprint. Stomatal density, total square micron size of the stomata including the aperture (stomatal area) and area of the aperture (pore) were measured

using ImageTool as described in section 3.3. Area of leaf cuticle platelets and thickness of cuticle layer were measured by ArcGis software (Esri, 380 New York Street, Redlands, CA, USA) in the Social Sciences Research Laboratory, The University of Saskatchewan, SK, Canada.

3.6 Drought stress application to excised leaves

To assess the role of the intact whole plant, drought stress responses were compared on excised (Arve et al. 2015) recovered turgid leaves from stems of DA and DS treated plants. Moisture loss was subsequently monitored over a 15 minute period. The leaf petiole was placed into vacuum grease in a small plastic container on an analytical balance (Appendix A3) at 19-22°C and 17-23% RH. Leaf weight was recorded over a 15 minute time course at 1 minute intervals and rate of moisture loss (mg min^{-1}) determined.

3.7 Tuber yield

Tubers harvested from the treatments grown in 11 L pots were grouped into five different weight classes (<5 g, 5-20 g, 20-50 g, 50-100 g and >100 g). Tuber yield (total mass per pot, g) and tuber number were recorded.

3.8 Thermal recovery from maximum drought stress

Adaxial leaf temperature of NAS and DAS treatments were monitored during the period from maximum Drought Stress (end of DS cycles) to full recovery (R cycle) through infrared thermography (ThermaCAM S60, FLIR Systems, USA) (Wisniewski et al. 2008). Images were analyzed using ThermaCAM QuickView (FLIR Systems, USA).

3.9 Statistical analysis

Data were analyzed with statistical software SPSS version 20.0 (IBM Corporation, 1 New Orchard Road, Armonk, NY, USA). Overall difference among genotypes and treatments and interactions between genotypes and treatments were determined by using the general linear model at 95% confidence interval. Pairwise comparison among genotypes and treatments was determined using the Tukey test.

4.0 RESULTS

4.1 Tuber yield and number

Tuber yield was measured as the total mean tuber mass per pot. Although as expected, drought stress treatments (DAS, NAS) did induce lower mean tuber yield, variation was high and there was no significant effect on yield (Fig. 4.1a). By contrast, there was a genotype-dependent response when pooled across treatments in that RB had higher ($p<0.05$) yield than Fv (Fig. 4.1a). V had the highest mean total tuber number per pot (Fig. 4.1b). Separation of total mean tuber number into specific weight classes revealed the significant contribution of the smallest weight class (<5 g) to the V response (Fig. 4.2). RB expressed lower numbers of tiny tubers in this weight class but more large tubers in the 50-100 g category compared to V (Fig. 4.2) and this was the main contributing factor to RB's higher yield.

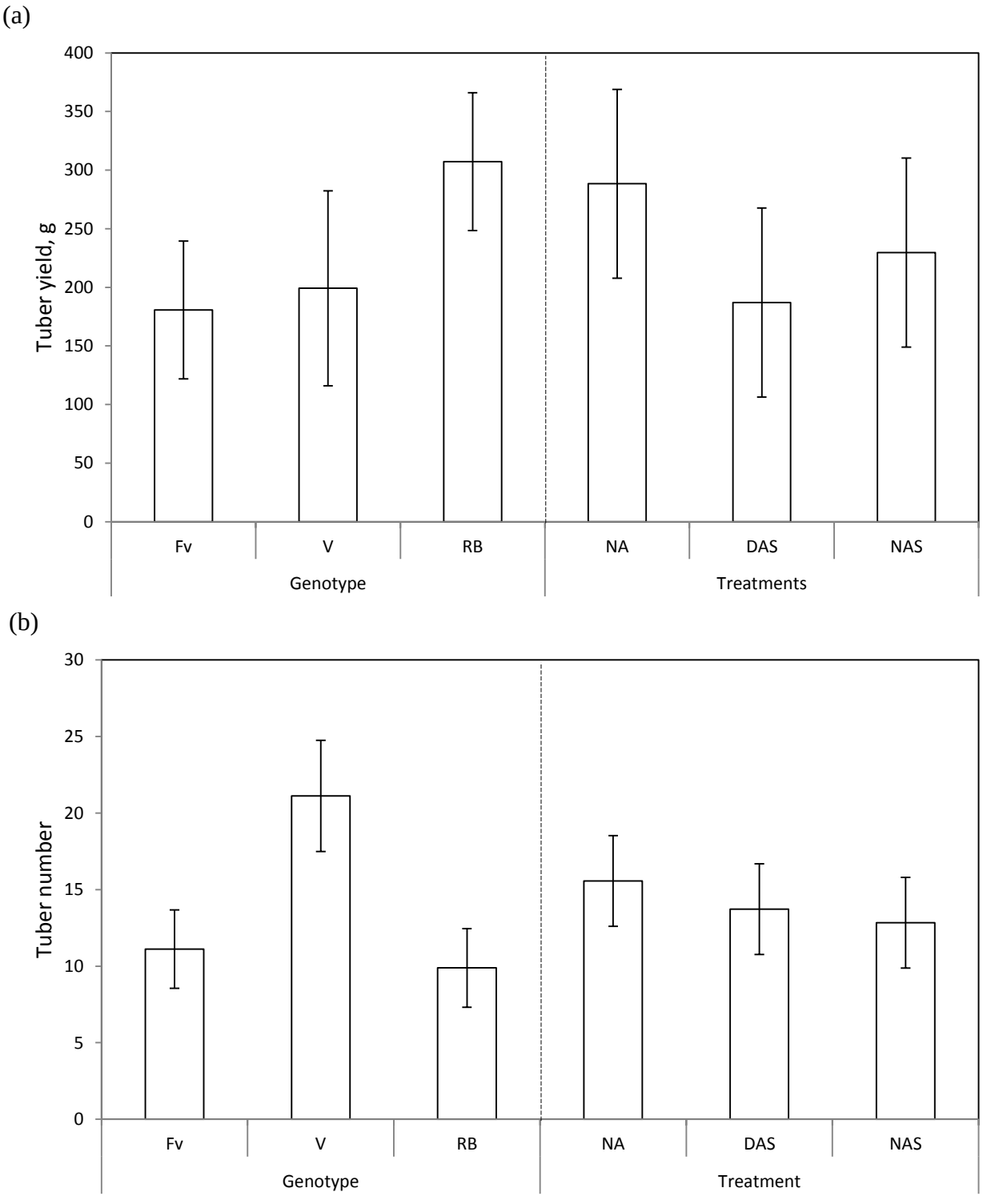


Fig. 4.1. Tuber yield and tuber number across three potato genotypes under drought stress. (a) tuber yield (g/pot) and (b) tuber number/pot in potato genotypes Fv, V and RB under NA, DAS and NAS treatments. Genotype data are pooled from all treatments and treatment data are pooled from all genotypes. Values represent means ± SE (*n* = 3-6 plants).

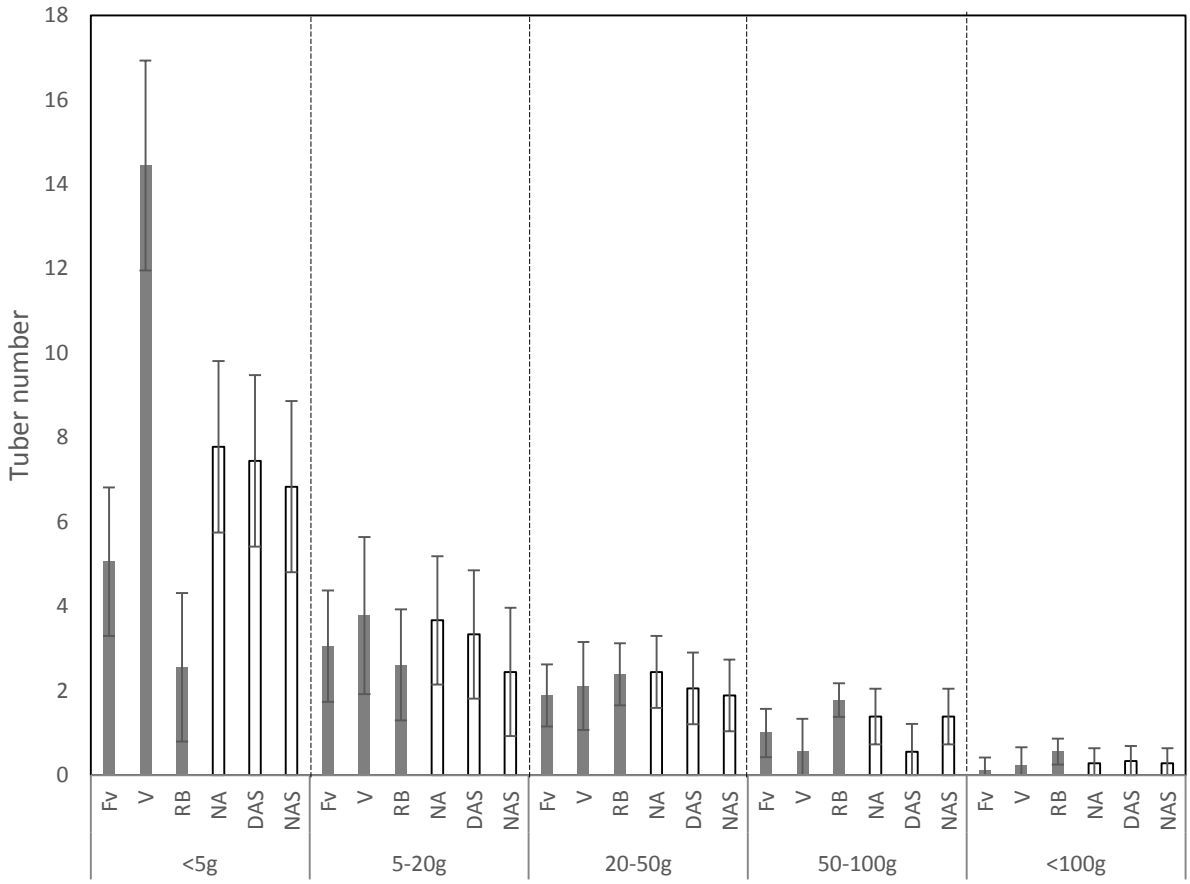


Fig. 4.2. Tuber number across different weight classes in three potato genotypes under drought stress. Tuber number/pot across different weight classes (<5g, 5-20g, 20-50g, 50-100g and >100g) in potato genotypes Fv, V and RB (grey bars) under NA, DAS and NAS treatments (white bars). Genotype data are pooled from all treatments and treatment data are pooled from all genotypes. Values represent means ± SE (*n* = 3-6 plants)

4.2 Drought stress application to intact leaves on stems

Leaf wilting is the most visual index of drought stress. Drought Acclimation reduced ($p<0.05$) leaf wilting under drought stress in the RB genotype but not in the Fv genotype (Fig. 4.3). Drought acclimation itself did not reduce %LWC of Fv compared to RB and V, but subsequent exposure to severe drought stress (1st and 2nd DS) did reduce %LWC of Fv compared to RB and V (Fig. 4.4). Fv was generally more sensitive to drought stress compared to RB and V. Unlike visual leaf wilting, measurement of %LWC did not distinguish an acclimation effect

under drought stress in RB or when pooled across genotypes (Fig. 4.4). Also, while the %LWC was reduced in drought stressed plants at the 1st stress, by the 2nd drought stress, %LWC increased in the DAS and NAS treated plants to equivalent values of the NA control.

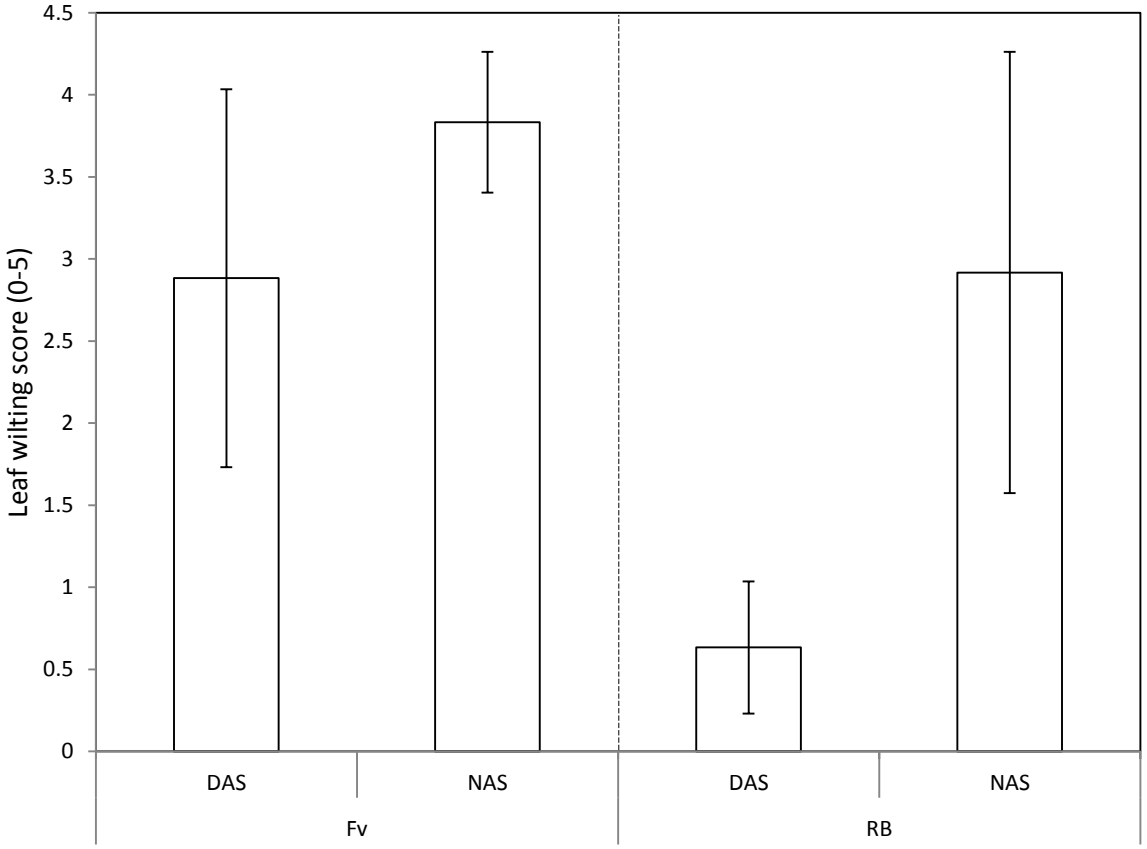


Fig. 4.3. Leaf wilting score across two potato genotypes after DS cycle. Leaf wilting score (0 = 0% leaf wilting to 5= 100% leaf + stem wilting) in potato genotypes Fv and RB under DAS and NAS treatments at the end of 2nd DS cycle. Values represent means ± SE (*n* = 6 plants).

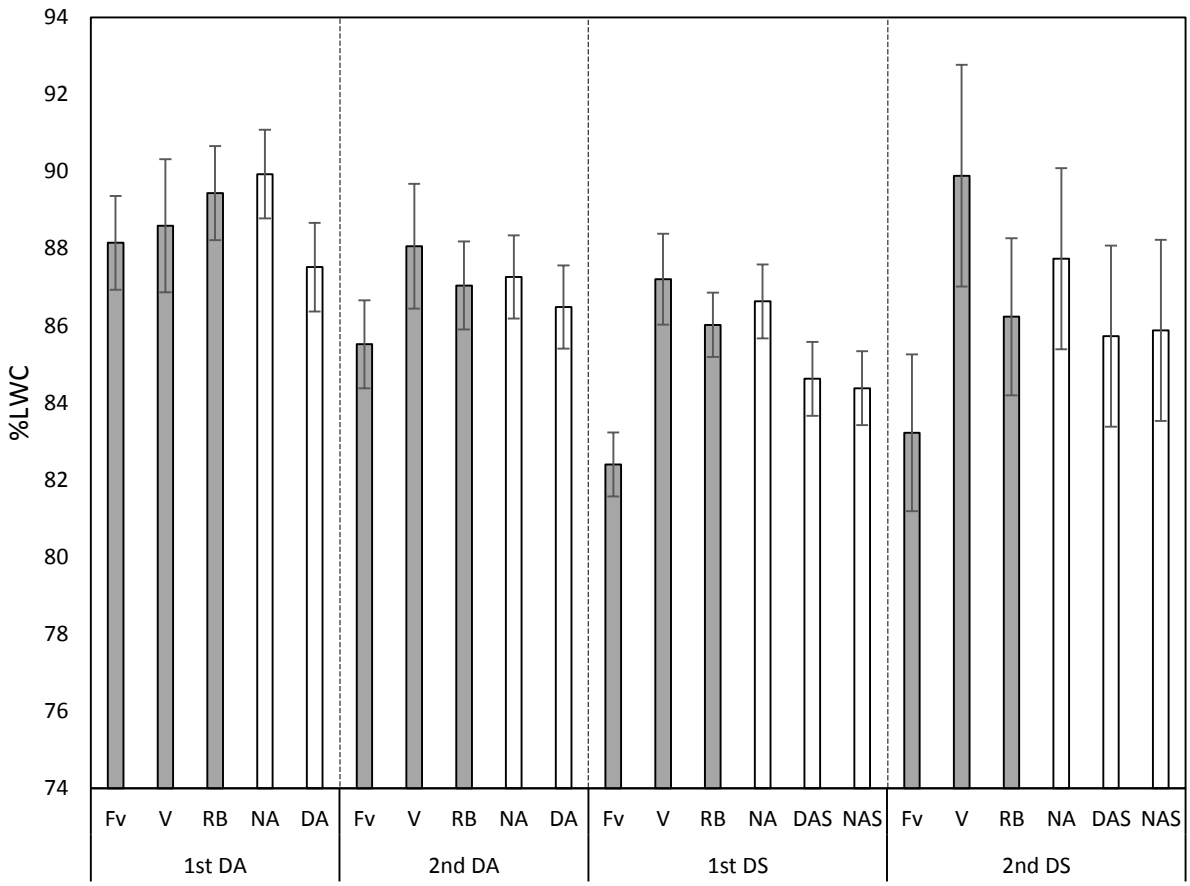


Fig. 4.4. Leaf water content across three potato genotypes after DA and DS cycles. Leaf water content (%LWC) in potato genotypes Fv, V and RB (grey bars) under NA, DAS and NAS treatments (white bars) at the end of both Drought Acclimation (1st and 2nd DA) and Drought Stress (1st and 2nd DS) cycles. Genotype data are pooled from all treatments and treatment data are pooled from all genotypes. Values represent means ± SE (*n* = 3-6 plants)

4.3 Drought stress application to excised leaves

Drought shock imposed by excision of turgid leaves after Recovery (R) from two cycles of Drought Acclimation (DA) and two cycles of Drought Stress (DS) revealed differences between genotypes and treatments (Fig. 4.5). However, these differences depended upon timing of the drought shock. When drought shock was imposed after Recovery from the 1st DA (1st DA-R), the acclimated germplasm (DA) lost moisture at a lower rate ($p<0.05$) than the Non-Acclimated germplasm (NA) but, during the 2nd DA-R, DA had a higher rate of moisture loss

than the NA treated germplasm. When drought shock was applied after Recovery from the 1st DS (1st DS-R), both Drought-Acclimated Stressed (DAS) and Non-Acclimated Stressed (NAS) germplasms had a lower rate of moisture loss from excised leaves compared to Non-Acclimated Controls (NA). But upon Recovery after a second Drought Stress (2nd DS-R), drought acclimation did not change the rate of leaf moisture loss (DAS vs NAS). Under drought shock, genotype V displayed the highest rate of moisture loss compared to the other genotypes at most sampling times.

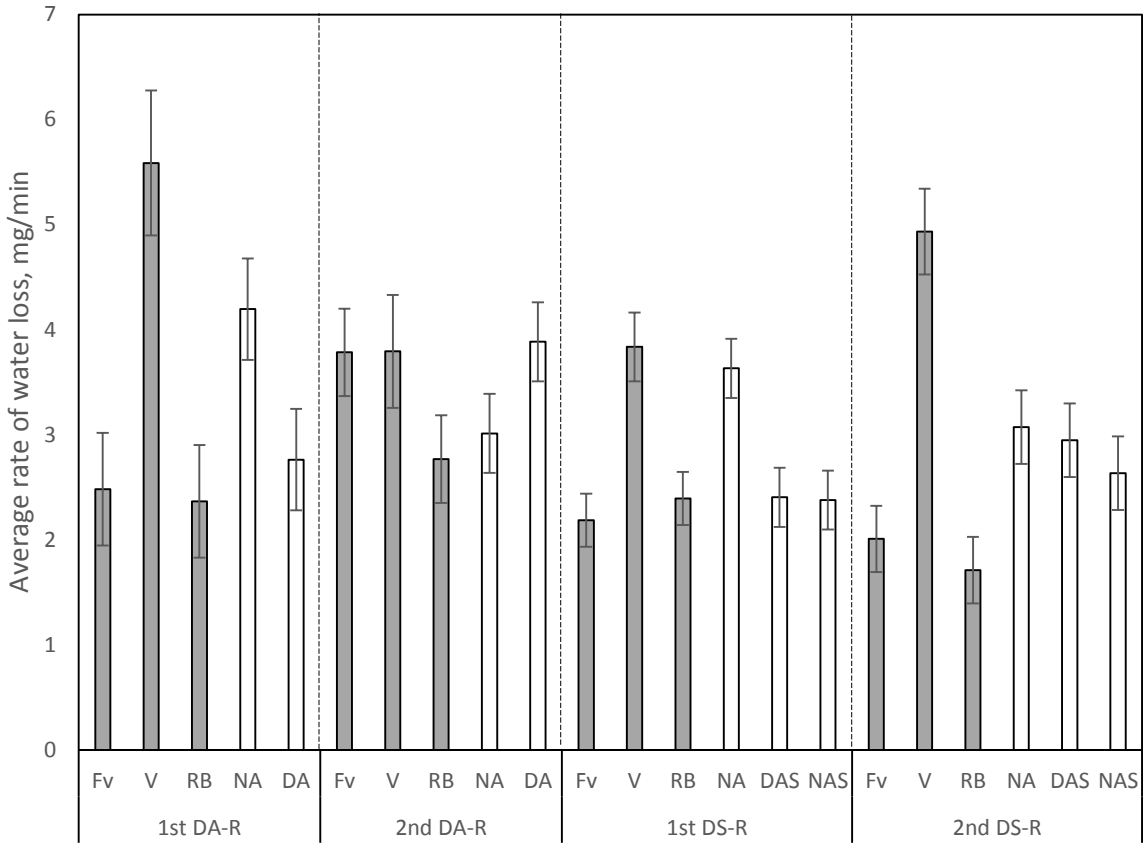


Fig. 4.5. Rate of leaf water loss across three potato genotypes during Recovery after DA and DS cycles. Average rate of water loss from leaf surface over 15 minutes in potato genotypes Fv, V and RB (grey bars) under NA, DAS and NAS treatments (white bars) at the end of both Drought Acclimation (during 1st and 2nd DA-R) and both Recovery (during 1st and 2nd DS-R) cycles. Genotype data are pooled from all treatments and treatment data are pooled from all genotypes. Values represent means ± SE (n = 3-5 plants).

4.4 Final shoot (leaf and stem) dry weight, plant height, stem diameter and stem number

There was no acclimation effect on final shoot dry weight, plant height, stem diameter and stem number. However, genotypes differed significantly for these traits (Fig. 4.6). RB had the greatest plant height while Fv induced highest final shoot dry weight. V had lowest final stem diameter and stem number.

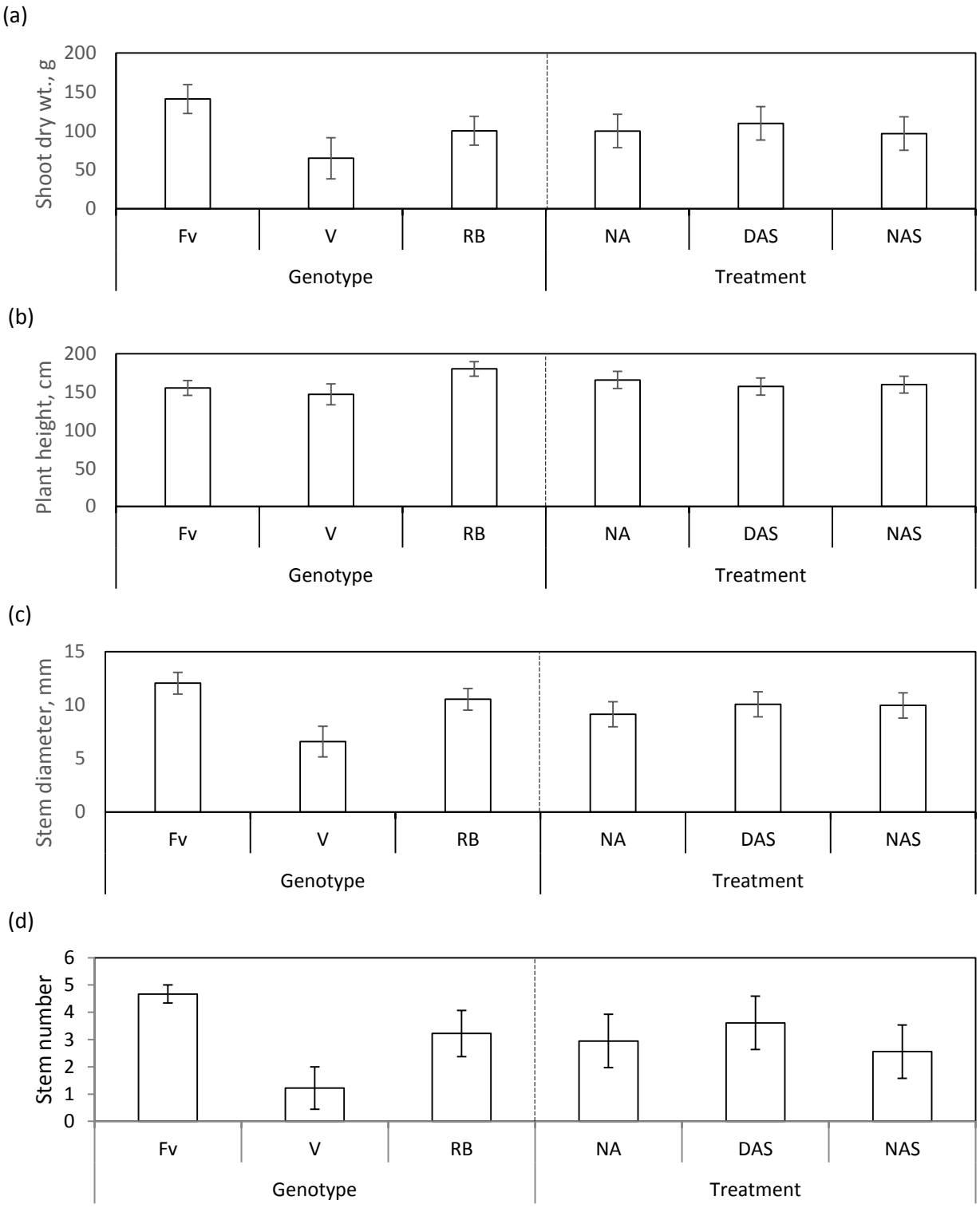


Fig. 4.6 Different physical parameters across three potato genotypes under drought stress. Final shoot dry weight (g), plant height (cm), stem diameter (mm) and stem number in potato genotypes Fv, V and RB under NA, DAS and NAS treatments before the harvesting. Genotype data are pooled from all treatments and treatment data are pooled from all genotypes. Values represent means ± SE (n = 3-6 plants).

4.5 Stomatal area, density and pore area on intact leaves on stems under drought stress

Since the 1st drought stress induced highest variation between treatments and genotypes in intact leaves on stems (Fig. 4.4), this stress point was used to assess stomatal density and size, as well as pore size. Drought Stress induced more ($p<0.05$) stomata but of smaller size (DAS and NAS vs NA, Figs. 4.7 and 4.8a) with smaller pore size (NAS vs NA, Fig. 4.9a) at the end of the 1st DS. Drought Acclimation induced more open pores after drought stress (DAS vs NAS, Fig. 4.9a). Drought Stress induced smaller stomata in the Fv genotype but stomatal size did not change in RB (Fig. 4.8b). When stressed, Acclimated germplasm had more open pores than Non-Acclimated germplasm in both Fv and RB genotypes (Fig. 4.9b).

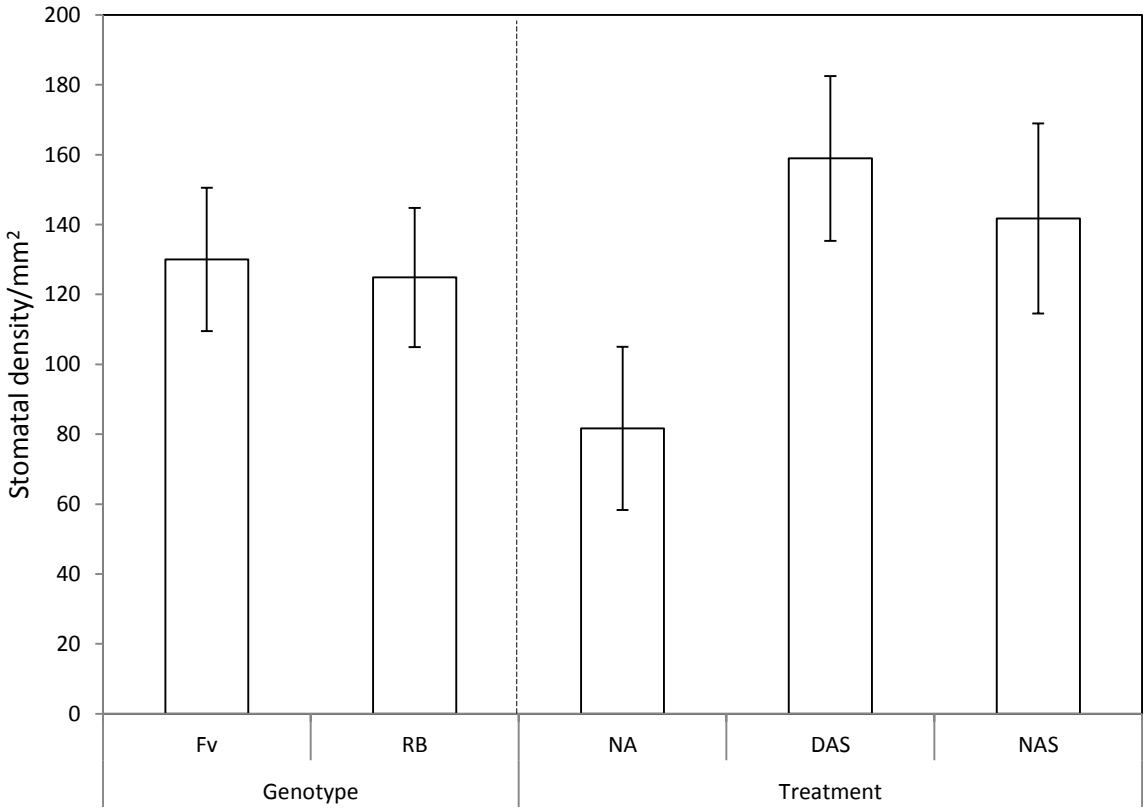
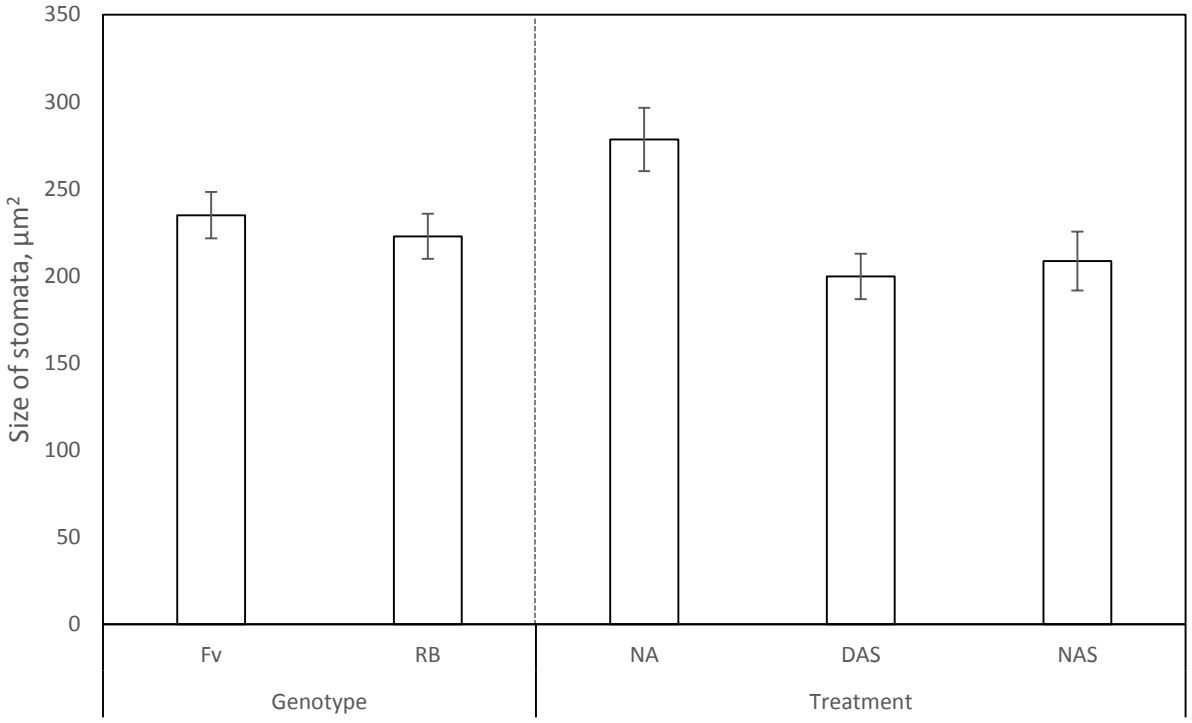


Fig. 4.7. Stomatal density across two potato genotypes after DS cycle. Stomatal density per square millimeter of leaf area in potato genotypes Fv and RB and under NA, DAS and NAS treatments at the end of first Drought Stress (1st DS) cycle. Genotype data are pooled from all treatments and treatment data are pooled from all genotypes. Values represent means ± SE (*n* = 3 plants)

(a)



(b)

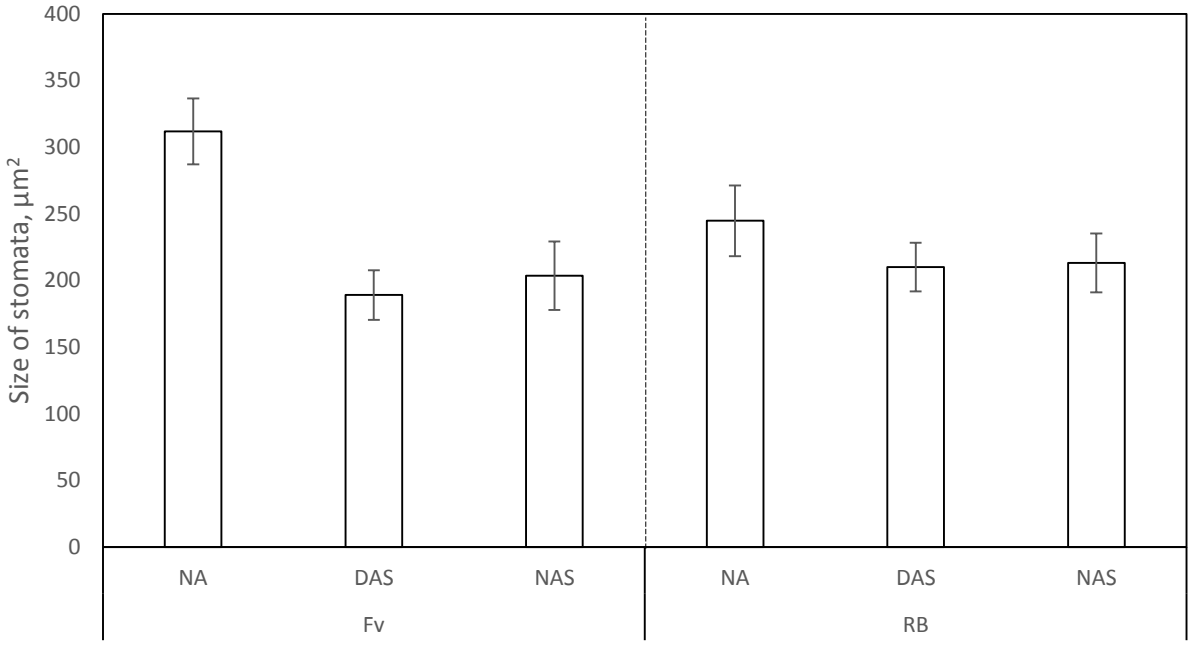
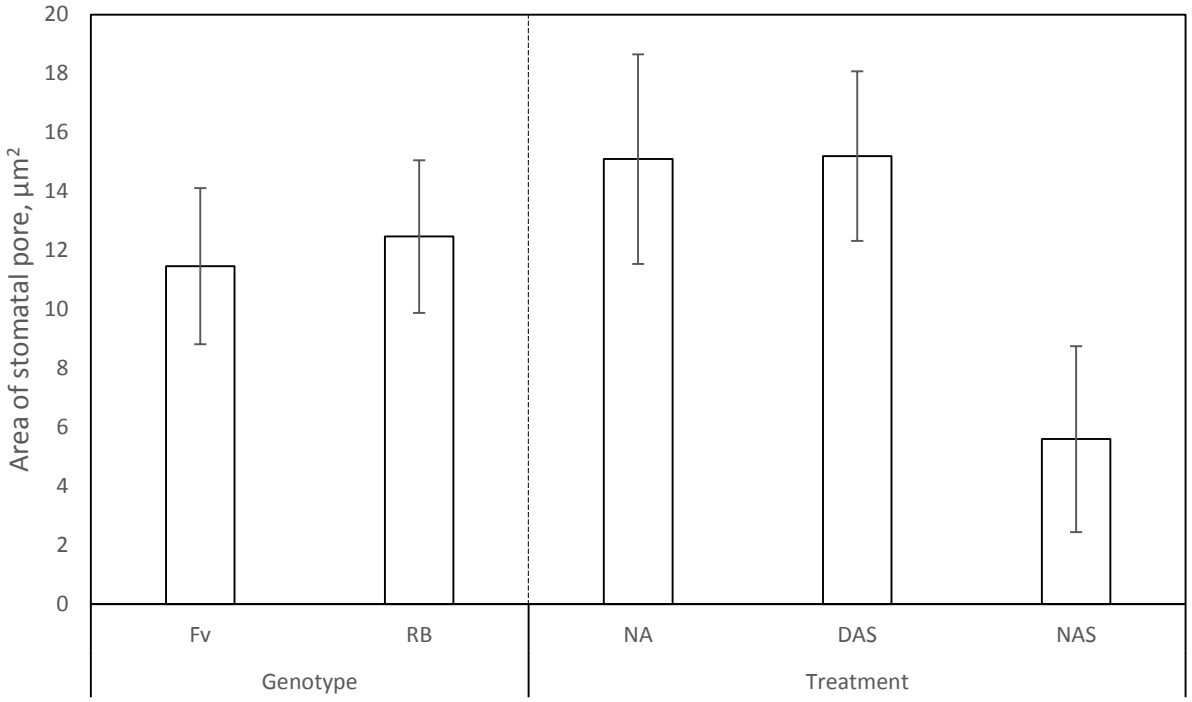


Fig. 4.8. Stomatal size across two potato genotypes after DS cycle. Stomatal size (a) in two potato genotypes Fv and RB under NA, DAS and NAS treatments. Genotype data are pooled from all treatments and treatment data are pooled from all genotypes. (b) in treatments within genotypes at the end of first Drought Stress (1st DS) cycle. Values represent means ± SE (n = 3 plants).

(a)



(b)

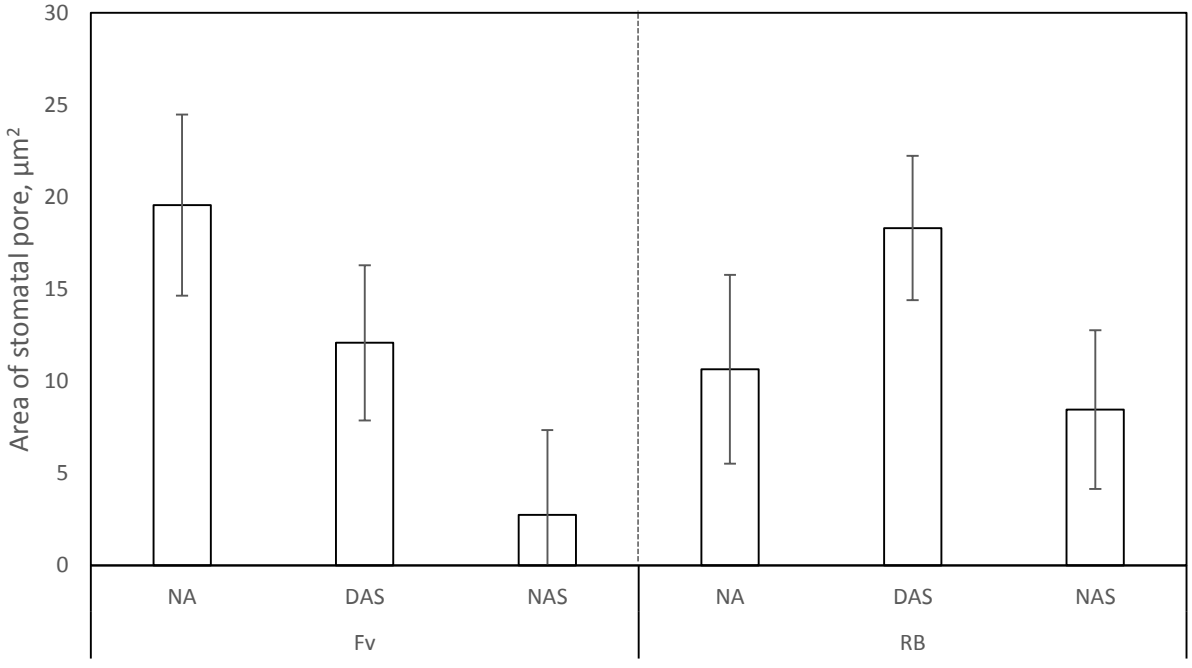


Fig. 4.9. Stomatal aperture across two potato genotypes after DS cycle. Area of stomatal pore (a) in two potato genotypes Fv and RB under NA, DAS and NAS treatments. Genotype data are pooled from all treatments and treatment data are pooled from all genotypes. (b) in treatments within genotypes at the end of first Drought Stress (1st DS) cycle. Values represent means ± SE (*n* = 3 plants).

4.6 Cuticle platelet size and thickness on intact leaves on stems under drought stress

Drought Stress induced smaller ($p<0.05$) cuticular platelets in both DAS and NAS treatments than in platelets from NA germplasm (Figs. 4.10 and 4.11) in both Fv and RB genotypes (Fig. 4.11). There was no effect of acclimation on this response. Drought Acclimation induced the thickest cuticle layer (DAS vs NAS and NA) across the genotypes (Fig. 4.12).

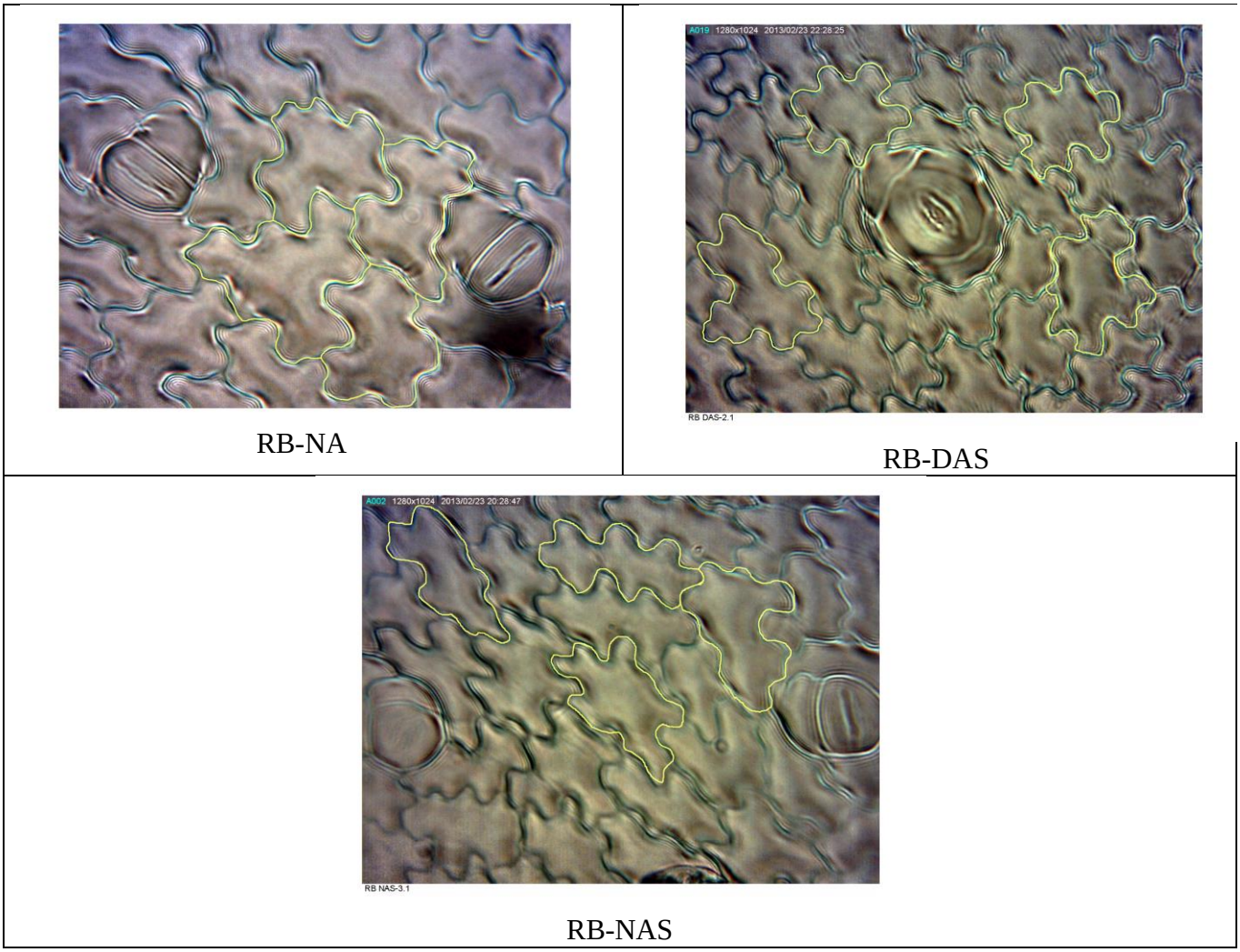


Fig. 4.10. Leaf cuticular platelets in one potato genotype after DS cycle. Leaf cuticular platelets in a potato genotype RB under NA, DAS and NAS treatments at the end of first Drought Stress (1st DS) cycle. Images are representative of each treatment response.

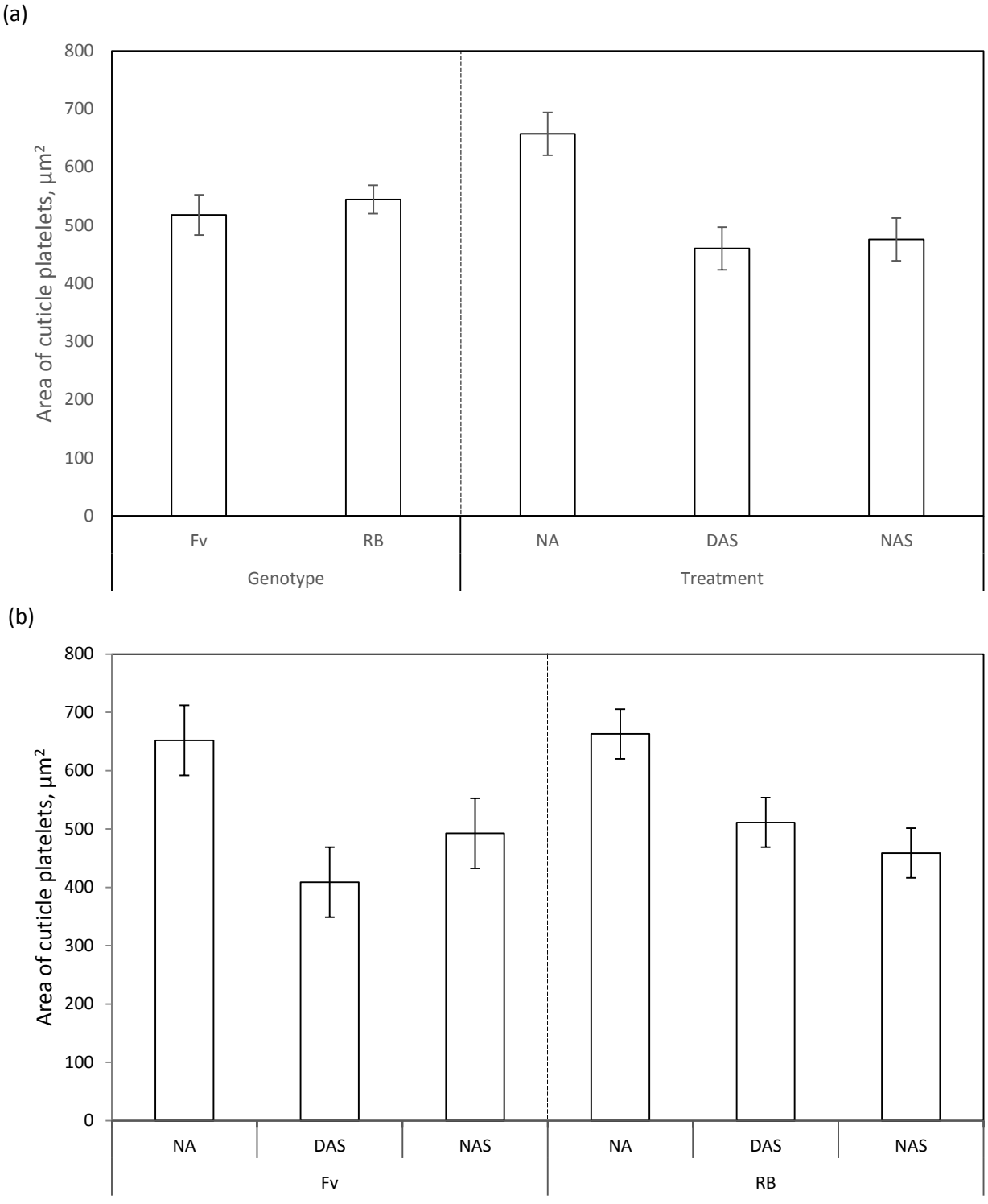


Fig. 4.11. Leaf cuticular platelets across two potato genotypes after DS cycle. Area of leaf cuticular platelets (a) in two potato genotypes Fv and RB under NA, DAS and NAS treatments. Genotype data are pooled from all treatments and treatment data are pooled from all genotypes. (b) in treatments within genotypes at the end of first Drought Stress (1st DS) cycle. Values represent means ± SE (n = 3 plants).

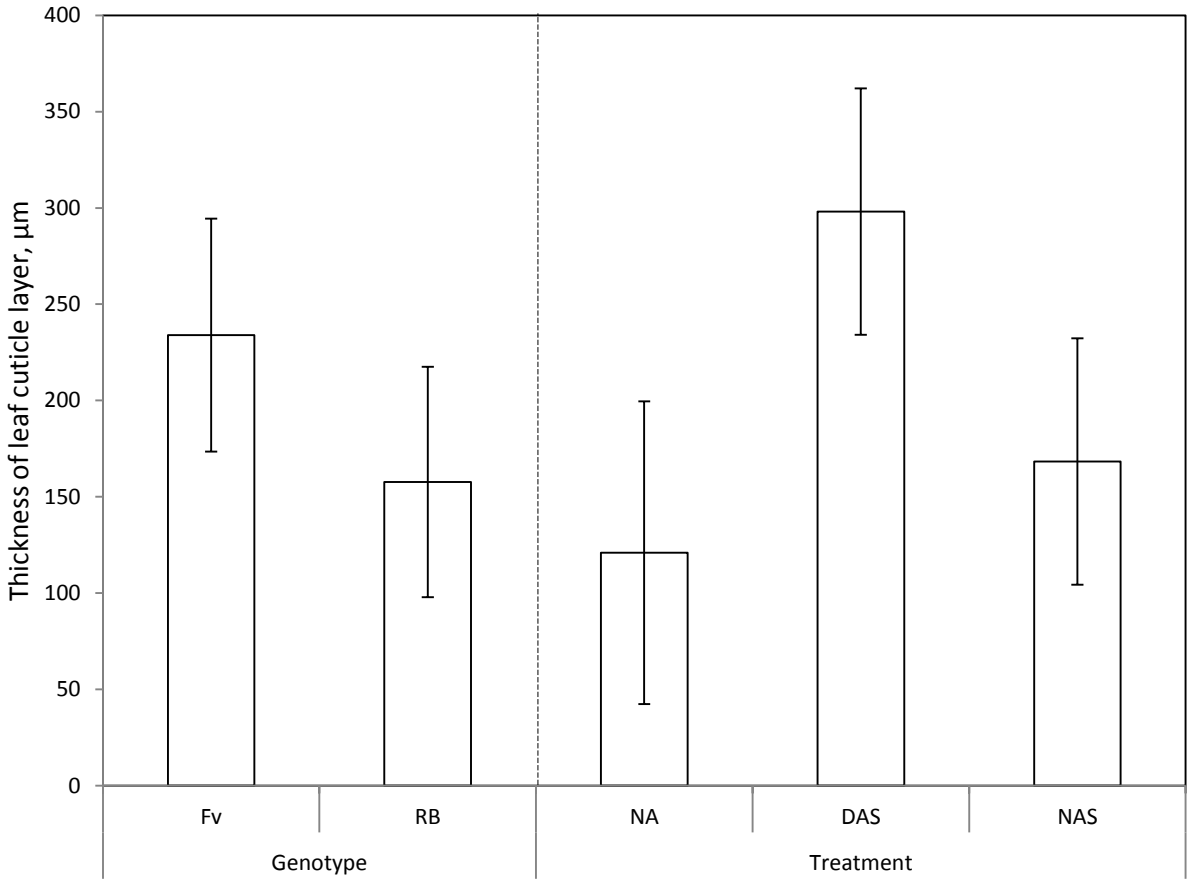
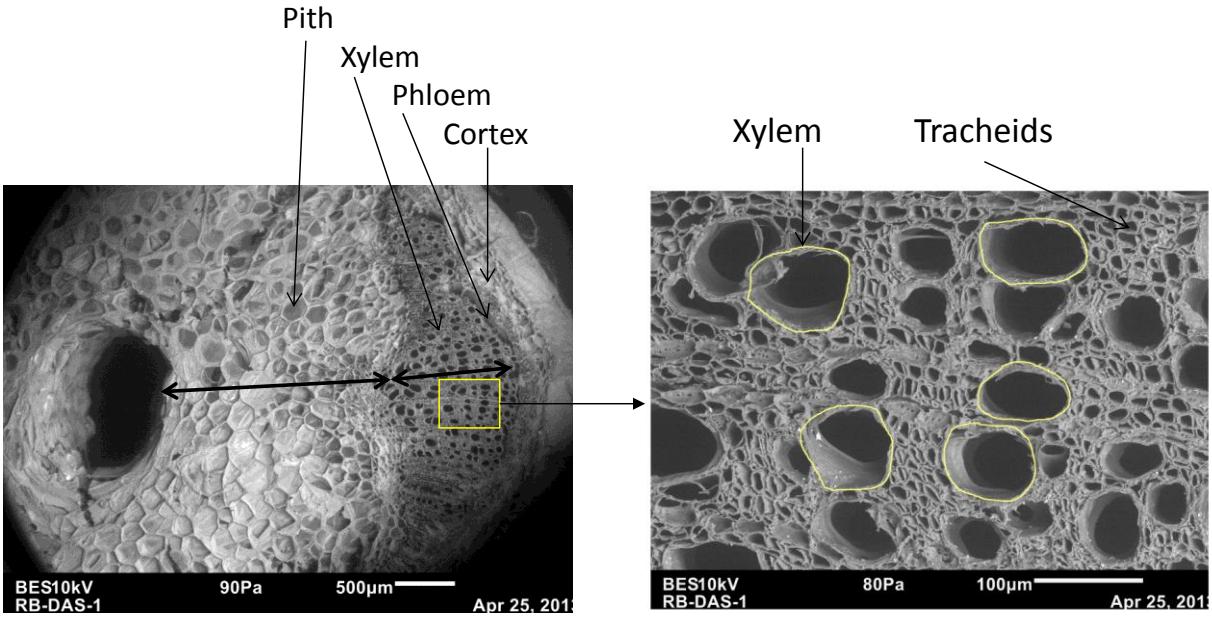


Fig. 4.12. Leaf cuticular thickness across two potato genotypes after DS cycle. Thickness of leaf cuticle layer in two potato genotypes Fv and RB under NA, DAS and NAS treatments at the end of first Drought Stress (1st DS) cycle. Genotype data are pooled from all treatments and treatment data are pooled from all genotypes. Values represent means ± SE (*n* = 3 plants)

4.7 Xylem vessel area and stem water content

Drought Acclimation did not induce a greater cross-sectional area of xylem vessels (Fig. 4.13) when the 5 largest xylem vessels were compared (DAS vs NAS and NA) in any of the three genotypes Fv, V and RB (Fig. 4.14a). The cross-sectional length of the entire xylem region measured perpendicularly from the stem perimeter towards the centre of the stem was significantly shorter than the pith in both Fv and V but there was no difference between the length of xylem and pith in RB (Fig. 4.14b). Drought Acclimation had no effect on this measurement.

A significant difference ($p<0.05$) in percentage stem water content (%SWC) was observed among the genotypes (Fig. 4.15a) and there was also an interaction effect (Fig. 4.15b). Genotype V expressed highest %SWC followed by RB and then Fv. Drought Acclimation induced lower %SWC in Fv (Fv-DAS vs Fv-NAS). Drought Stress also induced lower %SWC in RB-NAS vs RB-NA (Fig. 4.15b).



(a) Stem cross-section (25X) (b) Xylem cross-section (250X)

Fig. 4.13. Potato stem cross-section. Cross-sectional area of (a) stem (labelling from Esau 1977) and (b) the five largest diameter xylem vessels from fresh stem samples of RB observed with low vacuum scanning electron microscopy. Samples were taken after recovery from 2nd DS. Images are representative of the treatment response.

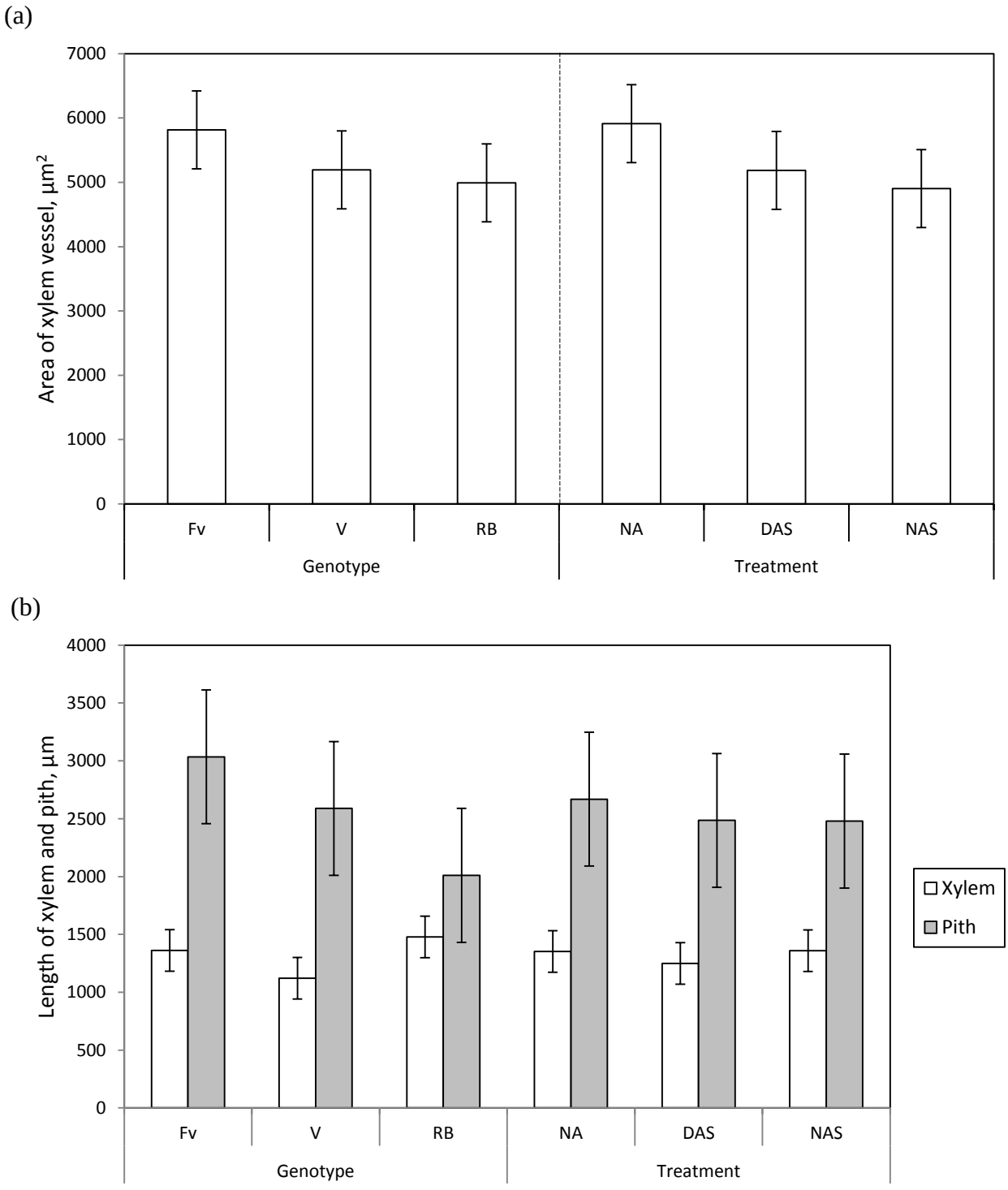


Fig. 4.14. Area of xylem vessels and length of xylem and pith across three potato genotypes under drought stress. (a) xylem vessels and (b) length of xylem and pith region measured perpendicularly from the stem perimeter towards the centre of the stem in potato genotypes Fv, V and RB under NA, DAS and NAS treatments before harvesting. Genotype data are pooled from all treatments and treatment data are pooled from all genotypes. Values represent means ± SE (n = 3 plants).

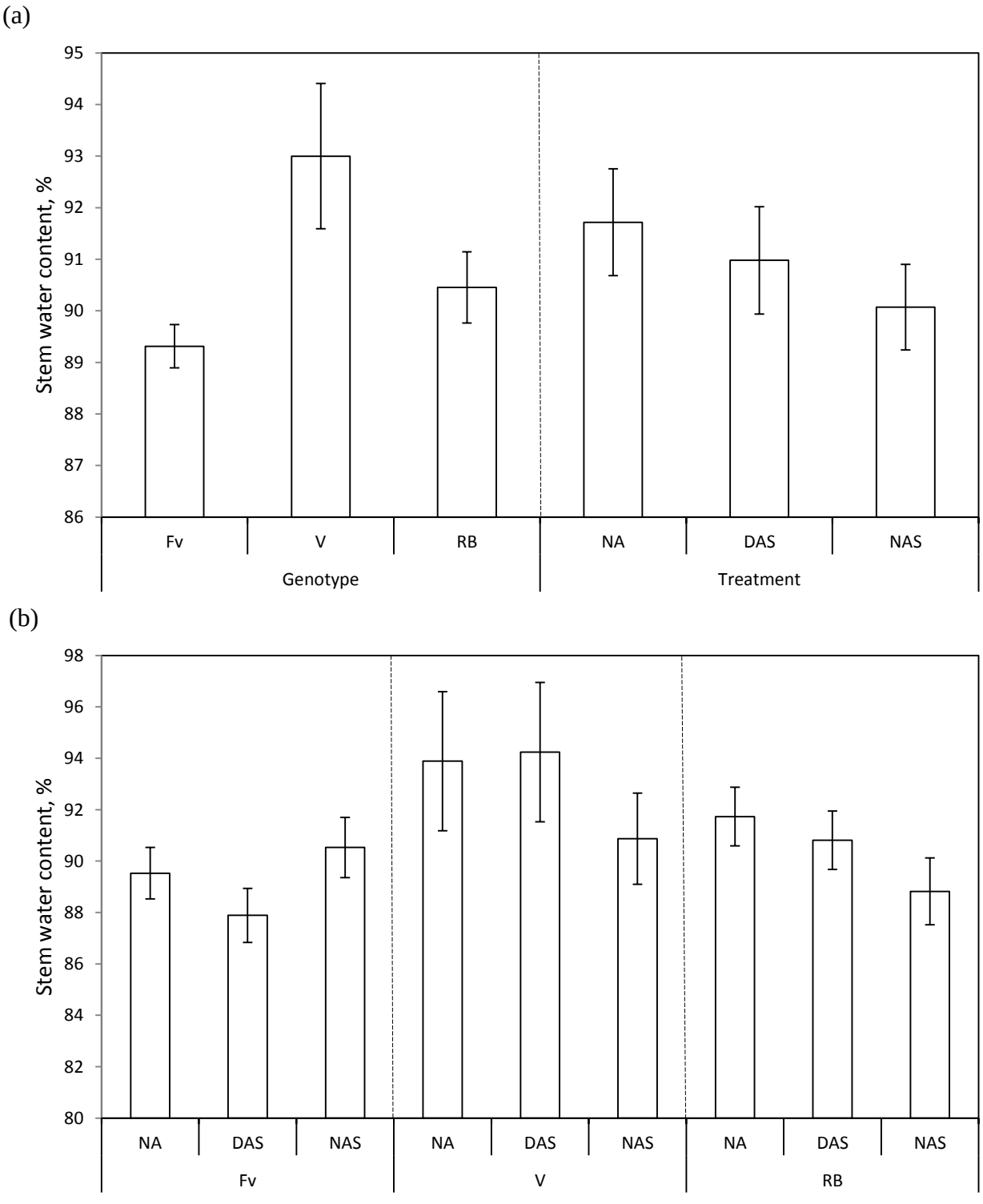


Fig. 4.15. Stem water content across three potato genotypes under drought stress. Stem water content (%) (a) in potato genotypes Fv, V and RB under NA, DAS and NAS treatments. Genotype data are pooled from all treatments and treatment data are pooled from all genotypes. (b) in treatments within genotypes before the harvesting. Values represent means ± SE (n = 3 plants).

4.8 Recovery time from maximum stress

Through infrared thermal imaging, leaf surface temperatures were monitored. Potato genotype Fv took the longest time (16 hrs, Fig. 4.16) to recover after re-watering the Drought Stressed plants at Stage 3 (Table 3.2) and subsequently recovered to temperatures of Stage 0 plants. By contrast, the other genotypes took less time to regain temperature at Stage 0 in that V took 12 hrs and RB took 5 hrs (Fig. 4.17).

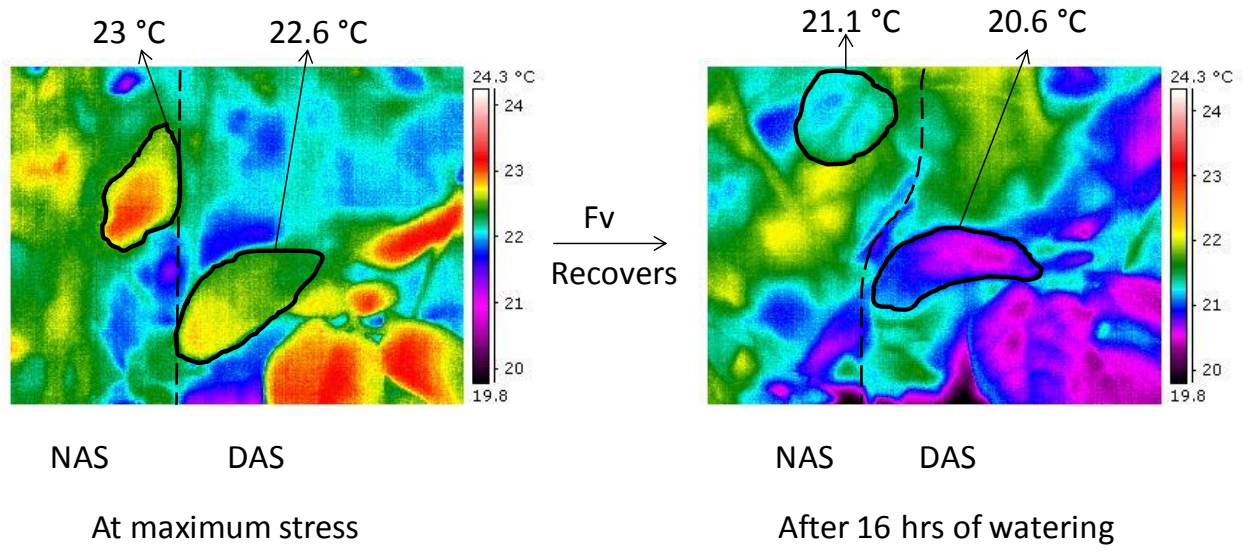


Fig. 4.16. Recovery time from drought stress in potato genotype Fv after DS cycle. IR Thermal images for recovery time after re-watering at maximum soil water deficit of potato genotype Fv under DAS and NAS treatments at the end of first Drought Stress (1st DS) cycle. One leaflet from a youngest fully expanded leaf from NAS (left of the dashed line) and DAS (right of the dashed line) plants were focused. Temperatures showing after 16 hrs of re-watering remained stable for 21 hours more.

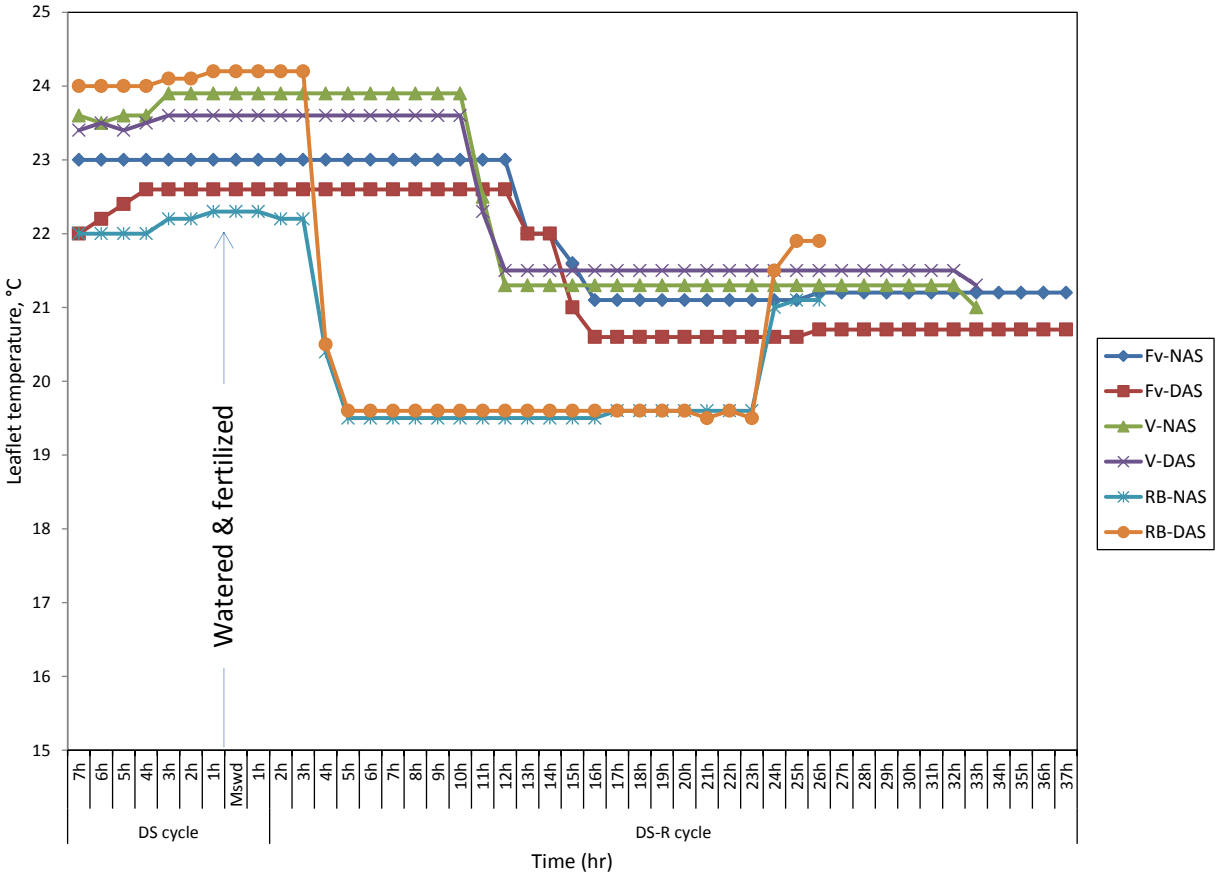


Fig. 4.17. Leaflet temperature profile from Drought Stress to Recovery across three potato genotypes. Changes in average leaflet temperature during maximum soil water deficit to full Recovery in potato genotype Fv, V and RB under DAS and NAS treatments at the end of Drought Stress (DS) cycle. Mswd: maximum soil water deficit.

5.0 DISCUSSION

In this study, acclimation had no significant effect on tuber number and yield responses. Tuber number and yield appeared to be genetically determined and were not influenced by Drought Acclimation or Stress. Pooled data from all three treatments (NA, DAS and NAS) indicated a genotype-dependent response such that RB had higher numbers of large tubers (50-100 g) and had higher yield (total tuber mass per pot) than Fv across all treatments. By contrast, V had the highest tuber number with more tiny tubers (<5 g). The higher tuber number of genotype V in the <5 g category was consistent across NA, DAS and NAS treatments of this genotype. Stage of development when stressed may be the most significant factor determining yield responses across cultivars (Stark et al. 2013). Since RB was late maturing while Fv and V had similar developmental rate, Fv and V were consequently stressed at the same tuber initiation stage considered to be the most critical drought stress stage (Costa et al. 1997). Although V and Fv were stressed at the same stage of development, there was a differential response in tuber number but not in tuber yield between these two genotypes. Thus, tuber number was a more sensitive index of genotype response than tuber yield in that V had the highest tuber number across the treatments and tuber number was significantly reduced in Fv across these same conditions. Tuber number is influenced by seed tuber characteristics (Lahlou et al. 2003) and number of stolons per stem (Haverkort et al. 1990). Moreover, there was no difference in tuber yield between V and Fv which further indicates tuber number to be a more sensitive index than tuber yield to distinguish these two genotypes.

Characterization of drought tolerance in potato cultivars is complicated by the fact that differential yield responses have not been consistently related to specific physiological or morphological traits (Stark et al. 2013). The indicators most used to measure drought stress

resistance are yield (Farshadfar and Elyasi 2012), leaf water content (Omae et al. 2005) and excised leaf water loss (Wang and Clarke 1993). Plant water management is a combination of increasing water uptake and reducing water loss during drought stress and is of obvious importance. While this study did not examine water uptake mechanisms, parameters related to reducing water loss were evaluated.

Stress was imposed both on intact leaves on stems and excised leaves to distinguish the effect of the main stem. When intact leaves on stems were stressed, V had higher %Leaf Water Content (%LWC) at maximum soil water deficit compared to Fv but there was no difference in %LWC between V and RB. Leaf water content is considered to be a more important indicator of water status than other water potential parameters under drought stress conditions (Sinclair and Ludlow 1985) and was proposed as a selection criterion for drought tolerance in barley (Matin et al. 1989), wheat (Schonfeld et al. 1988) and pigeonpea (Kimani et al. 1994). In this study, %LWC was recorded when the first plants (Fv-NAS) reached Stage 3 in order to sample a consistent stage of Drought Stress (Stage 3 is when 75% of leaves showed visible wilting, Table 2). Fv was more sensitive to wilting than V or RB and would reach Stage 3 before V and RB when Drought Stressed. In fact, when RB Drought Stressed pots were left in the greenhouse unwatered for 2 months, no visible wilting occurred (data not shown). Nevertheless, Fv Acclimation (DAS) did reduce visible wilting at both Drought Stress cycles (1^{st} and 2^{nd} DS) but it was only significant at 1^{st} DS (data not shown). By contrast even at 2^{nd} DS, the RB-DAS treatment significantly reduced visible wilting over RB-NAS. It is not clear why RB could avoid significant wilting compared to Fv but unlike Fv and V, the xylem cross section of RB was of similar length to its pith while the pith was significantly longer than the xylem cross section in both Fv and V. Also, root mass was not measured and it is not known if RB had a larger root

mass which could continue to uptake and extract more moisture from the soilless mix during Drought Stress. RB also had the fastest Recovery from Drought Stress of all the genotypes tested.

The higher %LWC in V at the time of sampling may be related to a higher % stem water content than Fv. Similar to RB, V recovered earlier than Fv from maximum Drought Stress which may be linked with the higher % stem water content of V and the acclimation response of V and RB but not Fv where %SWC of acclimated V and RB genotypes was equivalent to NA controls under drought stress. Stem water reservoir is able to augment water supply to the leaves and provides a substantial degree of protection against desiccation in tropical alpine rosette plants (*Espeletia* and *Senetio* species, Holbrook 1995). Stem water replaces transpirational losses in tropical forest canopy trees before water contained in the soil (Goldstein et al. 1998). The stem reservoir in white oak played a key role in supplying water for transpirational use especially as the soil reservoir depletes (Hinckley et al. 1974). The amount of water stored in the trunk of *Pinus pinaster* accounted for 12% of the daily transpiration when soil water was abundant, but increased to 25% of the daily transpiration following a period of drought (Loustau et al. 1996). The source of additional %SWC in our plants did not appear to be related to quantity of xylem and pith since there was no difference in the length of xylem and pith between Fv and V.

The potential importance of the main stem was further exemplified when the influence of the main stem was removed through leaf excision with only the petiole attached. In this case, V lost water by the highest rate from the excised leaf under drought shock conditions. By contrast, RB had consistently lower rates of water loss from excised-leaves without the main stem under these drought shock conditions which may have added to RB's ability to withstand severe drought conditions.

Stomatal regulation is the major mechanism controlling the water regime in higher plants (Egilla et al. 2005) and an acclimation response was detected. Drought Stress (in both DAS and NAS compared to NA Controls) did not change total stomatal size (area) in RB, but did change in Fv at maximum soil water deficit. Drought Stress (NAS and DAS) also induced higher stomatal density across the genotypes (Fv and RB) at maximum soil water deficit. In maize, drought resistant lines showed higher stomatal frequency and smaller stomatal apparatus (Ristic and Cass 1991).

When plants suddenly encountered drought, the most important quick response was stomatal closure (Yordanov et al. 2000). In this study, NAS (Non-Acclimated and Drought Stressed) responded to Drought Stress through greater stomatal closure than Acclimated and Stressed (DAS) plants. Under DAS, stomatal apertures were wider in both RB and Fv genotypes. Stomatal closure is correlated with a decline in leaf turgor as a consequence of low water potential (Kramer 1988). Stomata primarily control transpiration which in turn in part regulates greater WUE (Levy et al. 2013). Stomatal closure during soil drying is mediated by changes in root water status through chemical signals ascending from the root to the leaves and leads to the closure of stomata in concert with the level of soil water stress (Yordanov et al. 2000). There are many signals that induce stomatal closure. The best known signal is abscisic acid (ABA) (Wright 1969) along with secondary messengers, such as Ca_2+, H_2O_2 and NO (Arve et al. 2011). Passive loss of turgor pressure also results in stomatal closure. The stomatal aperture changes in response to humidity (Okamoto et al. 2009). Drought acclimation involves osmotic adjustment. By increasing the concentration of solutes in the symplast, turgor can be maintained at low tissue water potentials by enabling water to continue to be extracted from dry soil (Khalil and Grace 1992). The turgor allows stomatal opening and cell expansion, root growth and an increase in

productivity (Kozolowski and Pallardy 2002). Another response mechanism is the closure of stomata in response to a reduction in soil water content (Khalil and Grace 1992). When stomata are closed, the leaf cuticular layer plays a particularly fundamental protective role against water loss (Boyer et al. 1997, Jenks and Ashworth 1999, Yoo et al. 2009).

Drought Stress induced smaller cuticle platelets in RB and Fv at maximum soil water deficit. Interestingly, Drought Acclimated and Drought Stressed (DAS) germplasm had the greatest leaf cuticle layer thickness. The cuticular layer is known to be important in preventing additional water loss from transpiration (Jenks and Ashworth 1999). When potato cultivars were exposed to mild drought stress, the drought resistant cv. 'Raritan' doubled its epicuticular wax level whereas the drought sensitive cv. 'Shepody' increased its wax level by less than 20% (Coleman 1986). A study with maize (Ristic and Jenks 2002) revealed an inverse relationship between epidermal water loss and cuticle thickness in which a maize line with lower rates of water loss in shoots and detached leaves had thicker cuticles than a line with higher rates of water loss. An adaptation to plant life in dry environments includes thick cuticles and wax layers through reduced extra-stomatal transpiration (Arve et al. 2011). Plants adapted to dry environments have thicker cuticles and lower rates of transpiration through the cuticular layer than plants from less dry environments (Oppenheimer 1960). Many plants possess the ability to respond to water-limited environments by increasing the deposition of epicuticular waxes which likely increases the cuticle's ability to function as a hydrophobic barrier (Mamrutha et al. 2010). The role of wax components (organic compounds, mainly alkanes, alcohols, fatty acids and *n*-alkyl esters) is to protect plants from water loss (Halinski and Szafranek 2006) and fatty acids and glycerol were found to be most associated with preventing cuticular transpirational water loss (Zhang et al. 2013). In our study, Drought Acclimation induced more open stomata and

increased cuticular thickness, this result suggests that Drought Acclimation can both increase CO_2 uptake and prevent water loss through these two mechanisms.

Drought adapted plants lower tissue dehydration either by maintaining water potential or tolerating low tissue water potential (Vasquez-Robinet et al. 2008) and this can minimize water loss. Since maximum CO_2 fixation is essential to optimal crop production in potato (Blum 2009), restriction of transpiration through stomatal control may not be a realistic option for production systems (Levy et al. 2013). However, one factor that should be considered is the impact of cuticular thickness on reducing transpirational water loss. If a genotype through drought acclimation can both increase CO_2 uptake (stomata partially open during drought stress) while reducing transpirational water loss (thicker cuticles), an adapted plant may both gain carbon and reduce desiccation stress at the same time.

Finally, although the majority of the drought stress literature applies drought stress directly without acclimation, the importance of pre-treating potato plants to acclimation cycles was demonstrated in this study. Compared to application of drought stress directly (NAS), a pre-treatment of drought acclimation cycles followed by drought stress (DAS) reduced leaf wilting, induced thicker cuticular layer and more open stomata under stress. Without a DAS type approach, potentially key drought stress resistance mechanisms will be missed.

6.0 SUMMARY AND CONCLUSION

Potato plants are generally sensitive to drought stress. However, field potato crops undergoing mild water deficit conditions may acclimate to subsequent severe water deficits. This thesis examined the effects of drought acclimation on drought stress resistance in three potato genotypes Fv12246-6 (Fv), Vigor (V) and Russet Burbank (RB) in a low relative humidity (17-25%) greenhouse. Non-Acclimated and Non-Stressed (NA), Drought Acclimated and Drought Stressed (DAS) and Non-Acclimated and Drought Stressed (NAS) treatments were applied across genotypes.

Genotypes Fv and V were stressed at the same tuber initiation stage, considered to be the most critical stage affecting yield. Acclimation had no significant effect on increasing tuber number or yield under drought stress. Both genotypes expressed equivalent yield (total tuber mass) across treatments but V had higher tuber numbers than Fv. Thus, tuber number was a more sensitive index to distinguish V from Fv than tuber yield.

Genotypes V and RB had a higher % leaf water content (%LWC) at maximum drought stress compared to Fv. Leaves were stressed while intact on the stem and then leaves from all treatments were harvested for %LWC when the first genotype reached stage 3 drought stress (75% leaf wilting). Higher %LWC under maximum drought stress could be a result of reduced water loss and/or access to more stored water since the leaf was left intact on the stem during drought stress. V expressed the highest main stem water content followed by RB and Fv, respectively. Stem water might represent a water source to leaves during drought stress. The potential importance of the main stem was further examined by removing the influence of the main stem through leaf excision of turgid leaves, then imposing a drought shock and monitoring subsequent leaf water loss. In this case, V lost water by the highest rate. By contrast, RB and Fv

had consistently lower rates of leaf water loss when turgid leaves were excised from the main stem and subsequently exposed to drought shock conditions.

Relative cross-sectional length of xylem and pith tissue in the stem was compared across treatments and genotypes before harvesting. No acclimation response was observed, however, genotypic differences were detected. The xylem to pith ratio was lower in V and Fv but in RB, xylem tissue region diameter was higher and was equivalent to the pith. RB also recovered most quickly after drought stress followed by V and Fv, respectively.

More detailed measurements of leaf stomatal density, size, pore opening, as well as cuticular platelet size and thickness were examined on the adaxial surface in RB and Fv. Drought stress (NAS and DAS) induced higher stomatal density and reduced cuticular platelet size in both genotypes compared to NA. Size of stomata (stomatal area) was reduced under NAS and DAS in Fv but stomatal area did not change in RB. Drought acclimation added another dimension towards water conservation management mechanisms. Acclimation induced less visible wilting in RB, smaller stomata in Fv and also more open stomata in both RB and Fv when measured at maximum soil water deficit on leaves intact on the stem. Drought acclimation also increased leaf cuticular thickness in both RB and Fv genotypes.

Genotype dependent responses were pronounced in this study. Tuber yield and number were genetically determined and acclimation had no effect on increasing these components under drought stress. However, water conservation mechanisms based on leaf and stem characteristics were both genotype and treatment-dependent. Genotype V maintained a higher %LWC than Fv likely through a mechanism of water supply from water stored in the stem and delivered through continued leaf transpiration under drought stress. RB also had higher %LWC than Fv, and its

mechanism of water conservation appears to have involved a similar tool to V: higher % stem water content and continued supply of water through the transpiration stream via open stomata under stress. However, RB also has additional drought stress resistance tools: stomatal closure under drought shock, acclimation-induced thicker cuticular layer, equivalent xylem to pith ratio which may have enhanced the greater water uptake as observed under RB's fastest recovery from drought stress compared to the other genotypes. Fv was the most sensitive potato genotype according to highest degree of leaf wilting and lowest %LWC under drought stress. It also had the least number of drought stress resistance tools: smaller stomata and closure under drought shock, acclimation-induced thicker cuticular layer. Furthermore, Fv had the lowest % stem water content and slowest recovery time after drought stress. Future investigation of the stem as a reservoir of water during drought stress should be examined to identify key elements for drought stress survival and recovery in the potato plant.

Therefore, the hypotheses of: genotypic differences in drought stress resistance, that pre-exposure to water deficit will induce drought acclimation in potato genotypes and leaf & stem characteristics would distinguish genotypes (Table 6.1) and ability to acclimate to drought stress can be accepted for specific traits. Importantly, incorporating drought acclimation cycles into drought stress studies induces additional drought stress resistance mechanisms (Table 6.2) which will otherwise be missed when plants are directly drought stressed in the absence of acclimation. This approach can be applied as a deficit irrigation strategy in the field level potato production. The stem as a water reservoir also should be further investigated and an expansion of experiments to the field be conducted.

Table 6.1 Genotype-dependent responses

Genotype	Higher yield/ tuber number	Drought resistant tools								Type
		Reduced leaf wilting under stress	Higher % leaf water content under stress	Higher % stem water content	Smaller stomata under stress	Acclimation-induced more open stomata	Smaller epidermal cells under stress	Equivalent Pith and Xylem length	Faster recovery from maximum stress	
RB	√	√	√	√	X	√	√	√	√	Resistant
Fv	X	X	X	X	√	√	√	X	X	Sensitive
V	√	√	√	√	?	?	?	X	X	Moderately Resistant

Table 6.2 Drought Acclimation-induced responses

	Reduced leaf wilting	More open stomata under stress	Thicker leaf waxy layer under stress
Non-Acclimated and Drought Stressed (NAS)	X	X	X
Drought Acclimated and Drought Stressed (DAS)	√	√	√

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APPENDIX A

Relevant figures stated in the Methods of Chapter 3.0

Fig. A1. Stolon elongation and tuber initiation during first four flower blooming stage in potato genotype Vigor

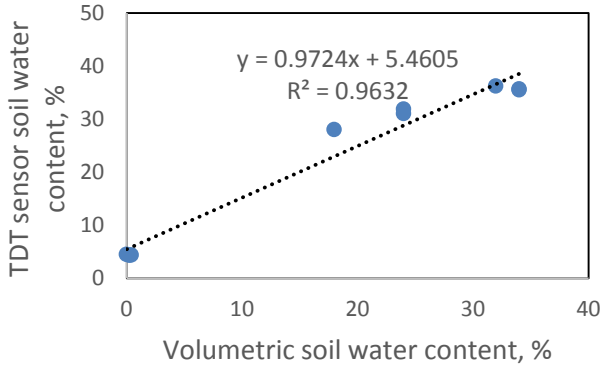


Fig. A2. Correlation between soil water content data measured by TDT sensors and volumetric method

Fig. A3. Setup for measuring leaf water loss over a 15 minute time course

APPENDIX B

Table B1 ANOVA table for the effects of genotypes and treatments on gross tuber yield

Source	DF	SS	MS	F	P
Genotype	2	158682.93	79341.46	3.607	0.036
Treatment	2	77569.36	38784.68	1.621	0.21
Genotype*Treatment					

Table B2 ANOVA table for the effects of genotypes and treatments on gross tuber number

Source	DF	SS	MS	F	P
Genotype	2	824.13	412.07	14.31	0.000
Treatment	2	52.09	26.01	0.90	0.414
Genotype*Treatment	4	32.53	8.13	0.282	0.887

Table B3 ANOVA table for the effects of genotypes and treatments on gross tuber number in different weight classes

Source	Wt Class	Df	SS	MS	F	p
Genotype	<5g	2	871.19	435.59	32.23	0.000
	5-20g	2	8.2	4.1	0.541	0.587
	20-50g	2	2.26	1.13	0.473	0.627
	50-100	2	10.44	5.22	3.662	0.036
	>100g	2	1.87	0.93	2.24	0.121
Treatment	<5g	2	6.19	3.09	0.229	0.796
	5-20g	2	10.78	5.39	0.711	0.498
	20-50g	2	2.19	1.09	0.46	0.635
	50-100	2	6.25	3.12	2.192	0.126
	>100g	2	0.03	0.01	0.033	0.967
Genotype*Treatment	<5g	4	66.31	16.58	1.227	0.317
	5-20g	4	8.4	2.1	0.277	0.891
	20-50g	4	12.311	3.078	1.291	0.292
	50-100	4	2.756	0.689	0.483	0.748
	>100g	4	0.733	0.183	0.44	0.779

Table B4 ANOVA table for the wilting score at the end of 2nd Drought Stress (2nd DS) Cycle

Source	DF	SS	MS	F	P
Between Fv and RB	1	15.04	15.04	9.695	0.005
Between DAS and NAS	1	15.68	15.68	10.301	0.004
Among Fv-DAS,Fv-NAS, RB-DAS and RB-NAS	3	33.39	11.13	14.103	0.000

Table B5 ANOVA table for %leaf water content at the end of Drought Acclimation and Drought Stress Cycles

Cycle	Source	DF	SS	MS	F	P
1st DA	Genotype	2	10.158	5.07	1.211	0.315
	Treatment	1	39.22	39.21	9.362	0.005
	Genotype*Treatment	2	19.19	9.59	2.291	0.123
2nd DA	Genotype	2	29.19	14.59	3.965	0.033
	Treatment	1	4.14	4.14	1.126	0.299
	Genotype*Treatment	2	0.801	0.4	0.109	0.897
1st DS	Genotype	2	182.62	91.31	30.129	0.000
	Treatment	2	41.2	20.6	6.797	0.003
	Genotype*Treatment	4	20.99	5.25	1.732	0.164
1st DS	Genotype	2	273.76	136.88	7.545	0.002
	Treatment	2	34.012	17.006	0.937	0.401
	Genotype*Treatment	4	41.84	10.46	0.577	0.681

Table B6 ANOVA table for excised-leaf water loss during Recovery cycles

Cycle	Source	DF	SS	MS	F	P
1st DA-R	Genotype	2	693.31	346.65	31.168	0.000
	Treatment	1	188.95	188.95	16.989	0.000
	Genotype*Treatment	2	34.62	17.31	1.556	0.212
2nd DA-R	Genotype	2	96.23	48.11	7.152	0.001
	Treatment	1	70.01	70.01	10.406	0.001
	Genotype*Treatment	2	180.67	90.33	13.426	0.000
1st DS-R	Genotype	2	253.1	126.55	33.716	0.000
	Treatment	2	189.18	94.59	25.201	0.000
	Genotype*Treatment	4	316.58	79.15	21.086	0.000
1st DS-R	Genotype	2	991.82	495.91	84.815	0.000
	Treatment	2	18.87	9.43	1.614	0.2
	Genotype*Treatment	4	29.55	7.39	1.264	0.283

Table B7 ANOVA table for final shoot dry weight, final plant height, final stem diameter and final stem number

Cycle	Source	DF	SS	MS	F	P
Shoot dry wt	Genotype	2	37258.17	18629.08	12.363	0.000
	Treatment	2	1237.44	618.72	0.411	0.666
	Genotype*Treatment	4	9092.53	2273.13	1.509	0.22
Plant height	Genotype	2	8716.8	4358.4	10.745	0.000
	Treatment	2	529.86	264.93	0.653	0.000
	Genotype*Treatment	4	3189.33	797.33	1.966	0.000
Stem diameter	Genotype	2	179.99	89.99	19.94	0.526
	Treatment	2	6.95	3.47	0.77	0.471
	Genotype*Treatment	4	25.27	6.32	1.4	0.306
Stem number	Genotype	2	72.13	36.07	11.49	0.121
	Treatment	2	7.69	3.85	1.226	0.254
	Genotype*Treatment	4	5.93	1.483	0.473	0.756

Table B8 ANOVA table for stomatal density, stomatal size and pore area

Cycle	Source	DF	SS	MS	F	P
Stomatal density	Genotype	1	705.26	705.26	0.127	0.722
	Treatment	2	127998.28	63999.14	11.569	0.000
	Genotype*Treatment	2	7196.68	3598.34	0.65	0.524
Stomatal size	Genotype	1	29709.91	29709.91	1.633	0.202
	Treatment	2	931277.74	465638.87	25.589	0.000
	Genotype*Treatment	2	289145.01	144572.51	7.945	0.000
Stomatal pore area	Genotype	1	182.85	182.85	0.243	0.623
	Treatment	2	20718.78	10359.39	13.743	0.000
	Genotype*Treatment	2	11399.89	5699.95	7.562	0.001

Table B9 ANOVA table for leaf cuticle plate area and thickness

Cycle	Source	DF	SS	MS	F	P
Area of leaf cuticle platelets	Genotype	1	22650.02	22650.02	1.538	0.217
	Treatment	2	1027702.44	513851.22	34.882	0.000
	Genotype*Treatment	2	103310.25	51655.12	3.507	0.033
Thickness of leaf cuticle layer	Genotype	1	59586.35	59586.35	3.308	0.077
	Treatment	2	239514.67	119757.33	6.648	0.003
	Genotype*Treatment	2	22901.79	11450.89	0.636	0.535

Table B10 ANOVA table for area of xylem vessels and length of xylem and pith region

Cycle	Source	DF	SS	MS	F	P
Area of xylem vessels	Genotype	2	16596269.88	8298134.94	1.968	0.144
	Treatment	2	24295730.47	12147865.23	2.881	0.06
	Genotype*Treatment	4	47870271.11	11967567.78	2.838	0.027
Length of xylem and pith vessels	Genotype					
	Xylem	2	592509.24	296254.62	4.5	0.026
	Pith	2	4746806.14	2373403.07	3.476	0.053
	Treatment					
	Xylem	2	67330.92	33665.46	0.511	0.608
	Pith	2	209915.98	104957.99	0.154	0.859
	Genotype*Treatment					
	Xylem	4	241013.02	60253.26	0.915	0.476
	Pith	4	1305957.88	326489.47	0.478	0.751

Table B11 ANOVA table for %stem water content

Source	DF	SS	MS	F	P
Genotype	2	134.3	67.15	11.976	0.000
Treatment	2	34.83	17.42	3.106	0.049
Genotype*Treatment	4	127.54	31.88	5.686	0.000

APPENDIX C

4.0 IMPACT OF PREVIOUS DROUGHT STRESS HISTORY ON SUBSEQUENT DROUGHT STRESS RESISTANCE

4.1 Hypothesis

The previous drought stress history of the genotype V1002 will affect subsequent drought stress resistance.

4.2 Materials and methods

4.2.1 Background information of the potato plants

During a previous study in our lab in 2010, six potato genotypes [V1002-2, Russet Burbank (RB), A90586-11, Cv97065-1, Fv12246-6 and Cv92028-1] were studied to determine their resistance to drought stress. The treatments were Non-Acclimated (NA) and Drought Acclimated (DA). DA plants were exposed to Drought Acclimation cycle for 13 times over 7 weeks period. The soil moisture content was maintained above 25% and let to drop down to 10% and go up to 25% by re-watering. Harvested tubers were kept in the cool room (4°C) for 5 months before transplantation. Plants were grown by using these tubers (described in details in Chapter 4.3.1) in a greenhouse (17/7 hours of light/dark, highest/lowest temperature as 25/22°C, RH 50% and light intensity 250-300 µmol/m^2/sec). After 6 weeks of transplantation, there were lots of variation in the growth stage and vigor of the plants in all genotypes except V1002. Therefore, plants of V1002 were selected for the experiment.

Tubers of V1002 from two different previous drought stress history were considered. These were Non-Acclimated (NA) and Drought Acclimated (DA). Tubers had been kept in the cool room (4°C) for 5 months before transplantation. The experiment was carried out during summer 2011 in the greenhouse, College of Agriculture and Bioresources, The University of Saskatchewan, Canada.

4.2.1.1 Genetic information of the tubers

To follow

4.2.2 Establishment of plants

Approximately 150 tubers (50-300g) were placed at the bottom of 11-litre plastic pots with the SM#4 (Sunshine Mix No. 4, Sungro Horticulture Canada Limited) mix just to cover the tubers. All the pots were placed in a greenhouse (17/7 hours of light/dark, highest/lowest temperature as 25/22°C, RH 50% and light intensity 250-300 µmol/m^2/sec). When plants grew up to 5cm high above the rim, all stems but the single main stem were removed. The pots were filled by additional SM#4 mix up to 2.5cm down the rim. Plants were watered to keep the soil moist. Plants were also fertilized twice a week by Plant Prod fertilizer (N:P:K, 20:20:20, 1g/litre of water, 200ml per pot).

4.2.3 Experiment layout

Nine plants from each of two treatments (Non-Acclimated, NA and Drought Acclimated, DA) were transferred to another greenhouse (17/7 hours of light/dark, highest/lowest temperature as

22/20°C, RH 17-25% and the light intensity 275 µmol/m^2/sec) where relative humidity was lower than the previous greenhouse. The treatments and replicates were arranged as in Table 4.1.

Table 4.1 Experiment layout during second generation of drought stress experiment.

Previous history	Treatments and codes in this experiment	
	Treatments	Codes
Non-Acclimated (NA)	Non-Acclimated and Non-Drought Stressed Controls (NA)	NA-NA
	Drought Acclimated and Drought Stressed (DAS)	NA-DAS
	Non-Drought Acclimated but Drought Stressed (NAS)	NA-NAS
Drought Acclimated (DA)	Non-Acclimated and Non-Drought Stressed Controls (NA)	DA-NA
	Drought Acclimated and Drought Stressed (DAS)	DA-DAS
	Non-Drought Acclimated but Drought Stressed (NAS)	DA-NAS

Pots were placed randomly into 60cm X 40cm X 21.5cm white plastic boxes. Two sensors were used for measuring the soil moisture. One sensor was placed into NA pot and the other was placed into the DAS pot. The minimum moisture content required for the growth of potato is 25% water content (Costa et al. 1997). The moisture level of NA pots was maintained at or above 30% water content and, accordingly, plants were irrigated as and when needed. Plants were fertilized twice a week.

Application of Drought Acclimation, Drought Stress and Recovery cycles and taking different response measurements were described in Chapter 3.3.3 – 3.3.10. This experiment was repeated twice. In the second set, the experiment layout was as in Table 4.2.

Table 4.2 Experiment layout during third generation.

1st generation	**2nd generation**	**3rd generation**
NA (Non Acclimated, non-drought stressed controls)	NA-NA (Non-Acclimated and Non-Stressed Controls)	NA-NA-NA-1
		NA-NA-NA-2
		NA-NA-NA-3
		NA-NA-NAS-1
		NA-NA-NAS-2
		NA-NA-NAS-3
		NA-NA-DAS-1
		NA-NA-DAS-2
		NA-NA-DAS-3
	NA-DAS (Non-Acclimated and Non-Drought Stressed – Drought Acclimated and Stressed)	NA-DAS-NA-1
		NA-DAS-NA-2
		NA-DAS-NA-3
		NA-DAS-NAS-1
		NA-DAS-NAS-2
		NA-DAS-NAS-3
		NA-DAS-DAS-1
		NA-DAS-DAS-2
		NA-DAS-DAS-3
DA (Drought acclimated)	DA-DAS (Drought Acclimated – Drought Acclimated and Stressed)	DA-DAS-NA-1
		DA-DAS-NA-2
		DA-DAS-NA-3
		DA-DAS-NAS-1
		DA-DAS-NAS-2
		DA-DAS-NAS-3
		DA-DAS-DAS-1
		DA-DAS-DAS-2
		DA-DAS-DAS-3

4.3 Results

Drought stress history in plants during 1st, 2nd and 3rd generations and drought acclimation increased drought stress resistance.

4.3.1 Tuber Yield

Germplasm with no previous acclimation history in the first generation (NA and DA) induced equivalent tuber yield (total mass per pot, g) (Figure 4.2a). Similarly, in the second generation, the NA-NA and NA-DAS treatments were not significantly different (Figure 4.2b). But the NA-NAS treatment had significantly lower tuber yield compared to the NA-NA controls. Furthermore, germplasm with an acclimation history in the 1st generation (DA-NAS) induced significantly higher ($p<0.05$) yield compared to germplasm having no previous history (NA-NAS). Similarly, drought acclimation in two successive generations (DA-DAS) induced higher yield when compared to germplasm with drought acclimation in one generation (NA-DAS).

Consistent with the 1st generation, there was no difference in yield in drought acclimation among treatments in the 2nd generation. In the third generation, there was no difference among treatments (Figure 4.2c).

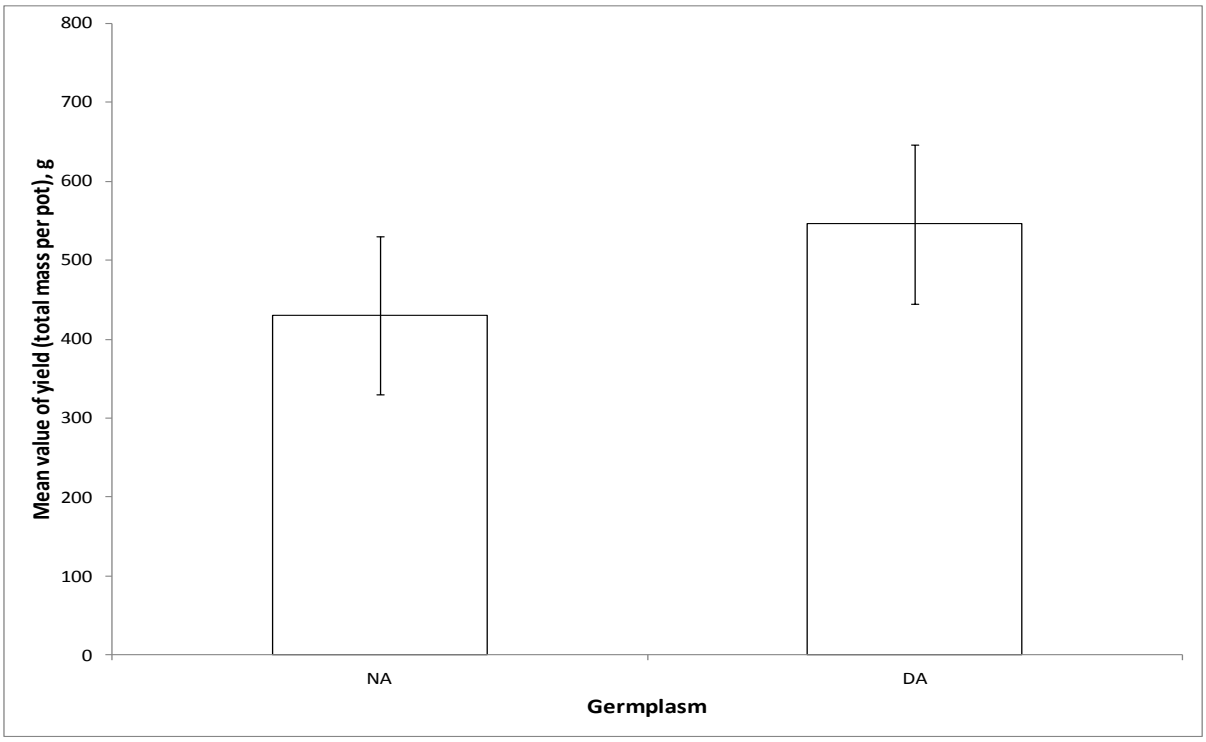


Figure 4.2a Mean yield (total mass per pot, g) in V1002 in the first generation.
NA = Non-Acclimated Controls and DA = Drought-Acclimated.

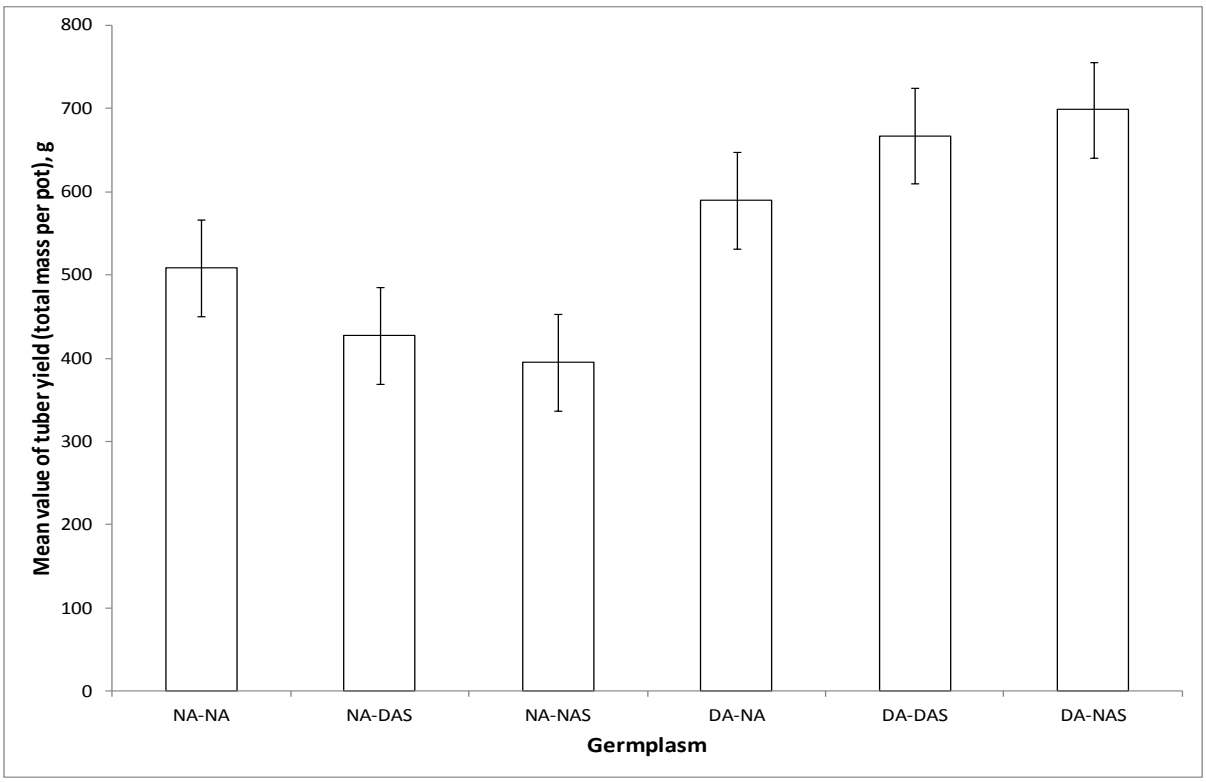


Figure 4.2b Mean yield (total mass per pot, g) in different germplasm in the second generation. Germplasm names contain two generations of history (eg. NA-DAS means Non-Stressed Controls (NA) in the first generation and Drought-Acclimated and Stressed (DAS) in the second generation).

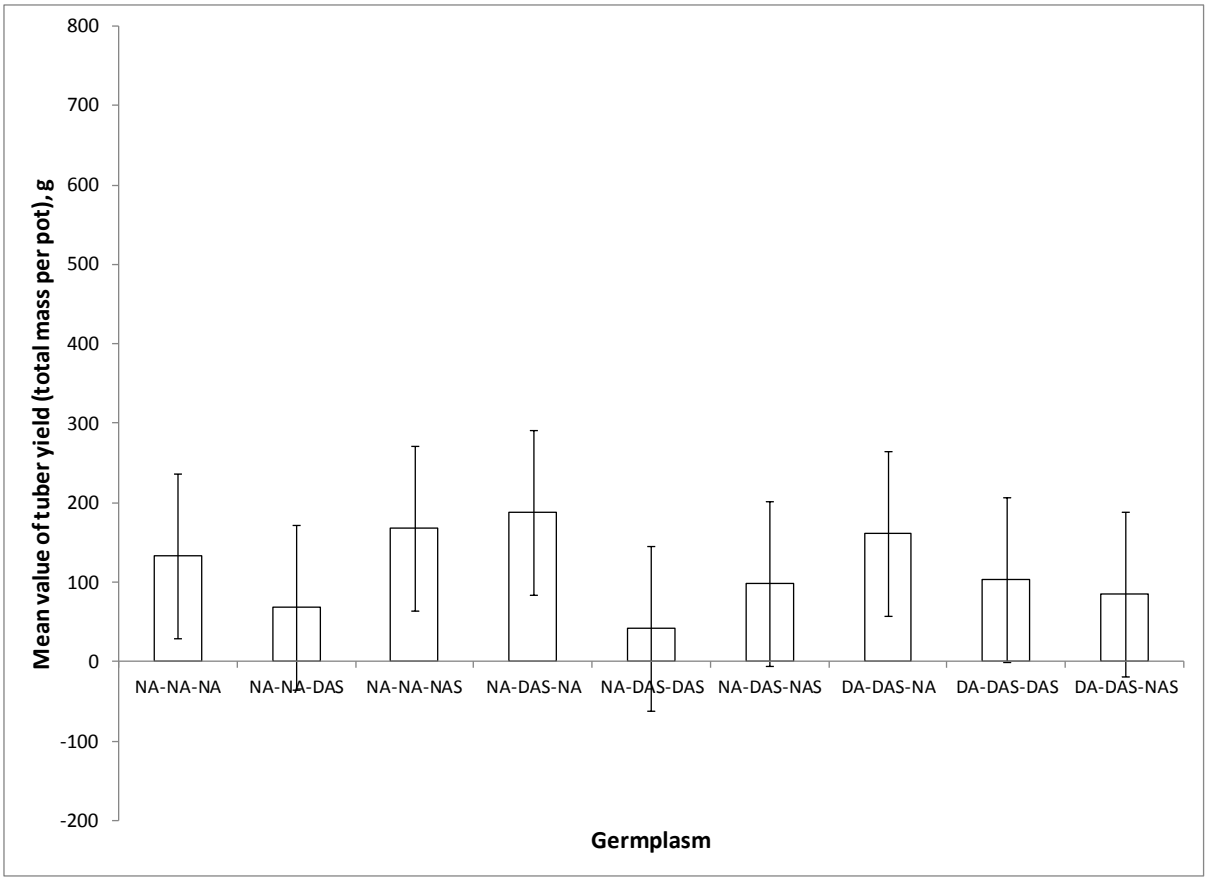


Figure 4.2c Mean yield (total mass per pot, g) in different germplasm in third generation. Germplasm names contain three generations of history (eg. NA-DAS-NAS means Non-Stressed Controls (NA) in first generation, Drought-Acclimated and Stressed (DAS) in second generation and Non-Acclimated and Stressed (NAS) in current generation).

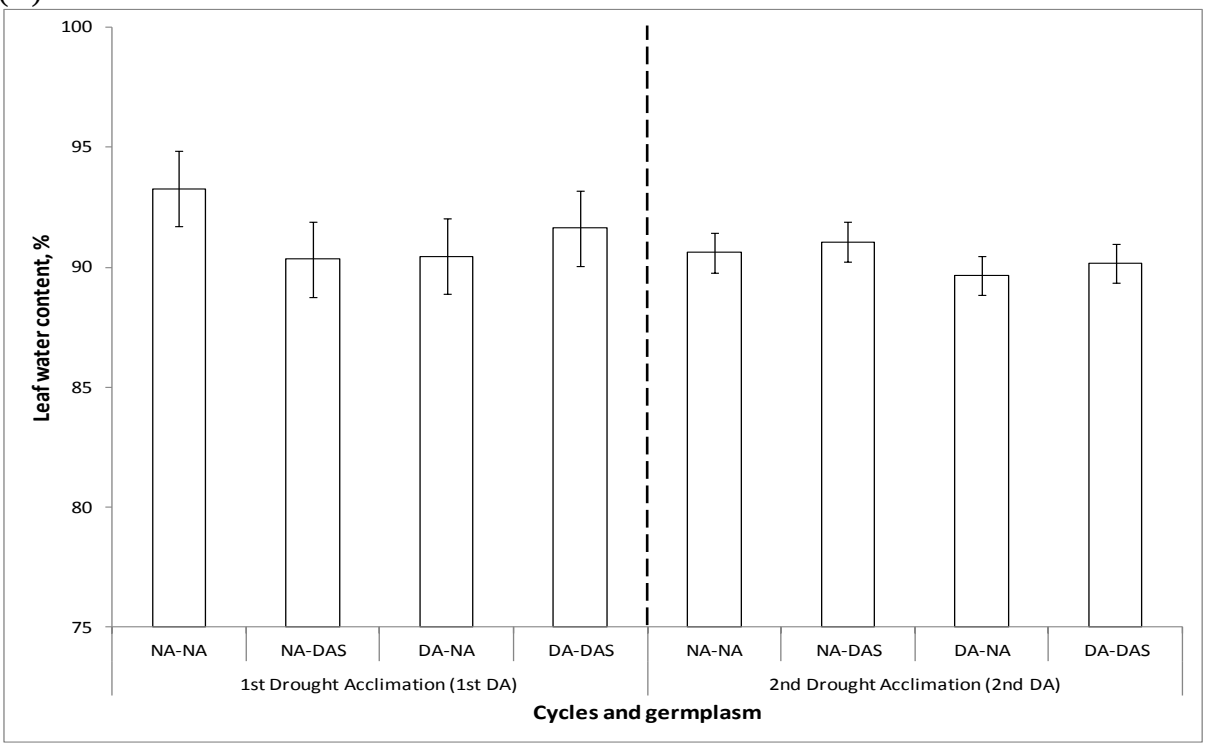
4.3.2 Leaf water content at low soil moisture levels

Leaf water content (% LWC) varied upon exposure to Drought-Acclimation (DA) and Drought Stress (DS) cycles and was influenced by the generations (Figs. 4.3 and 4.4).

In the second generation at DS1, NA-NAS % LWC was reduced compared to NA-NA and NA-DAS. However, with one generation of drought acclimation history (DA-NAS), a significant increase in % LWC was observed (Fig. 4.3B). By three generations of drought acclimation and stress history, there were more pronounced significant differences among the acclimation treatments.

Once severe water deficit was imposed (DS1, DS2), greater impact of the acclimation treatments and generations was revealed by the third generation. At DS1, similar to two generations, at three generations, % LWC was reduced in NA-NA-NAS compared to Non-Stressed Control plants (NA-NA-NA) (Fig 4.4b When NAS was preceded by either one or two generations of DAS history (NA-DAS-NAS or DAS-DAS-NAS), % LWC was increased when measured under severe moisture deficit. In addition, when DAS (NA-NA-DAS) was preceded by one generation of drought acclimation history (NA-DAS-DAS), % LWC increased.

(A)



(B)

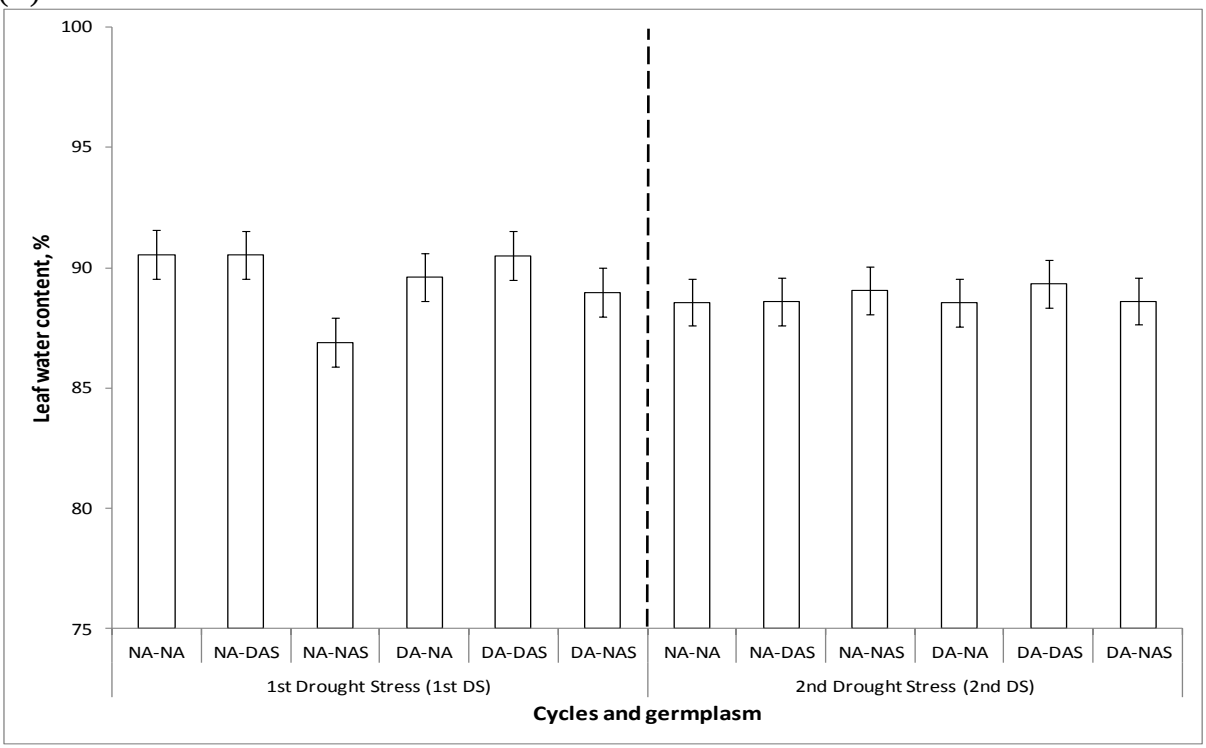
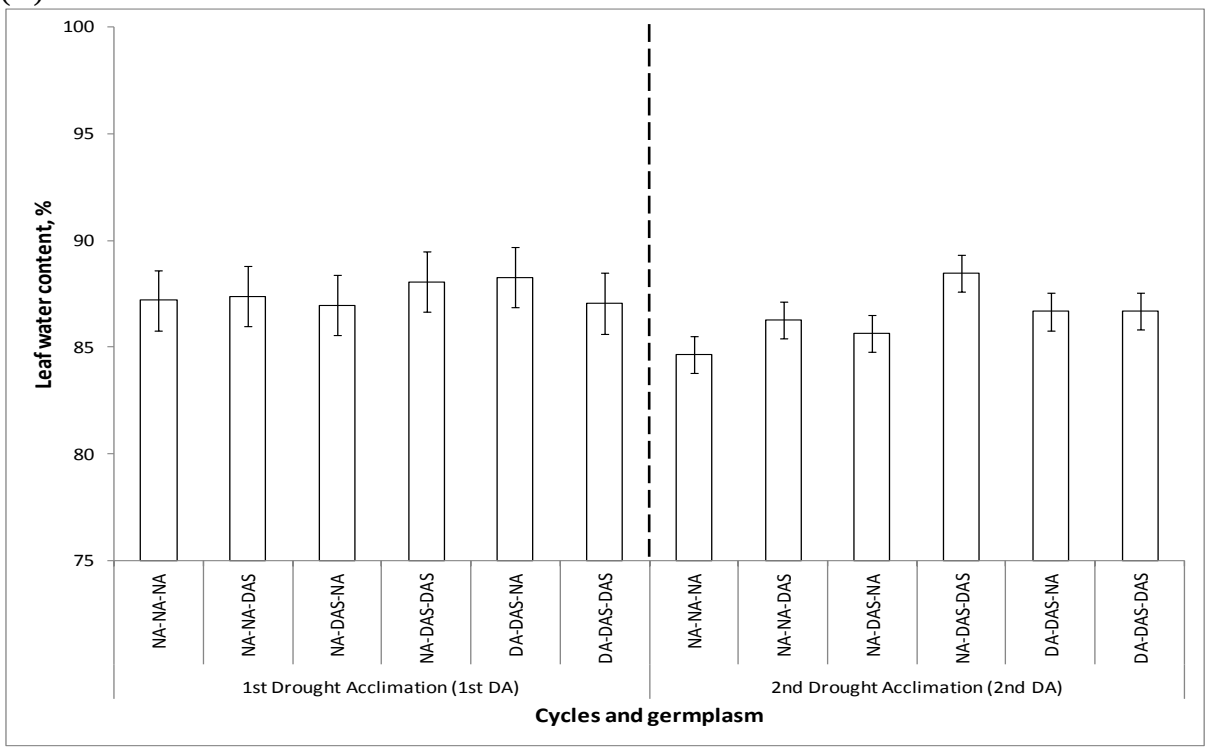


Figure 4.3 Two Generation response, leaf water content (%) at the end of (A) first and second Drought Acclimation cycles (1st and 2nd DA) and (B) first and second Drought Stress cycles (1st and 2nd DS).

(A)


100
95
90
85
80
75
Leaf water content, %
NA-NA-NA
NA-NA-DAS
NA-DAS-NA
NA-DAS-DAS
DA-DAS-NA
DA-DAS-DAS
NA-NA-NA
NA-NA-DAS
NA-DAS-NA
NA-DAS-DAS
DA-DAS-NA
DA-DAS-DAS
1st Drought Acclimation (1st DA)
2nd Drought Acclimation (2nd DA)
Cycles and germplasm

(B)

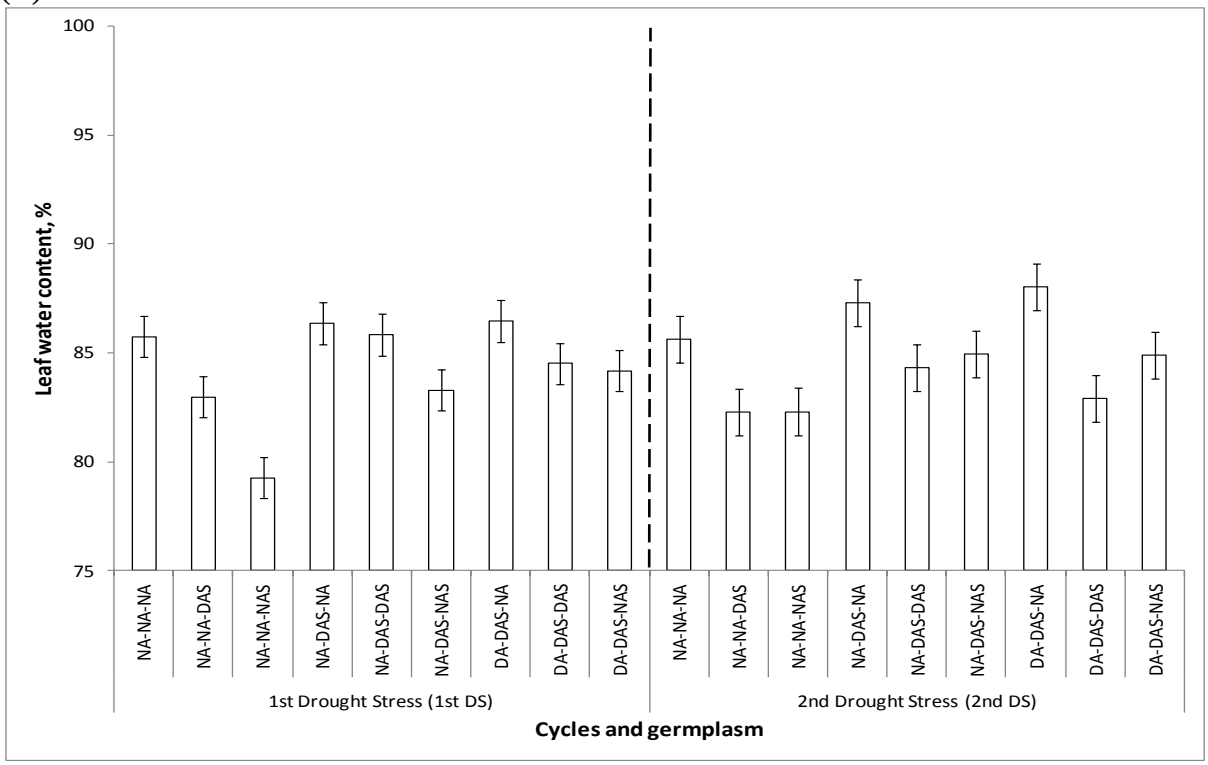

100
95
90
85
80
75
Leaf water content, %
NA-NA-NA
NA-NA-DAS
NA-NA-NAS
NA-DAS-NA
NA-DAS-DAS
NA-DAS-NAS
DA-DAS-NA
DA-DAS-DAS
DA-DAS-NAS
NA-NA-NA
NA-NA-DAS
NA-NA-NAS
NA-DAS-NA
NA-DAS-DAS
NA-DAS-NAS
DA-DAS-NA
DA-DAS-DAS
DA-DAS-NAS
1st Drought Stress (1st DS)
2nd Drought Stress (2nd DS)
Cycles and germplasm

Figure 4.4 Three Generation response, leaf water content (%) at the end of (A) first and second Drought Acclimation cycles (1st and 2nd DA) and (B) first and second Drought Stress cycles (1st and 2nd DS).

4.3.3 Excised leaf moisture loss (drought shock) over a fifteen minute time course

Drought stress history and drought acclimation had impact on leaf water loss over a 15 minute time course (Table 4.3). Upon excision of the first fully expanded leaf during the early vegetative stages (prior to the tuber initiation stage, within 7 weeks of growth), germplasm having a previous acclimation history (DA-DAS-NA) lost water at a higher rate ($p<0.05$) than the germplasm with no history (NA-NA-NA). However, after that stage (tuber initiation and tuber bulking), excised leaves of germplasm with a previous drought acclimation/stress history had a lower to equal rate of water loss compared to the germplasm with no history.

Acclimation (DA-DAS-DAS) induced a higher ($p<0.05$) rate of water loss from excised leaves compared to Non-acclimated controls (NA-NA-NA) after the first acclimation (1st DA) and during the first Recovery, 1st R (after DS1) (Table 4.3). However, upon the second exposure to acclimation (2nd DA) and during the second Recovery, 2nd R (after DS2), the rate of water loss from excised leaves of acclimated plants was equivalent to excised leaves of non-acclimated controls.

Table 4.3 Average rate of water loss from the leaf surface over 15 minutes in three different treatments (NA-NA-NA, DA-DAS-DAS and DA-DAS-NA) at the end of acclimation cycles (end of 1st and 2nd DA) and during recovery cycles (1st and 2nd R) after each drought stress 1st DS and 2nd DS, respectively.

Cycles	Treatments	Average rate of water loss, mg/min	p value by Mixed Model Analysis	
			Contrast	p value
1st DA	NA-NA-NA	1.44	DA-DAS-DAS vs NA-NA-NA	0.0057
	DA-DAS-NA	5.41	DA-DAS-NA vs NA-NA-NA	0.0001
	DA-DAS-DAS	3.17	DA-DAS-DAS vs DA-DAS-NA	0.0014
2nd DA	NA-NA-NA	2.84	DA-DAS-DAS vs NA-NA-NA	0.6694
	DA-DAS-NA	3.73	DA-DAS-NA vs NA-NA-NA	0.0079
	DA-DAS-DAS	2.98	DA-DAS-DAS vs DA-DAS-NA	0.0253
1st R	NA-NA-NA	5.91	NA-NA-NA vs DA-DAS-DAS	0.0269
	DA-DAS-NA	3.88	NA-NA-NA vs DA-DAS-NA	0.0187
	DA-DAS-DAS	7.82	DA-DAS-DAS vs DA-DAS-NA	0.0001
2nd R	NA-NA-NA	5.2	NA-NA-NA vs DA-DAS-DAS	0.3630
	DA-DAS-NA	3.91	NA-NA-NA vs DA-DAS-NA	0.1319
	DA-DAS-DAS	4.42	DA-DAS-DAS vs DA-DAS-NA	0.5493

4.3.4 Leaf cuticle area

At maximum drought stress [at the end of first Drought Stress (1st DS) in the third generation], germplasm with no previous acclimation history (NA-NA-NAS) expressed the smallest leaf

cuticle platelet area (Figure 4.5). But germplasm with an acclimation history in the previous two successive generations (DA-DAS-NAS) induced larger ($p<0.05$) leaf cuticle platelet area than NA-NA-NAS and equivalent area to Non-Stressed Controls (NA-NA-NA) at the same maximum drought stress condition.

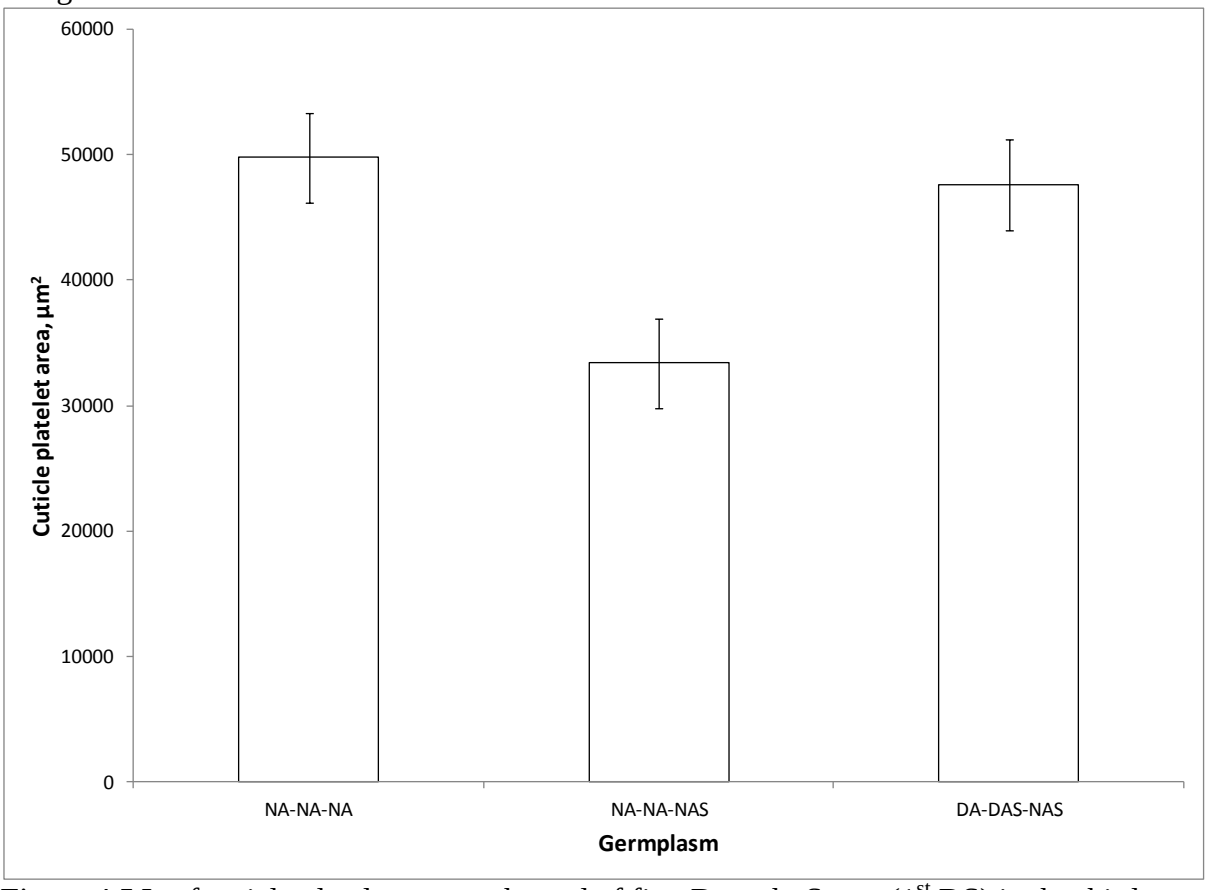
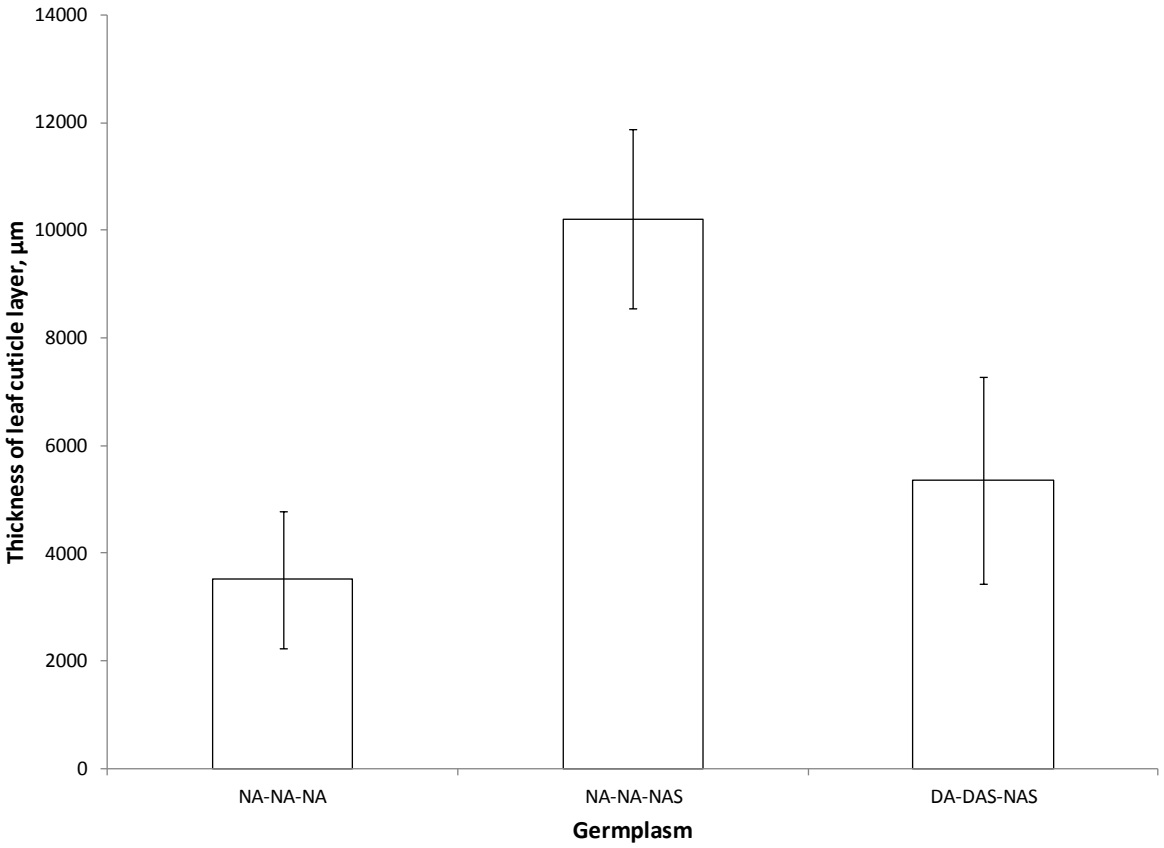


Figure 4.5 Leaf cuticle platelet area at the end of first Drought Stress (1st DS) in the third generation.


14000
12000
10000
8000
6000
4000
2000
0
Thickness of leaf cuticle layer, µm
NA-NA-NA
NA-NA-NAS
DA-DAS-NAS
Germplasm