Drought-Induced Changes in Leaf Water Relations of Canadian Hard Red Spring Wheat Cultivars

by

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Abstract

Drought-induced soil water limitations are a limiting factor in wheat yield; therefore, the expected increased frequency in drought events make it a vital topic of research. For wheat, sustained crop yield at limited soil water availability (i.e., drought tolerance) has been linked to osmotic adjustment (OA) as the main driver to minimize drought-induced reductions in leaf hydration status and growth. Hard red spring wheat (HRSW) cultivars are typically grown in rainfed areas of western Canada with milder climates, but ongoing climate change has increased the frequency and intensity of drought events during the summer months, which raises questions about how successful HRSW cultivars are in tolerating drought. The extent of OA and its relation to stomatal behavior, leaf rolling, and kernel development under periods of drought remains unknown for HRSW. For several commercially used cultivars (‘Superb’, ‘Stettler’, ‘AAC Viewfield’), data indicates that OA does not contribute to drought tolerance. In contrast, I found that sustained kernel weight during periods of relatively low soil water content was linked to ‘tight’ stomatal behavior (i.e., efficient transition from onset to full stomatal closure) and ‘early’ leaf rolling (i.e., reductions in flag leaf width). Among cultivars, ‘Superb’ was most successful in employing these strategies which also prolonged the onset of severe leaf dehydration under drought to a soil relative water content (i.e., % of field capacity) as low as 36% (defined as threshold $\Theta_{RWC}$); ‘Stettler’ at a $\Theta_{RWC}$ of 48%, and ‘AAC Viewfield’ at a $\Theta_{RWC}$ of 51%. Moreover, $\Theta_{RWC}$ marked the onset of drought-induced losses in kernel weight in all three cultivars. Leaf epicuticular waxes exhibited differences in chemical composition between cultivars, which is discussed in the context of leaf water loss beyond stomatal regulation under drought. In conclusion, hard red
spring wheat lacks OA but both leaf stomatal behavior and leaf rolling aid in securing leaf hydration status and kernel weight under drought.
Lay Summary

Wheat is an important crop for Canada and contributes approximately $11B annually to the country’s economy in addition to being a fundamental food source. Due to the reliance of wheat on rainwater for irrigation (especially in the prairies) increased frequency and severity of drought events caused by ongoing climate change are predicted to inflict a severely negative impact on crop production. To address some of these challenges, this study focused on three Canadian hard red spring wheat cultivars) to investigate the impact of decreasing soil water availability on leaf water relations and kernel weight, which are important measures of production. I found no evidence of drought-induced osmotic adjustment (i.e., physiological strategy for maintaining leaf hydration status under drought) in any of the three cultivars. However, leaf stomatal behaviour and leaf roll was found to aid in maintenance of leaf hydration status and kernel weight under limited soil water availability.
Preface

All the research presented in this thesis was conducted at the Horticultural Greenhouse at the University of British Columbia, Point Grey campus. Support for setting up and maintaining the greenhouse compartment was provided by the greenhouse staff. Threshing of wheat spikes was partially conducted by Cheyenne Chu (undergraduate assistant in the Plant Water Relations lab). The analysis of epicuticular waxes using the GC-MS was developed and conducted by Yifan Yan and Lufiani Lina Madilao (UBC Wine Research Centre).

All the remaining work is original, unpublished, independent work by the author, Gopal Sharma. I was the lead investigator and designed the research experiment in consultation with Drs. Knipfer (Co-Supervisor) and Brar (Co-Supervisor), collected the data, analysed the data, and wrote the manuscript together with Dr. Knipfer. Dr. Knipfer helped in the experimental design, data analysis, writing of the manuscript, and provided funding for research. Dr. Brar provided advice on cultivar selection, experimental design, funding for research, and revised the manuscript.
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<td>Flag leaf width</td>
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<td>Gs</td>
<td>Stomatal conductance</td>
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<td>HRSW</td>
<td>Hard red spring wheat</td>
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<tr>
<td>Leaf RWC</td>
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<td>Leaf $\Psi$</td>
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<td>OA</td>
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<td>Piecewise linear regression</td>
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<td>TKW</td>
<td>Thousand kernel weight</td>
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<td>Soil RWC</td>
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Chapter 1: Introduction

Section 1.1 Importance of wheat in Canadian agriculture

Wheat (Triticum aestivum L.) is one of the most important field crops in the world (Awika, 2011). Wheat is of economic importance to Canada, a major exporter of wheat (Food and Agricultural Organization, 2020). Within Canada, the agriculture industry provides $31.9 billion to the country’s Gross Domestic Product (GDP) (Agriculture and Agri-Food Canada, 2022). The primary agriculture industry consists of 190,000 farms that occupy over 62.2 million hectares of land (6.3% of Canada) (Agriculture and Agri-Food Canada, 2022). Crops such as wheat help contribute $26.3 billion of the Gross Domestic Product (GDP) of Canada (Agriculture and Agri-Food Canada, 2021). In 2022, Canada produced 34.7 million metric tonnes of wheat of which 75% (26.1 million metric tonnes) was spring wheat (Statistics Canada, 2022b). It is estimated that 85.3% of wheat produced in 2015 was exported (Statistics Canada, 2019).

Section 1.2 Climate change and drought conditions in western Canada

Climate change is driving an increase in mean annual temperatures (Cubasch, et al., 2001). Mean annual temperatures in the Canadian prairie provinces (major wheat growing region of the country, see below) have risen 1.6°C on average since 1895 (Sauchyn and Kulshreshtha, 2008). In addition, precipitation patterns are changing. In the context of the Canadian wheat growing regions, precipitation is following expected to continue to change in Canada is following a trend of more heavy precipitation but with decreased frequency during the summer months (Stone et al., 1999; Dore, 2005; Sauchyn and Kulshreshtha, 2008). Snow is a major source of replenishment of soil
water on the prairies (de Jong and Stepphun, 1983) but historically the snow depth has been reported to decrease in the prairies (Brown and Braaten, 1997). As wheat cultivation is primarily dependent on rainwater irrigation, reduced precipitation during the growing season and decreased snow depth puts traditionally used Canadian wheat cultivars under the risk of drought stress caused by limited soil water availability. In wheat, drought conditions are linked to decreased yield (Farooq et al., 2014; Pradhan et al., 2012; Qaseem et al., 2019). This is concerning as in some regions of the world, wheat yield is already not increasing at the historical rate (reviewed in Curtis and Halford, 2014). For example, in the UK wheat yield rose from 3.5 t ha\(^{-1}\) to 7.6 t ha\(^{-1}\) between 1961 and 1984 but it plateaued soon afterwards, and the yield was only 7.7 t ha\(^{-1}\) in 2011 (Curtis and Halford, 2014). Researchers have found that yield potential is closely linked to plant performance, and a variety of plant agronomic traits, including thousand kernel weight, panicle or ear length are known to be affected by drought (Zhang et al., 2018).

Within Canada, most of the wheat is grown in western Canada. Of the 34.7 million metric tonnes wheat produced in Canada in 2022, 92.7% is from western Canada most of which is spring wheat (Statistics Canada, 2022a). Western Canada consists of the provinces of British Columbia, Alberta, Saskatchewan, and Manitoba. The region is also more prone to droughts compared to the rest of Canada due to its topography (Bonsal et al., 2011). The lee of the mountains in western Canada creates a rain shadow effect on the prairies (region with most wheat grown) which leads to decreased precipitation (Bonsal et al., 2011).
Different soil types have different characteristics which can affect how much water is available for the roots. Canada has 10 different soil orders consisting of Brunisolic, Chernozemic, Cryosolic, Gleysolic, Luvisolic, Organic, Podzolic, Solonetlic, and Vertisolic (Soil Classification Working Group, 1998). Within western Canada the majority of the soil consists of Chernozemic soils (Fuller, 2010). Chernozemic soils are defined as soil which drains imperfectly to well and contain organic matter which causes them to darken (Soil Classification Working Group, 1998; Fuller, 2010). Brown, Dark Brown, Black, and Dark Grey groups make up the order of Chernozemic soils and are present in western Canada (Soil Classification Working Group, 1998). Brown, Dark Brown, and Black soil groups make up most of the southern prairies’ region where most of the spring wheat is grown in Canada (Fuller, 2010). In this region water deficit is the limiting factor and within these soil groups, the highest mean annual water deficit occurs in the Brown soil region followed by Dark Brown and Black soil regions (Pennock et al., 2011). Meanwhile, the lowest mean annual water deficit occurs in the Dark Grey soil regions (Pennock et al., 2011). As a majority of the spring wheat growing region consists of the highest mean annual water deficit soil group types, any decreases in precipitation can lead to devastating droughts.

The wheat growing regions of western Canada have had a history of droughts (Wheaton et al., 2008). One of the worst droughts in Canadian history occurred during 2001 and 2002 (Wheaton et al., 2008). The duration, severity, and the wide affected area of the drought resulted in a 25-year record low wheat production in Saskatchewan and Alberta (Wheaton et al., 2008). A more recent drought in 2021 resulted in a 37.1%
decrease in Prairie wheat production (Statistics Canada, 2022b). Considering the sensitivity of this region’s economy to drought, it is imperative to study drought tolerance of Canadian wheat cultivars and identify strategies to mitigate negative effects of drought on wheat production.

Section 1.3 Impact of drought on leaf water relations

Section 1.3.1 Leaf relative water content

The ability of a crop plant to maintain its hydration status during water stress (i.e., dehydration avoidance) is a key strategy to attain drought tolerance (Blum, 2010). Physiological measurements such as leaf relative water content (RWC\textsubscript{leaf}) and leaf water potential (leaf Ψ, see below) are commonly used to assess plant hydration status (Blum, 2010; Mullan and Pertragalla, 2012). The RWC\textsubscript{leaf} was first used by Weatherley (1950) as a way of quantifying the balance between leaf water uptake and water loss by transpiration of a cotton leaf in the field. Weatherley (1950) found that accuracy could be increased by using measures relative to the maximum amount of water that can be held by the leaf than measures relative to the dry weight of leaf. Using the dry weight for the ratio is less clear as to whether a decrease is caused by a decrease of water in the leaf or increase in leaf dry weight due to growth (Weatherley, 1950). Another advantage of using RWC\textsubscript{leaf} is that it does only require a scale for weights and no other special instruments to measure.

In the past, studies have shown that RWC\textsubscript{leaf} decreases with soil water availability in a variety of crops such as beets (Shaw et al., 2002), potatoes (Soltys-Kalina et al., 2016),
and cereals (Fu and Huang, 2001) in addition to wheat (Schonfeld et al., 1988; Siddique et al., 2000, Keyvan, 2010). For wheat, Salim et al. (1969) found that when plants had a RWC\text{leaf} above the threshold of 25%, they were able to recover full saturation of the leaf (following drought). A decline in RWC\text{leaf} ranging from 10 to 20% was seen between well-watered and drought stressed wheat in the field (Schonfeld et al., 1988). Siddique et al. (2000) noted a difference of more than 40% in RWC\text{leaf} between well-watered and drought stressed wheat plants and emphasized that plants experiencing stress during the anthesis stage performed worse (lower RWC\text{leaf}) than those that were stressed during the vegetative stage. Consequently, Atlaf et al. (2021) claimed that RWC\text{leaf} in wheat is a good criterion for selecting for drought tolerance due to its sensitivity to drought, which is similar to what Matin et al. (1989) reported in barley. Furthermore, reduced yield has been noted in wheat plants with lower RWC\text{leaf} (Schonfeld et al., 1988; Tahara et al., 1990). In a winter wheat study, Tahara et al. (1990) reported that the plants which had a low RWC\text{leaf} (during anthesis) consistently had a low yield at the end of the experiment. Moreover, Schonfeld et al (1988) found that a drought resistant wheat cultivar had a higher RWC\text{leaf} and yield in both well-watered and drought conditions.

Section 1.3.2 Leaf water potential

Leaf Ψ provides an estimate of the magnitude of the negative pressure inside xylem vessels (Scholander et al., 1965), which are responsible for long-distance transport of water. Reduction in soil water availability by drought and/or increases in transpiration causes a reduction in leaf Ψ (Vesala et al., 2017). The reduction of leaf Ψ (up to -2 MPa
decrease) in wheat experiencing drought conditions have been noted in the past (Seropian and Planchon, 1984; Siddique et al., 2000; Zhu et al., 2005; Subrahmanyam et al., 2006). Furthermore, midday values of leaf $\Psi$ have been found to be more than -2 MPa more negative than predawn values (Johnson et al., 1983) for wheat plants which had water withheld from them. In addition, Jongdee et al. (2002) reported that a less negative leaf $\Psi$ measured during water deficit conditions is linked to higher yield in rice. Similarly, Gupta et al. (2001) performed a correlation study in wheat and found leaf $\Psi$ to be linked to yield. Quarrie and Jones (1979) found that leaf $\Psi$ varied by cultivar when water was withheld for spring wheat and the less negative leaf $\Psi$ could indicate drought avoidance. Moreover, isohydric plants are able to effectively maintain $\Psi$ throughout the day while anisohydric plants are incapable to maintain $\Psi$ under increasing drought conditions (Tardieu and Simonneau, 1998; Sade et al., 2012). However, the effect of drought by limited soil water availability on leaf $\Psi$ of Canadian wheat cultivars remains unknown.

**Section 1.3.3 Leaf osmotic adjustment**

Osmotic adjustment (OA) is a physiological mechanism which aids in the maintenance of cell hydration status and is related to increased yield potential during drought conditions (Morgan, 1980; Blum, 2017). It occurs as a plant becomes more water stressed, causing cells to accumulate solutes thereby decreasing their solute potential ($\Psi_S$) (Jones and Turner, 1978). The $\Psi_S$ is dependent on the amount of osmotically active solutes within a cell (Nobel, 1983). Through this, the lower leaf $\Psi_S$ allows the
cells to retain their water by lowering their total \( \Psi \) and thereby maintaining its turgor pressure \( (\Psi_p) \) (Jones and Turner, 1978).

Osmotic adjustment has been studied extensively in the past. Hsiao (1973) reviewed the topic and discussed evidence for OA in maize. Evidence for OA has also been found in wheat lines: Morgan (1980) discovered that wheat which had water withheld displayed OA in spikelets and leaves because leaf \( \Psi_s \) declined while changes in RWC\(_{\text{leaf}}\) were negligible. Abid et al. (2018) found that leaf \( \Psi_s \) (and leaf \( \Psi \)) decreased during drought stress due to OA. Furthermore, wheat plants exhibiting OA were found to have higher yield (Blum et al., 1999; Fisher et al., 2005; Silvia et al., 2006; wheat and others, Blum, 2017). In drought conditions, Blum et al. (1999) found that plot yield was higher (0.76 kg/plot) for a wheat cultivar exhibiting high OA (0.63 MPa) compared to a cultivar (0.32 kg/plot) with lower OA (0.39 MPa). Fischer et al. (2005) suggested that wheat yield stability (as defined by the yield of a specific cultivar compared to the mean yield at that soil moisture) could be achieved through OA and \( \Psi_p \) maintenance. This result was corroborated by Mahmood et al. (2020) who found a positive correlation between OA and thousand kernel weight (TKW).

Section 1.3.4 Leaf stomatal behavior

Leaf water loss depends largely on stomatal regulation (Buckley, 2005). Stomatal closure decreases transpiration and is a mechanism that aids in the prevention of plant dehydration under drought (Tardieu et al., 2018). The stomata are controlled by guard cells (Nobel, 1983). Guard cells are flaccid at night (closed stomata); daylight causes
the uptake of K ions which decreases the cells’ water potential, consequently increasing water flow into the cell and causing them to become turgid (thereby opening the stomata) (Nobel, 1983). Historically it was theorized that abscisic acid (ABA, i.e., a plant hormone) was synthesized in the roots and transported to the shoots of the plants signalling the stomata to close under drought conditions (Busk et al., 1999). Although recently it has been argued that ABA is created in the leaves for drought stressed plants rather than transported (McAdam et al., 2016; McAdam and Brodribb, 2017). The signal leads to an outflow of ions and water, causing the guard cells to lose $\Psi_P$ and close (Hsu et al., 2020). The stomatal conductance ($G_S$) measures the opening and closing of the stomata and has been used to estimate the hydration status of a plant (Giménez et al., 2013). Studies in wheat have shown that $G_S$ decreases under drought stress (Quarrie and Jones, 1979; Shah and Paulsen, 2003; Siddique et al., 2000; Wang et al., 2015). Shah and Paulsen (2003) withheld water from wheat plants (drought treatment) after the anthesis stage (50% florets visible on the wheat spike) which caused the $G_S$ to drop from 0.21 to 0.14 mol m$^{-2}$s$^{-1}$ after one week. By the second week of withholding water, the control plants had a $G_S$ of approximately 0.14 mol m$^{-2}$s$^{-1}$ while wheat plants in the drought treatment were down to 0.06 mol m$^{-2}$s$^{-1}$ (Shah and Paulsen, 2003). By the third week, the $G_S$ of drought plants had hit 0 mol m$^{-2}$s$^{-1}$ with minimal decrease in the control plants (Shah and Paulsen, 2003). Quarrie and Jones (1979) discovered differences between cultivars with certain cultivars (‘Chinese Spring’ and ‘El Gaucho’) having a higher stomatal conductance than others (such as ‘H8810/47’) as soil dried. Furthermore, differences in cultivars have been found for stomata width and areas (Mehri et al., 2009). The cultivars which were more tolerant were noted to have
lower stomata widths and areas which allowed for less water to be used by the plant (Mehri et al., 2009).

Section 1.3.5 Leaf rolling

Leaf rolling can occur due to decreased water availability as well as irradiation and increased temperature (Kadioglu and Terzi, 2007). Leaf rolling in wheat starts from the midrib and forms a cylinder under stress (Sirault et al., 2015). Two types of cells are responsible for leaf rolling. Water loss in bulliform cells (upper epidermis of a leaf) or the shrinking of hypodermis cells (under epidermis of the leaf) can cause leaf rolling (Kadioglu and Terzi, 2007). Bulliform cells are the most common cause of leaf rolling in wheat (Merriu...
measure of leaf rolling) decreased as the leaf Ψ became more negative but resistant lines decreased more rapidly at a less negative leaf Ψ (-2.25 MPa) than susceptible lines (-2.5 MPa). The delay of leaf rolling has also been noted due to OA in rice cultivars (Mahmood et al., 2020). Moreover, leaf rolling has been used as a visual indicator of water stress in the past (spring wheat; Jones, 1979), but Clarke (1986) has argued that the subjective manner of estimating leaf rolling made it ill-suited for obtaining consistent results of plant water status.

Section 1.3.6 Leaf epicuticular waxes

Epicuticular waxes are a layer of waxes present on the adaxial and abaxial sides of wheat leaves which have a glaucous (blue-green) appearance (Mohammed et al., 2018). It has been theorized that increased waxes decrease transpiration rates to help the plant perform better under drought stress by reducing cuticular water loss (Araus et al., 1991). Epicuticular waxes in wheat are typically composed of alkanes, aldehydes, alcohols, acids, esters, and β-diketones (Bianchi and Figini, 1986); although it has been noted that alcohol levels decrease when β-diketones are present (Tulloch et al., 1980). In general, increased formation of leaf epicuticular wax is found during drought stress in wheat (Uddin and Marshal, 1988; Willick et al., 2018). While changes in waxes occur under drought stress, Bi et al. (2017) determined that glaucousness was not enough to determine drought tolerance. Instead, the structure of the wax crystals and changes in amount and composition of wax were found to be better indicators of drought tolerance in wheat (Bi et al., 2017). The existence of increased β-diketones for wheat plants (under water stress) helped reduce water loss (Bi et al., 2017). The epicuticular wax
load has also been found to have a positive relationship with barley yield (González and Ayerbe, 2010). Barley grain yield ranged from 100 to 350 g m\(^2\) during drought while the epicuticular wax load ranged from 0.35 to 0.50 g cm\(^2\) with the higher wax load being related to higher yield (González and Ayerbe, 2010).

**Section 1.3.7 Leaf physiological thresholds**

Physiological responses to reductions in soil water availability commonly do not follow a simple linear relationship and instead exhibit a distinct threshold at which the response abruptly intensifies. A piecewise linear regression (PLR) model can be used to extract the associated threshold value (Toms and Lesperance, 2003), thus making it practical for classification of response dynamics along a spectrum of decreasing soil water availability under drought. For example, Ahumada-Orellana et al. (2019) used a PLR model to identify the range of stomatal conductance and associated threshold where CO\(_2\) assimilation was maintained under drought conditions. Knipfer et al. (2020) used a PLR model on woody perennials to extract information on plant dehydration stages under drought from measurements of stem water potential. Furthermore, PLR models have been used to analyze thresholds of crop production by quantifying yield in different environments accounting for temperature and precipitation (Obeme et al, 2021). While PLR has been used in woody perennials, it has not been utilized to classify the impact of drought stress on water status (i.e., relative water content) on wheat.
Section 1.4 Rationale of the study

‘Superb’, ‘Stettler’, and ‘AAC Viewfield’ are hard red spring wheat (HRSW) cultivars which are classified as Canadian Western Red Spring market class and make up to 70% of the production in western Canada (Carson and Edwards, 2009). Historically, these cultivars have been adapted to the wheat-growing regions of the Canadian prairies (DePauw et al. 2009; Townley-Smith et al., 2010; Cuthbert et al., 2018). However, studies suggest that the commercially used HRSW cultivars ‘Superb’ (drought sensitive) and ‘Stettler’ (moderate drought tolerant) differ in their performance under drought (Ashe et al., 2017; Willick et al., 2018). ‘AAC Viewfield’ has also been noted to perform better in the field during drought conditions (Hucl, personal communication). However, the physiological mechanisms at work that aid in securing hydration status at leaf level remain elusive and physiological traits providing for improved drought tolerance need to be identified to assist future wheat breeding efforts.

Section 1.5 Objectives of the study

The overarching goal of this study was to investigate drought tolerance mechanisms of three commercially used Canadian HRSW cultivars (‘Superb’, ‘Stettler’ and ‘AAC Viewfield’) during early anthesis in order to elucidate the impact of reductions in soil water availability on leaf water relations (i.e., relative water content, water potential, solute potential, stomatal conductance, rolling, wax composition) and thousand kernel weight. Specific objectives were to:
i) Investigate leaf OA in response to drought and its impact on leaf relative water content,

ii) Characterize leaf stomatal behavior, epicuticular wax composition, and rolling in response to drought and their role in maintaining leaf relative water content, and

iii) Elucidate the link between kernel weight loss and leaf relative water content.

According to existing literature (as cited above), my general hypothesis was that OA is the main driver for sustaining kernel weight (or yield in actual farm conditions) when soil water availability becomes limiting under drought. Furthermore, OA can aid in future selection of wheat genotypes with improved physiological performance under conditions of drought stress.
Chapter 2: Leaf hydration status under drought is predominantly linked to stomatal regulation and leaf rolling but not osmotic adjustment in hard red spring wheat

Section 2.1 Introduction

Limitations in soil water availability during drought has become one of the most important constraints to agriculture globally with devastating impacts on wheat production (Henson et al., 1989; Xoconostle-Cazares et al., 2011). In northern regions of the world with mild summers, HRSW (hard red spring wheat) is commonly grown in rainfed cropping systems (De Jong et al., 2008). However, ongoing climate change has resulted in unprecedented drought events in these regions resulting in suboptimal soil water availability during the growing season and restricting wheat productivity (Campbell et al., 2007). For wheat, the ability to maintain grain yield at limited soil water availability (i.e., drought tolerance) has been found to be linked to osmotic adjustment, or OA (Morgan and Codon, 1986; Fisher et al., 2005; Blum, 2017; Mahmood et al., 2020). Osmotic adjustment minimizes cell water loss through active solute accumulation. A decline in xylem pressure is considered as the trigger for this process (Turner and Jones, 1980; Kramer and Boyer, 1995; Turner, 2017). A major benefit of OA is maintenance of growth under drought by $\Psi_p$ maintenance (Kramer and Boyer, 1995). Solute concentration effects by cell dehydration need to be distinguished from ‘active’ solute accumulation to obtain reliable estimates of OA (Babu et al., 1999, Bagatta et al. 2008). Differences in OA among wheat cultivars have been described (Morgan, 1977; Blum et al., 1999), and several wheat cultivars (such as ‘Capelle Desprez’ and ‘Condor’) only exhibit partial or no OA in response to a decline in soil
water availability (Morgan, 1980; Turner and Jones, 1980; Blum et al., 1990). This raises the question: is drought-tolerance of HRSW cultivars linked to OA?

Leaf stomatal regulation plays a key role in securing plant hydration status in response to a decline in soil water availability. A drought-induced reduction in stomatal conductance reduces water loss by transpiration through stomata, which can minimize the buildup of negative leaf xylem pressures (i.e., leaf \( \Psi \)) under drought (Kramer and Boyer, 1995; Tardieu et al., 2018). Plants can be categorized into ‘isohydric’ plants that are able to maintain a nearly constant leaf \( \Psi \) until showing signs of severe dehydration and ‘anisohydric’ plants that exhibit significant reductions in leaf \( \Psi \) in response to a decline in soil water availability (Tardieu and Simoneau, 1998). According to Henson et al. (1989), wheat cultivars (‘Gamenya’ and ‘Warigal’) exhibit anisohydric behavior because they are not able to maintain leaf \( \Psi \) and stomatal conductance under a decline of soil water content. Therefore, the question can be asked: do those HRSW cultivars that lack OA have the ability to maintain leaf \( \Psi \) under drought (i.e., isohydric behavior) through effective stomatal behavior under drought? Although other definitions of isohydricity have been used in the past, such as describing early or late stomatal closure under increasing drought stress, in this study I will refer to isohydricity in the context of leaf \( \Psi \) maintenance (Hochberg et al., 2018).

Leaf rolling and epicuticular waxes are other ways that plants can minimize water loss from the leaf surface. Leaf rolling reduces transpiration through the creation of a microclimate of higher humidity allowing stomata to remain in an open state and to
assimilate CO₂ (Oppenheimer, 1960; Kadioglu and Terzi, 2007); epicuticular waxes decrease cuticular transpiration by increasing the diffusion resistance of water from leaf surface into atmosphere which aids maintenance of plant hydration status under drought stress (Araus et al., 1991). For wheat, it has been demonstrated that increased formation of leaf epicuticular wax during drought stress prevents excessive water loss (Uddin and Marshal, 1988; Willick et al., 2018). Generally, a combination of alkanes, aldehydes, alcohols, acids, esters, and β-diketones are present in the wheat epicuticular waxes (Bianchi and Figini, 1986). During drought, an increase in β-diketones has been noted previously (Bi et al., 2017; Willick et al., 2018), which has been suggested as the cause of the glaucous appearance in wheat and barley (Adamski et al., 2013; Zhang et al., 2013; Bi et al., 2016). Increased glaucousness in wheat has been linked to lower transpiration rates to help a plant during drought (Richards et al., 1986). For HRSW cultivars, Willick et al. (2018) reported that improved drought tolerance of cultivar ‘Stettler’ compared to ‘Superb’ is associated with greater leaf rolling, leaf relative water content, epicuticular wax thickness and composition. However, the authors studied leaf rolling between cultivars following excision of leaves from the plant, which excludes any possible conclusions on the coordination of leaf rolling, stomatal regulation, and OA in the intact plant.

For commercially used HRSW cultivars ‘Superb’, ‘Stettler’ and ‘AAC Viewfield’, the goal of this study was to elucidate the impact of water stress by drought (i.e., reduction in soil water content) on leaf water relations (i.e., relative water content, water potential, solute potential, stomatal conductance, rolling, wax composition) and thousand kernel weight.
The data shown here highlight cultivar-specific differences in drought responses depending on the level of soil water content plants experiencing during drought.

Section 2.2 Material and methods

Section 2.2.1 Plant material and growth

Three Canadian HRSW cultivars ‘Superb’ (Townley-Smith et al., 2010), ‘Stettler’ (DePauw et al., 2009) and ‘AAC Viewfield’ (Cuthbert et al., 2018) were grown in large 10.6-L cylindrical pots. Pots were constructed from PVC tubes (15 cm x 60 cm; diameter x height, respectively) and a metal mesh (pore size around 4 mm²) was secured to the bottom end with a hose clamp to retain the soil medium. Each pot was equally filled with soil mix (two parts industrial sand with particle size ranging from 0.2 to 0.6 mm and one part greenhouse potting mix consisting of 75% peat and 25% perlite) up to a height of around 56 cm. This type of pot size and soil medium combination was chosen to minimize spatial inhibitions to root system growth and facilitate drainage; pots were placed on the ground and raised on plastic platforms (around 4 cm in height) to aid drainage following watering. For each pot, two seeds per cultivar were placed about 2 cm below the topsoil layer to initiate germination. One week after germination, one seedling was left in each pot and the other was pulled by hand. Two weeks after germination, about 20 g of slow-release fertilizer (Osmocote 14-14-14) was added to each pot to provide a mix of macro-/micronutrients facilitating plant growth. From time of seeding until early anthesis, plants were maintained well-watered by watering with tap water every 2-3 days to about 90% soil relative water content (soil RWC; for details see below) until the early anthesis stage (Zadoks 61, heads started to gain florets at around
60 days after seeding). When a majority of the heads had started flowering, the plant was considered to be in early anthesis. Consequently, when over 90% of the plants reached early anthesis, drought experiments were initiated. Any plants which had not headed were removed from the experiment.

Two consecutive drought experiments were performed, i.e., drought experiment 1 (duration of 19 days, seeding in early June 2021, initiation of experiment in early August 2021) and experiment 2 (duration of 17 days, seeding in mid-February, initiation of experiment in late April 2022) (see Appendix 1 for environmental conditions and Appendix 2 for plot design). During drought experiment 1, daily minimum and maximum temperature ranged from 18°C to 28°C, relative humidity from 50% to 90%, and vapor pressure deficit (VPD) from 0.30kPa to 1.50kPa (Appendix 1); during drought experiment 2, temperature ranged from 18°C to 23°C, relative humidity from 35% to 65% (relative humidity), and VPD from 0.75kPa to 1.65kPa during the drydown (Appendix 1). For both drought experiments plants were arranged following a randomized block design (see Appendix 2). In drought experiment 1, control plants were well-watered (n=6 per cultivar) and drought plants were subjected to a progressive drydown by not watering (n=6 per cultivar, Appendix 5 for soil RWC values over the drydown). The main purpose of drought experiment 1 was to determine leaf water status and associated threshold $\Theta_{RWC}$ (=onset of severe leaf dehydration) and the ability of plants for leaf osmotic adjustment in response to a progressive decline in soil RWC. Parameters measured (daily) during experiment 1 were soil RWC, leaf RWC, leaf $\Psi$, and leaf $\Psi_s$. Following experiment 1, and using information on $\Theta_{RWC}$, for drought
experiment 2 plants (n=3 plants per cultivar and watering treatment) were maintained for 17 days at 90%, 60% and 45% soil RWC (Appendix 4 for soil RWC values over the drydown); the main purpose was to determine the effect of a limited soil water availability on factors that determine leaf water loss and kernel weight. Parameters measured (every 1-3 days) during experiment 2 were soil RWC, Gs, flag leaf width (FLW), epicuticular waxes, and TKW. Considering daily time constraints when collecting data and logistical reasons, it was decided to rather measure a selected suite of parameters for each experiment and inform experiment 2 from observations in experiment 1 (for details on measured parameters see below).

Section 2.2.2 Soil relative water content

Soil RWC was determined from measurement of total pot weight (Eq. 1; Knipfer et al. 2020b):

\[
\text{Soil RWC} = \frac{m_{\text{total}} - m_a - m_b - m_c}{m_{\text{FC}} - m_a - m_b - m_c} = \frac{m_{\text{H}_2\text{O}} - m_{\text{H}_2\text{O--FC}}}{m_{\text{H}_2\text{O--FC}}} \times 100\%, \quad \text{Eq. 1}
\]

For each plant, \(m_{\text{total}}\) (total pot weight) was measured daily (between 12 to 2 pm) using a digital balance (Uline Industrial Platform Scale) and following measurements of leaf water relations (as described below). For determination of \(m_{\text{FC}}\) (total pot weight at field capacity), the pot was fully saturated at day-0 of the drought experiment, allowed to drain for 1-2h, and measured at field capacity. Parameters of \(m_a\) (plant weight, on average 100g of \(n=10\) plants), \(m_b\) (weight of plastic pot, on average 2.05kg), and \(m_c\) (soil dry weight) were treated as constants. Increases in plant weight of the
experimental period were less than 750g which only contributed to a change in soil RWC of less than 7%. Soil dry weight was calculated from \( m_{\text{H2O-FC}} \) (water weight at field capacity) and soil water holding capacity at field capacity (\( WC_{\text{FC}} = 0.36 \)); \( WC_{\text{FC}} \) was measured by weighing (Ohaus TP600S) soil samples at field capacity of approximately 500g in size (\( m_{\text{FC}}' \)), drying soil samples in the oven at 70°C for 24-h, weighing again (\( m_{\text{dry}}' \)), and calculated according to \( (m_{\text{FC}}' - m_{\text{dry}}') / m_{\text{dry}}' \) (i.e., soil gravimetric water content at field capacity). In addition to soil RWC, soil gravimetric water content (soil GWC) was determined from soil core samples at 3 different depths (15, 30, 45 cm from top) of the pot during experiment 1 (Appendix 5) to investigate soil water distribution along the length of the pot at a given soil RWC (Appendices 6 and 7). Soil core samples were extracted horizontally using a metal straw and placed in a 20 mL sealed scintillation vial; soil cores ranged from 20g to 25g and were stored in a cooler until they were transported to lab and measured within 2-h after sampling.

**Section 2.2.3 Leaf water potential**

Leaf water potential was measured on a flag or penultimate leaf (see Appendix 8 for measurement procedure). Prior to excision from the leaf base, the leaf was bagged (>30min) in a sleeve made from aluminum foil to equilibrate leaf apoplast with symplast; measurements of an unbagged leaf results in errors caused by ongoing transpiration and disequilibrium conditions (Turner, 1981). Immediately after excision, the leaf was sealed in a second plastic bag to maintain equilibrium conditions, stored in a cooler filled with ice (i.e., avoid evaporation from leaf), and transported to the laboratory. Within 1-h of excision, leaf \( \Psi \) was measured using a Scholander-type pressure chamber (PMS...
Model 615 Pressure Chamber Instrument). For measurement of leaf Ψ, the top portion of the leaf was cut (length of 6 to 10 cm) and inserted through the chamber lid (i.e., bottom portion was used for leaf RWC and solute potential measurements as described below). The pressure in the chamber was raised slowly and the balancing pressure was recorded when water menisci formed on excised vascular bundles (see Appendix 8 for example). A stereomicroscope (Bausch & Lomb 0.7-3x magnification) was installed above the chamber lid to accurately detect water menisci. Since canopy transpiration differs between night and day conditions and impacts leaf Ψ, leaf Ψ was measured on the same plant when the whole canopy was bagged (i.e., non-transpiring conditions as during night period) or non-bagged (i.e., transpiring conditions as during day period). To do so, I followed the following procedure (see Appendix 8): The evening (between 6 PM to 9 PM) prior to the measurement of leaf Ψ, two leaves were individually covered in aluminum sleeves (as described above) and subsequently the entire canopy was covered under a black plastic bag. The next day (between 8 AM to 9 AM), the first leaf was excised for measurement of leaf Ψ ‘bagged canopy’. Subsequently, the black plastic bag was removed from the canopy and the second leaf was excised for measurement of leaf Ψ ‘non-bagged canopy’ during the same day (between 12 PM to 2 PM). Iso/anisohydric behavior was determined from slope values of the relationship of leaf Ψ and soil RWC prior to the onset of severe leaf dehydration (ΘRWC). Isohydric behavior was defined for slope a at $P > 0.05$ (i.e., not significantly different from zero), and vice versa for anisohydric behavior.
Section 2.2.4 Leaf relative water content

Leaf relative water content (leaf RWC) was measured on the same leaf as used for measurement of leaf Ψ ‘bagged canopy’ associated with negligible plant water loss (see Appendix 8). A 3 cm long bottom portion of the leaf was excised prior measurement of leaf Ψ (as described above), and leaf fresh weight ($m_{\text{fresh}}$) was measured using a digital balance (VWR-214B2). Subsequently, the leaf portion was cut into 0.5 cm small pieces and placed into a petri dished filled with water to facilitate full leaf hydration. After 48-h, leaf pieces were gently blotted dry to remove excess surface water and weighed again to obtain leaf saturated weight ($m_{\text{sat}}$). Afterwards, leaf pieces were dried in an oven at 70°C temperature for 24-h and measured again to obtain leaf dry weight ($m_{\text{dry}}$). Leaf RWC was calculated according to $(m_{\text{fresh}} - m_{\text{dry}}) / (m_{\text{sat}} - m_{\text{dry}}) \times 100\%$ (Barr and Weatherley, 1962). Subsequently, the onset of severe leaf dehydration (i.e., associated with substantial leaf browning) in response to a decline in soil RWC was determined using a two-segment piecewise linear model and the predicted boundary was defined as $\Theta_{\text{RWC}}$. The effectiveness of maintaining leaf RWC prior reaching $\Theta_{\text{RWC}}$ was determined from the steepness of the corresponding slope value.

Section 2.2.5 Leaf solute potential

Leaf solute potential (leaf Ψ$_s$) was measured on the same leaf as used for measurements of leaf Ψ ‘non-bagged canopy’ (see Appendix 2). A 3 cm long bottom portion of the leaf was excised prior measurement of leaf Ψ, inserted into a 2-ml Eppendorf tube stored on ice (i.e., to minimize solute concentration effects by
evaporation), and transferred to a -80°C freezer within 1-h of sampling. For analyses of leaf $\Psi_S$, samples (stored for 4-12 weeks) were thawed at room temperature for 15 mins and centrifuged (Eppendorf Centrifuge 5417C) at 11,000 rpm for 14 mins to extract bulk leaf sap. A volume of 10 $\mu$L of extracted sap was placed in an osmometer (VAPRO Vapor Pressure Osmometer 5600) and osmolality (in mOsmol kg$^{-1}$) was measured. Subsequently, leaf $\Psi_S$ (in MPa) was calculated according $[(0.1\text{MPa} \times \text{‘osmolality’}) / 40.75 \text{mOsmol kg}^{-1}]$ (Barrios-Masias et al., 2018). Using linear regression analyses, drought-induced changes in leaf $\Psi_S$ prior to $\Theta_{RWC}$ were determined from steepness of slope values of the relationship of leaf RWC and soil RWC.

Section 2.2.6 Leaf stomatal conductance

A porometer (LICOR LI-600) was used to determine stomatal conductance (Gs) of the flag leaf every 1-3 days of each plant (between 12 to 1PM). In the greenhouse, the porometer was equilibrated to the surrounding environment, clamped to the flag leaf, and Gs was recorded when readings stabilized (less than 20 seconds). Severely dehydrated leaves were not evaluated for Gs. A 3-parameter sigmoidal non-linear regression model was fitted to data of Gs and soil RWC to determine the onset of drought-induced reductions in stomatal conductance at 95% of maximum Gs ($G_{s95}$) and soil RWC corresponding to negligible stomatal conductance at 5% of maximum Gs ($G_{s5}$; i.e., full stomatal closure). The difference between $G_{s95} - G_{s5} = \Delta G_s$ was calculated to quantify the effectiveness of the drought-induced stomatal response. For convenience, I will use the adjectives ‘tight’ and ‘slack’ to refer to a relatively small and large $G_s$, respectively.
Section 2.2.7 Flag leaf width

Flag leaf width (FLW) was measured every 1-3 days using a digital caliper (Aurora TLV181) at the midpoint of the leaf. Reductions in FLW were assigned to drought-induced leaf rolling (between 1 PM to 2 PM). For each plant, FLW was measured on three leaves and averaged. The extent of leaf rolling prior to the onset of severe leaf dehydration was quantified from the steepness of slope values of the relationship of average FLW and soil RWC (i.e., slope of zero at $P > 0.05 = \text{no leaf rolling}$).

Section 2.2.8 Leaf epicuticular waxes

Flag leaves were excised (between 10 AM and 11 AM), put into a cooler and transported to the lab. A single flag leaf was taken per plant and 4 plants per cultivar. Samples were weighed (VWR-214B2) and returned to the cooler to keep the leaves fresh. In the lab, leaves were removed from the cooler one at a time and the waxes were extracted (within 2-h of excision) following a modified protocol described by Li et al. (2008). Briefly, leaves were dipped into chloroform (CHCl$_3$) and stirred in a beaker to extract the waxes from the leaves. A pipette was used to extract 10 mL of the solution containing the waxes and placed in a test tube; another 3 mL was used to clean wax solution off the beaker walls. An internal standard (tetrocosane, C24 alkane) was added to the samples in the test tube. The internal standard allowed to calibrate the curve for the GC-MS. Samples were then dried using a LABCONCO RapidVap Vertex Dry Evaporator for 2-h and reconstituted using chloroform and derivatized (addition of pyridine, BFSTA, and TMCS). Reconstituted samples were dried again under nitrogen
gas until they were completely evaporated. Samples were once again reconstituted (using 500 μL CHCl₃) and filtered. This was followed by the samples being run in the GC-MS (Agilent, Mass Spectrometry Core Facility, UBC) to identify wax compounds including alcohols, aldehydes, alkanes, β-diketones, and fatty acids. Data were analyzed using the Agilent Masshunter software to determine total wax amount and composition. Peaks were identified in the data and a NIST Special Database 2.0 was used to determine the compound for that peak. The area under the peak was then calculated and summed to quantify the amount of wax present on a flag leaf.

Section 2.2.9 Kernel weight

Wheat spikes from plants (which did not have flag leaves harvested) were harvested at maturation after the end of experiment 1 and 2. Excised wheat spikes were placed in paper bags and transported to the lab within 2-h. Spikes were threshed using a hand thresher and counted. Following the procedure of Qu et al. (2022), the TKW of the plants was measured by counting 100 seeds, weighing them (Ohaus TP600S), and then multiplying by 10. In the cases where the plants did not produce 250 seeds, the average weight per seed was determined and multiplied by 1,000 to get the TKW.

Section 2.2.10 Data analyses

Data were analyzed using R Statistical Software (R Core Team, 2022) in the platform RStudio (RStudio Team, 2022) using package ‘lme4’ (Bates et al., 2015). SigmaPlot (Version 8.0) was used to create graphs and for the regressions (PLR and linear regressions). Slope values for the PLR model (leaf RWC) were identified before (slope
a) and after (slope \( b \)) \( \Theta_{\text{RWC}} \). Linear regressions were run on the leaf \( \Psi_S \), FLW, transpiring and non-transpiring leaf \( \Psi \) data prior to \( \Theta_{\text{RWC}} \) and slope \( c \) was determined. A linear mixed effects model was used to analyze leaf RWC, leaf \( \Psi_S \), FLW, transpiring and non-transpiring leaf \( \Psi \). Cultivar was included in the models as a fixed factor and individual as a random factor (to account for repeated measures) was used with soil RWC as the covariate in the model. F and p-values were found for each parameter using a Type III ANOVA. Statistically significant differences in means were defined as \( P < 0.05 \). If interaction effects (of cultivar and individual) were not found to be significant, a Type II ANOVA was conducted. If any significance was found, a post-hoc Tukey’s t-test was run to determine which differences in means were statistically significant. For epicuticular waxes, an ANOVA was conducted for each wax group to determine if differences between cultivars were statistically supported. A Type III ANOVA was also conducted for the TKW data to determine if cultivar, treatment (soil RWC group), or an interaction effect existed. A post-hoc Tukey’s analysis was done after to determine which groups differed statistically. Statistical significance was defined as \( P < 0.05 \). The assumptions of normality of residuals and equal variances were tested prior to running statistics using the Shapiro-Wilk test and the Bartlett test of homogeneity of variance. All data met the assumptions. All statistical results are found in the Appendix.

Section 2.3 Results

Compared to ‘Stettler’ and ‘AAC Viewfield’, ‘Superb’ successfully maintained leaf RWC and to lower levels of soil RWC under progressing drought stress (Figure 1). For ‘Superb’ (Figure 1A), \( \Theta_{\text{RWC}} \) corresponded to a soil RWC of 36% and no significant change in leaf RWC was detected during leaf hydration phase I (slope \( a \) at \( P = 0.76 \)). In
contrast, for ‘Stettler’ (Figure 1B) and ‘AAC Viewfield’ (Figure 1C), ΩRWC was reached at a higher soil RWC of 48% and 51%, respectively, and slope a was $P \leq 0.05$ during leaf hydration phase I. During phase I, there was a significant ‘cultivar’ effect ($P = 0.04$, Appendix 9) with a statistically significant difference between ‘Superb’ and ‘AAC Viewfield’ ($P = 0.02$, Appendix 10). Following ΩRWC, severe leaf dehydration and a substantial drop in leaf RWC was detected in all three HRSW cultivars. For ‘Superb’ (Figure 1A), this reduction was statistically significant (slope b at $P < 0.01$). For ‘Stettler’ and ‘AAC Viewfield’, slope b ($P > 0.05$) was shallower (Figure 1B and 1C).

During leaf hydration phase I, leaf Ψ was differentially affect under non-transpiring and transpiring conditions when soil RWC declined (Figure 2). For a bagged (non-transpiring) canopy (Figures 2A to 2C) leaf Ψ was maintained at approximately -0.85 MPa, -1 MPa, and -0.7 MPa for ‘Superb’, ‘Stettler’, and ‘AAC Viewfield’ respectively. Reductions in leaf Ψ in response to a decline in soil RWC were negligible in all three HRSW cultivars and corresponding slope c (slope of data in phase 1) was at $P > 0.05$. Analysis of the differences in means indicated a significant ‘cultivar’ effect was found for non-transpiring canopy in phase I (Appendix 11). The means of ‘AAC Viewfield’ and ‘Stettler’ were found to be statically different (Appendix 12). For a transpiring canopy (Figures 2D to 2F), a significant decline in leaf Ψ with soil RWC was detected in all three HRSW cultivars (slope c at $P < 0.05$). For a transpiring canopy, there was a significant ‘cultivar’ effect in phase I (Appendix 11) and a statistically significant difference in the mean was predicted between ‘AAC Viewfield’ and ‘Superb’ (Appendix 13).
Leaf $\Psi_s$ remained at a similar level (approximately 1.15 MPa) in response to reductions in soil RWC during phase I for ‘Superb’ (slope $c = 0.001$, $P > 0.05$, Figure 3A) and ‘Stettler’ (slope $c = -0.001$, $P > 0.05$ Figure 3B). For ‘AAC Viewfield’, my data indicate a slight reduction in leaf $\Psi_s$ (from -1.05 to -1.25 MPa) during phase I (slope $c = 0.004$, $P < 0.05$, Figure 3C). Further analysis showed that there was no predicted no significant ‘cultivar’ effect in phase I (Appendix 11). Following $\Theta_{RWC}$, leaf $\Psi_s$ dropped substantially to more negative values in all three HRSW cultivars (Figures 3A to 3C).

Stomatal regulation in response to soil RWC differed among the three HRSW cultivars (Figure 4). For ‘Superb’ (Figure 4A), $G_{s95}$ and $G_{s5}$ corresponded to a soil RWC of 72% and 48% respectively, resulting in a $\Delta G_{si}$ of 24%. For ‘Stettler’ (Figure 4B), $G_{s95}$ and $G_{s5}$ were 82% and 46% respectively, giving a $\Delta G_{si}$ of 36%. For ‘AAC Viewfield’ (Figure 4C), $G_{s95}$ and $G_{s5}$ were at 99% and 38% respectively, and $\Delta G_{si}$ was 61%. In addition, $G_{s5}$ was reached prior to $\Theta_{RWC}$ in ‘Superb’ (Figure 4A). On the other hand, $G_{s5}$ coincided with $\Theta_{RWC}$ in ‘Stettler’ and occurred after $\Theta_{RWC}$ in ‘AAC Viewfield’ (Figures 4B and 4C).

During phase I, a decline in FLW by leaf rolling was detected in ‘Superb’ but not for ‘Stettler’ and ‘AAC Viewfield’ (Figure 5). For ‘Superb’ (Figure 5A), FLW declined significantly from on average 16 to 10 mm at $\Theta_{RWC}$ (slope $c = 0.138$, $P < 0.05$). For ‘Stettler’ ($c = 0.0024$, $P > 0.05$ Figure 5B) and ‘AAC Viewfield’ ($c = 0.0013$, $P > 0.05$, Figure 5C), regression analyses predict no significant reduction in FLW during phase I.
The FLW for both ‘Stettler’ and ‘AAC Viewfield’ was maintained at approximately 13 mm in phase I. Statistical analyses showed a ‘cultivar’ effect during phase I (Appendix 9), and a statistically significant difference in means was determined between ‘AAC Viewfield’ and ‘Superb’ ($P < 0.05$) (Appendix 14).

Analysis of leaf epicuticular waxes did not indicate a significant difference between the three HRSW cultivars for a given wax group (Table 1; Appendix 15), but in all three cultivars the highest wax groups present were the alkane (‘Stettler’ at 63%; ‘AAC Viewfield’ at 59%; ‘Superb’ at 55%). For all three cultivars, the mean of alkane group was statistically different from the other wax groups (Appendix 16). While other wax groups were present, the alkane group was the only wax group which had a mean statistically different from all other wax groups. The means of the β-diketone group were statistically different from esters and fatty acids for ‘Stettler’ and ‘AAC Viewfield’ but not for alcohols and aldehydes.

TKW was maintained during phase I in all three HRSW cultivars, and reductions in TKW occurred when threshold Θ_{RWC} was passed (Figure 6). For ‘Superb’, a significant reduction in TKW from 33 to 9g was detected in the treatment group of 30% soil RWC during phase II following Θ_{RWC} (Figure 6A). In ‘Stettler’, TKW dropped significantly at 30% soil RWC that followed Θ_{RWC} of 48% from 35 to 6g (Figure 6B). In ‘AAC Viewfield’, TKW dropped significantly at <60% soil RWC and, again, after reaching Θ_{RWC} of 58% from 36 to 13g (Figure 6C). In general, ‘Superb’ exhibited better TKW compared to ‘Stettler’ and ‘AAC Viewfield’ under normal soil moisture (90%). Statistical support was found for differences between cultivars and treatments (Appendix 17). Differences in
means for treatments were found to be statistically supported (Appendix 18). Moreover, the means of ‘AAC Viewfield’ and ‘Superb’ were found to be statistically difference (Appendix 19).

Soil core samples were extracted to estimate soil water heterogeneity and root water uptake. A statistical analysis of the means of the cores shows that there were no statistically significant differences between cultivar and soil depths (Appendices 6 and 7). The soil GWC in ‘Superb’ starts between 8% to 16% for well-watered plants above 90% soil RWC at all three depths. By the time ‘Superb’ hit 30% soil RWC, the soil GWC was close to 0% at all three depths (Appendix 5B). Similarly, ‘Stettler’ starts between 6% and 19% soil GWC for well-watered plants and hits close to 0% soil GWC at approximately 35% soil RWC (Appendix 5C). ‘AAC Viewfield’ followed a similar trend where it started between 4% and 17% soil GWC for well-watered plants while it was close to 0% soil GWC for plants at approximately 35% soil RWC (Appendix 5D). While there is a general trend downwards in soil GWC of soil cores from top to bottom of pot these changes are not statistically significant (Appendices 6 and 7). Furthermore, representative pictures of root system did not indicate substantial differences in maximum rooting depth between the three HRSW cultivars (Appendix 20). The roots of each cultivar surpassed the depth of their pots (60 cm).
Section 2.4 Discussion

In this study I showed that HRSW cultivars ‘Superb’, ‘Stettler’, and ‘AAC Viewfield’ exhibit negligible OA in response to drought and that sustained kernel weight in these cultivars is linked to ‘tight’ stomatal regulation, effective leaf rolling, and higher alkane in leaf cuticle. Although all three HRSW cultivars exhibited anisohydric behavior under transpiring canopy conditions, I found that this behavior was not sufficient to trigger OA. The onset of reductions in TKW was closely linked to threshold $\Theta_{\text{RWC}}$, which suggests $\Theta_{\text{RWC}}$ as a predictor of yield losses under drought conditions. Compared to ‘Stettler’ and ‘AAC Viewfield’, ‘Superb’ exhibiting tight stomatal regulation and effective leaf rolling during phase I was most effective in maintaining TKW and as reflected by its $\Theta_{\text{RWC}}$ thresholds at a relative low soil RWC of 36%. While the slopes in phase II all decreased after $\Theta_{\text{RWC}}$ there was no statistical support for the slopes for ‘Stettler’ and ‘AAC Viewfield’ which can most likely be explained by the lack of data points after the threshold. Regardless, my study highlights physiological characteristics that may improve drought tolerance and serve as future breeding targets for HRSW cultivars. An overview of measured cultivar-specific physiological traits is given in Table 2 that may serve as a reference for future studies into drought tolerance of HRSW cultivars.

Leaf OA minimizes the extent of drought-induced cell dehydration due to its positive effect on lower cell water potential and is typically the result of organic solutes accumulating inside the cell that do not interfere with enzyme activities even at high concentrations (Turner and Jones, 1980; Turner, 1986; Kramer and Boyer, 1995). No significant reduction in leaf $\Psi_S$ was observed in ‘Superb’ and ‘Stettler’ until reaching $\Theta_{\text{RWC}}$. This suggests that no OA occurs in both HRSW cultivars as the leaf $\Psi_S$ does not
decrease and therefore no accumulation of solutes occurs while leaf RWC is relatively stable during phase I. I speculate that the slight, but significant, reduction in leaf $\Psi_s$ of ~0.05MPa for ‘AAC Viewfield’ was most likely linked to the reduction in leaf RWC until reaching $\Theta_{RWC}$ and a solute concentration effect (Figure 1). Interestingly, for ‘Superb’ leaf RWC was maintained during phase I even in the absence of OA which points to other physiological mechanisms supporting this behavior. Moreover, the method described in Babu et al. (1999) was used to evaluate OA. The solute accumulation effect under decreasing soil RWC was estimated by measured leaf $\Psi_s$ minus leaf $\Psi_{S0}$ (leaf $\Psi_s$ due to concentration effect) at 80% soil RWC. In all three HRSW cultivars, this method predicted a relatively small solute accumulation effect – if any (‘Superb’ at -0.10MPa; ‘Stettler’ at -0.18MPa and ‘AAC Viewfield’ at -0.20MPa) supporting the previous findings (Appendix 21). Overall, active solute accumulation (i.e., OA) played a negligible role in all three HRSW cultivars.

Drought induced stomatal closure is a mechanism that prevents excessive plant water loss (Tyree and Sperry, 1988; Brodribb and McAdam, 2011). My data points to cultivar-specific differences in stomatal regulation with respect to $G_{s95}$ and $G_{s5}$. Stomata started closing ($G_{s95}$) the earliest in ‘AAC Viewfield’. Meanwhile, both ‘Superb’ and ‘Stettler’ had a much later initiation of stomatal closure, so the plants were able to transpire fully for longer than ‘AAC Viewfield’. Although this was the case, the stomata did close much earlier ($G_{s5}$) in ‘Superb’ and ‘Stettler’ than it did in ‘AAC Viewfield’. For ‘Superb’, the margin of $G_{s95}$ (onset of stomatal closure) to $G_{s5}$ (stomatal closure) was relatively small (i.e., soil RWC from 72% to 48%), which indicates a relatively ‘tight’ stomata control. In
contrast, my data for ‘AAC Viewfield’ exhibited the opposite response where stomatal closure was initiated early on (Gs$_{95}$) when soil RWC declined and Gs$_5$ was reached at soil RWC of 38%. This relatively ‘slack’ stomatal control may be interpreted as a sign of drought intolerance from a physiological perspective because the plant does not adjust its stomata quickly in response to drying and continues to transpire (Tardieu et al., 2018). ‘Stettler’ played an intermediate role but behaved more similarly to ‘Superb’. My data suggest that a ‘tight’ stomata control under drought and prior to reaching $\Theta_{\text{RWC}}$ is linked to maintenance of leaf RWC under drought in the absence of OA. On the other hand, relatively late onset of stomata closure in ‘Superb’ can be interpreted as a beneficial trait to maintain leaf gas exchange (i.e., CO$_2$ assimilation) under water limiting conditions that has positive impacts on grain development. However, my data highlight that cultivar-specific stomatal regulation in response to progressive drought did not affect the degree of isohydricity. For example, although ‘Superb’ and ‘AAC Viewfield’ differed substantially in their stomatal response they showed both showed anisohydric behavior under transpiring canopy conditions. This suggests that anisohydric behavior associated with reduction in leaf $\Psi$ does not trigger OA in HRSW cultivars.

Bi et al., (2017) found that the structure of wax crystals and changes in amount and composition of the wax layer were indicators of leaf water loss. Bianchi and Figini (1986) suggested that the β-diketones could be responsible for redistribution of incident radiation in wheat. β-diketones have also been linked to glaucousness (Adamski et al., 2013; Zhang et al., 2013; Bi et al., 2016) which can help with lower photosynthesis and transpiration rates during drought (Richards et al., 1986). While previous research has
shown the presence of higher levels of β-diketones in wheat, my data does not indicate a statistically significant difference in the means from other groups. Instead, the means of the alkane group were statistically different from the other groups. It has been previously suggested that alkanes are important for drought performance in wheat and other plants (as reviewed in Rahman et al., 2021). Based on these observations it is hard to determine if the percentage of β-diketone is solely responsible for the differences in TKW noted in this study. Furthermore, differences in means between cultivar for each wax group were not statistically significant suggesting that differences in wax could not explain observed differences in TKW.

Leaf rolling has several benefits for maintaining hydration status including minimizing transpirational water loss while stomata can remain in an open state and protecting the leaf from high radiation (Kadiogiu and Terzi, 2007). My data on drought-induced changes in FLW by leaf rolling indicate that leaf rolling happens prior to $\Theta_{\text{RWC}}$ in ‘Superb’ but not in ‘Stettler’ and ‘AAC Viewfield’. This suggests that leaf rolling in ‘Superb’ plays a part in maintaining its leaf RWC. Moreover, ‘Superb’ has the lowest $G_{s95}$ of the three HRSW cultivars, which could be due to the leaf rolling seen in ‘Superb’ but not in the other two cultivars. Visual approaches or sustained yield under drought have been used by breeders to select breeding material with improved drought tolerance. This is so because they can be much faster and cheaper compared to physiological tests (Jones, 1978). In this context my data indicate that leaf rolling, or observation of leaf rolling are not necessarily a sign of drought stress in HRSW but can acts as a mechanism providing for improved drought tolerance in specific cultivars.
The homogeneity of soil water in deep pots such as the one used in this project plays a big part in drought studies. Rewatering plants from the top causes water to remain in the top of the pot (Turner, 2019) which could make estimates of soil RWC inaccurate as they are taken as an average of the pot. My data indicate that there was no statistical difference between soil GWC at different depths suggesting that possible gradient in soil moisture along the pot profile were negligible. Furthermore, all three HRSW cultivars grew root systems that reached the bottom soil layer of pots indicating that roots had access to all soil layers (Appendix 5). This falls in line with previous research as wheat roots stop growing after the heading stage (Hurd, 1964) so the wheat roots are already established and at the bottom of the pot by the time the drydown was applied. This suggests that the differences in drought performance seen in the HRSW cultivars were not due to rooting depth. Nevertheless, this highlights that further research on ‘Superb’, ‘Stettler’, and ‘AAC Viewfield’ should target a detailed investigation of root physiological responses and root architecture to drought.

Wheat TKW decreases as soils become drier in part due to the lack of water availability causing plants to form grains in a shortened time frame (Poudel et al., 2020). Similar results were found in this study on HRSW cultivars. However, the reduction in TKW seen between cultivars was closely linked to $\Theta_{\text{RWC}}$. In turn, this points towards the importance of this threshold as a predictor for TKW for HRSW cultivars under water limited conditions. Leaf RWC provides information on plant water status (Mullan and Pertragalla, 2012). In turn, a reduction in leaf RWC is linked to reductions in yield and
TKW (Schofeld et al., 1988; Tahara et al., 1990). Consequently, a HRSW cultivar with a lower $\Theta_{RWC}$ would perform better in drought as it would be able to manage itself to conserve water. HRSW cultivars with tighter stomatal control (‘Superb’ and ‘Stettler’) close their stomata quickly when water availability decreases (Busk et al., 1999) thereby decreasing transpiration and preventing severe leaf dehydration (Tardieu et al., 2018).
Section 2.5 Tables and figures

Table 1. Leaf epicuticular wax composition of three wheat cultivars (‘Superb’, ‘Stettler’, and ‘AAC Viewfield’) under well-watered conditions. The mean percentage (n=12 plants) and standard error of the mean (SEM) of wax groups is calculated for each cultivar. Statistical analysis can be found in Appendices 15 and 16.

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<tr>
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<th></th>
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<tr>
<td></td>
<td>Superb</td>
<td>Stettler</td>
<td>AAC Viewfield</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>Alcohol</td>
<td>3.56%</td>
<td>0.61%</td>
<td>2.42%</td>
<td>0.92%</td>
<td>3.72%</td>
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<tr>
<td>Aldehyde</td>
<td>12.88%</td>
<td>8.02%</td>
<td>3.95%</td>
<td>3.95%</td>
<td>9.88%</td>
</tr>
<tr>
<td>Alkane</td>
<td>54.56%</td>
<td>10.19%</td>
<td>63.38%</td>
<td>10.57%</td>
<td>59.18%</td>
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<tr>
<td>β-diketone</td>
<td>23.74%</td>
<td>7.16%</td>
<td>26.88%</td>
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<td>26.70%</td>
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<tr>
<td>Ester</td>
<td>0.82%</td>
<td>0.70%</td>
<td>2.11%</td>
<td>2.11%</td>
<td>-</td>
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<tr>
<td>Fatty Acid</td>
<td>4.45%</td>
<td>2.78%</td>
<td>2.27%</td>
<td>1.76%</td>
<td>0.52%</td>
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Table 2. General overview of leaf physiological traits observed in this study for three wheat cultivars (‘Superb’, ‘Stettler’, and ‘AAC Viewfield’) under water stress by drought during phase I (prior to reaching \( \Theta_{RWC} \)). Signs indicate relative differences in characteristics between cultivars; ‘better’ (+), ‘same’ (o), or ‘absent’ (-)

<table>
<thead>
<tr>
<th>Response Trait</th>
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<tr>
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<td>Iso-/anisohydric behavior</td>
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<tr>
<td>Osmotic adjustment</td>
<td>-</td>
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<tr>
<td>Leaf epicuticular waxes</td>
<td>o</td>
</tr>
<tr>
<td>Stomatal behavior</td>
<td>+</td>
</tr>
<tr>
<td>Leaf rolling</td>
<td>+</td>
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</table>
Figure 1. Effect of water stress by drought on leaf relative water content of three wheat cultivars (panel A ‘Superb’, panel B ‘Stettler’, panel C ‘AAC Viewfield’). Each symbol represents a single measurement of an individual plant. The solid line is a two-segment piecewise linear regression model fitted across data points (for $R^2$ and p values, see bottom left in each panel); the vertical dashed line indicates the predicted threshold of onset of severe leaf dehydration ($\Theta_{RWC}$). Roman numerals I and II indicate the corresponding phases of leaf hydration status prior and after $\Theta_{RWC}$. Parameters a and b are slope values of phase I and II, respectively, with corresponding p-values and coefficient of determination ($R^2$).
Figure 2. Effect of water stress by drought on leaf xylem pressure (leaf Ψ) under non-transpiring (panels A to C) and transpiring (panels D to F) of three wheat cultivars (panels A/D ‘Superb’, panels B/E ‘Stettler’, panels C/F ‘AAC Viewfield’). Each symbol represents a single measurement of an individual plant. The solid line is a simple linear regression fitted across datapoints of phase I; parameter c is the slope value with corresponding p-values and coefficient of determination (R²). The vertical dashed line is the superimposed threshold Θ_{RWC} (see Figure 1). Roman numerals I and II indicate the corresponding phases of leaf hydration status prior and after Θ_{RWC}.
Figure 3. Effect of water stress by drought on leaf solute potential of three wheat cultivars (panel A ‘Superb’, panel B ‘Stettler’, panel C ‘AAC Viewfield’). Each symbol represents a single measurement of an individual plant. The solid line is a simple linear regression fitted across datapoints of phase I; parameter $c$ is the slope value with corresponding $p$-values and coefficient of determination ($R^2$). The vertical dashed line is the superimposed threshold $\Theta_{RWC}$ (see Figure 1). Roman numerals I and II indicate the corresponding phases of leaf hydration status prior and after $\Theta_{RWC}$. 
Figure 4. Relation of soil relative water content and stomatal conductance of three wheat cultivars (panel A ‘Superb’, panel B ‘Stettler’, panel C ‘AAC Viewfield’). Each symbol represents a single measurement on an individual plant. The solid line is a non-linear sigmoidal three-parameter regression fitted across datapoints. The vertical blue dashed linear are the thresholds of 95% (Gs95, onset of stomatal closure) and 5% (Gs5, full stomatal closure) of maximal stomatal. Parameter \( \Delta G_s_i \) is the difference between Gs95 and Gs5. The green colored dashed line is the superimposed threshold \( \Theta_{\text{RWC}} \) (see Figure 1). Roman numerals I and II indicate the corresponding phases of leaf hydration status prior and after \( \Theta_{\text{RWC}} \).
Figure 5. Effect of water stress by drought on flag leaf width of three wheat cultivars (panel A ‘Superb’, panel B ‘Stettler’, panel C ‘AAC Viewfield’). Each symbol represents a single measurement of an individual plant. The solid line is a simple linear regression fitted across datapoints of phase I; parameter c is the slope value with corresponding p-values and coefficient of determination ($R^2$). The vertical dashed line is the superimposed threshold $\Theta_{RWC}$ (see Figure 1). Roman numerals I and II indicate the corresponding phases of leaf hydration status prior and after $\Theta_{RWC}$. 
Figure 6. Thousand kernel weight at different levels of soil RWC of three wheat cultivars (panel A ‘Superb’, panel B ‘Stettler’, panel C ‘AAC Viewfield’). Each bar is the mean ± standard error of mean of n=3 plants. Different letter indicates statistically significant differences at \( P < 0.05 \) (posthoc Tukey test).
Chapter 3: Conclusion and future studies

Section 3.1 Conclusion

Selection of plants during crop domestication was based on promoting a variety of beneficial traits increasing productivity and quality, but tolerance to limitation in soil water availability was historically not of great importance (Xoconostle-Cazares et al., 2011). For wheat, reductions of tiller number, leaf area, and leaf gas exchange in response to drought stress are common phenomena that negatively impact yield potential and plant survival (Muasick and Dusek, 1980; Innes et al., 1981; Trillo and Fernandez, 2005; Cai et al., 2018, Nyugen et al. 2022). With increasing world population and climate change causing unprecedented drought events, it is of great concern that yield from wheat is no longer growing as fast of a pace as it once did in certain regions of the world (reviewed in Curtis and Halford, 2014). Knowledge about plant-water relations of currently used wheat cultivars, and especially of those that have been grown traditionally in rainfed areas, provides crucial information regarding their true potential to sustain yield under drought conditions. The results of this study on HRSW cultivars ‘Superb’, ‘Stettler’ and ‘AAC Viewfield’ provides new insights into their leaf water relations and indicate a close link to reductions kernel weight under drought. All cultivars showed drought-induced reductions in TKW that coincided with leaf hydration threshold $\theta_{RWC}$. This suggests that $\theta_{RWC}$ serves as a predictor of grain quality under limited soil water availability. Data indicated that superior maintenance of leaf hydration (i.e., phase I) of ‘Superb’ could be associated with leaf rolling which was linked to prolonged stomatal opening (see $G_{s5}$ threshold) and leaf gas exchange at relatively low levels of soil RWC. In addition, while all three cultivars showed anisohydric
behavior under transpiring conditions, OA was absent and could be excluded as a mechanistic trait providing for improved drought performance. While differences in the composition of leaf epicuticular waxes existed, they were not statistically supported and therefore could also be excluded as the reason for difference in drought performance. Overall, this study of Canadian wheat cultivars indicates that leaf roll and stomatal behavior are important physiological traits to consider when selecting for cultivars with improved drought tolerance.

Section 3.2 Future Studies

While OA has been studied extensively, there are still many questions regarding its application in plant breeding. Historically, only one wheat cultivar (‘Mulgara’) has been released even though many studies have been conducted in the field (Turner, 2018). This could be partly due to the lack of a standard method to identify OA in cultivars as seen by the variety of methods conducted by Babu et al. (1999). Due to this, a comparison of results is hard to conduct as the method of interpreting OA can be different between studies. To help overcome this, the use of a known cultivar exhibiting OA (such as ‘Mulgara’) would allow for the comparison and validation of different methodologies. A future study with the inclusion of ‘Mulgara’ in comparison to ‘Superb’, ‘Stettler’ and ‘ACC Viewfield’ could provide for an importance reference to better assess OA.

Root development could also be responsible for the increases in yield due to OA (Serraj and Sinclair, 2002). In the past, Hurd (1974) found that root weight differed between
wheat cultivars and root density differed at different depths. The maximum depth of wheat roots has also been found to vary between cultivars but is also dependent on the soil conditions (Gregory, 1994). This is supported by previous wheat studies conducted in soil from the prairies (Hurd, 1964; Hurd, 1968). A look at extracted roots of all three HRSW cultivars used here showed that the rooting depth was similar for all three cultivars (Appendix 20). In the field, there is typically no growth restrictions as there is no pot surrounding the growing medium. To better understand roots and water uptake, the root growth and root hydraulics should be studied in detail. The use of a soil and sand medium together makes the extraction of roots more difficult; a future study with the use of sand would help overcome this issue. The use of sand would allow for easier extraction of roots in both well-watered and dry conditions. While no differences in root depth were seen from extracted samples, a closer look at root growth, architecture, and root hydraulics could provide more information on water uptake potential of roots. It would also help to determine if performance at leaf level is associated with root depth or density.

Currently over 100 Canada Western Red Spring (Canadian wheat classification) wheat cultivars exist in Canada with a small number of those cultivars making up a majority of the production in the class (Statistics Canada, 2022). These numerous cultivars provide a large pool to assess drought performance on, and ‘drought profiles’ for each cultivar should be established to identify those cultivars which are better suited for drier environments. A similar study (with the same measures) to the one conducted in this thesis but instead on more cultivars would help achieve this.
The use of PLR to determine $\Theta_{RWC}$ provides a small range in which the threshold is present. The verification of this threshold and its link to TKW can be supported further by the application of multiple different soil RWC treatments. By having multiple soil RWC treatments (e.g., 20%-30%, 30%-40%, 40%-50%, ..., 80%-90% soil RWC) the range in which TKW falls can be better ascertained and consequently verify threshold range. Additionally, the incorporation of field trials to determine yield results of cultivars can be used to further evaluate the validity of the data presented and provide support for the importance of $\Theta_{RWC}$. A comparison of yield of wheat plants which are irrigated and those that are not, would help to determine if the results measured in the greenhouse would translate in the field as well.
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Appendices

**Appendix 1.** Daily temperature (panels A and D), relative humidity (panels B and E), and VPD (panels C and F) during experiments 1 (panels A-C, *June to September 2021*) and 2 (panels D-F, *February to June 2022*). The drydown is indicated by the shaded blue region. The imposed red line represents when the plants were harvested.
**Appendix 2.** Experimental randomized block design for (panel A) experiment 1 and (panel B) experiment 2 including cultivars and treatments.

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### Lines
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<tr>
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<tr>
<td>St</td>
<td>Stettler</td>
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<tr>
<td>Su</td>
<td>Superb</td>
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### Soil Relative Water Content Treatments
- **90%**
- **60%**
- **45%**
- **Not Watered**
Appendix 3. Temporal changes of soil relative water content for experiment 1 (Figure 1 to 5). Watered plants (90% soil relative water content) and not watered plants (water withheld) are shown by the blue and red shaded regions, respectively. Each pot is represented by a unique symbol and line color. Each symbol is a single pot measure (panel A ‘Superb’, panel B ‘Stettler’, panel C ‘AAC Viewfield’).
Appendix 4. Temporal changes of soil relative water content measured for experiment 2 (Figures 6 and 7). The 90%, 60%, 45% soil relative water content, and not watered treatments are represented by black, dark grey, light grey and white symbols respectively. Each dot point is the average value of the plants in the treatment (panel A ‘Superb’, panel B ‘Stettler’, panel C ‘AAC Viewfield’).
Appendix 5. Representative image of wheat plants growing in large cylindrical plastic pots inside the greenhouse compartment (panel A). Relationship of soil relative water content and gravimetric water content measured from soil cores at top (panel B), middle (panel C) and bottom (panel D) location along pots. Triplets of data points at a given soil relative water content come from the same pot. The top, middle, bottom soil layers are represented by circle, triangle, and square shapes respectively.
**Appendix 6.** Statistical comparison of soil gravimetric water content (Appendix 5) using ANOVA analyses (mixed effects linear regression model). Values are the respective degrees of freedom (df), F and p-values (Type II) for ‘cultivar’ effect and ‘soil relative water content’ effect. The asterisk (*) indicates $P < 0.05$.

<table>
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<td>179.1754</td>
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**Appendix 7.** Statistical comparison of soil gravimetric water content (Appendix 5) using ANOVA analyses (mixed effects linear regression model). Values are the respective degrees of freedom (df), F and p-values (Type II) for 'depth' effect (soil layer) and 'soil relative water content' effect. The asterisk (*) indicates $P < 0.05$.

<table>
<thead>
<tr>
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Appendix 8. Overview of experimental procedure for measurement of leaf relative water content, solute potential, and water potential under non-transpiring (left hand panels) and transpiring (right hand panels) conditions.
**Appendix 9.** Statistical comparison of a mixed effects linear regression among cultivars using ANOVA analyses (Type III). Analyses of leaf parameters for leaf hydration phase I (i.e., range between 100% to $\Theta_{RWC}$). Values are the respective degrees of freedom (df), F and p-values (Type III) for ‘cultivar’ effect, ‘soil relative water content’ effect, and its interaction effect. The asterisk (*) indicates $P < 0.05$.

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<tr>
<td></td>
<td>Cultivar x Soil Relative Water Content</td>
<td>2</td>
<td>15.366</td>
<td>6.406e-07*</td>
</tr>
</tbody>
</table>
**Appendix 10.** Statistical comparison of mixed effects linear regressions among cultivars using a post-hoc Tukey’s analysis (corresponding to Appendix 9). Data shown are for soil relative water content and leaf relative water content in phase I (i.e., range between 100% to $\Theta_{RWC}$). The corresponding mean estimate of the differences, standard error of means (SEM), t values and p-values are given for each comparison. The asterisk (*) indicates $P < 0.05$.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Mean</th>
<th>SEM</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAC Viewfield – Stettler</td>
<td>1.22</td>
<td>1.10</td>
<td>1.109</td>
<td>0.5097</td>
</tr>
<tr>
<td>AAC Viewfield – Superb</td>
<td>2.90</td>
<td>1.08</td>
<td>2.680</td>
<td>0.0220*</td>
</tr>
<tr>
<td>Stettler – Superb</td>
<td>1.69</td>
<td>1.06</td>
<td>1.591</td>
<td>0.2526</td>
</tr>
</tbody>
</table>
Appendix 11. Statistical comparison of a mixed effects linear regression among cultivars using ANOVA analyses (Type II). Data shown are for soil relative water content corresponding to phase I (i.e., range between 100% to $\Theta_{RWC}$). Values are the respective degrees of freedom (df), F, and p-values (Type II) for ‘cultivar’ effect and ‘soil relative water content’ effect. The asterisk (*) indicates $P < 0.05$.

<table>
<thead>
<tr>
<th>Leaf parameter</th>
<th>Factor</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solute potential</td>
<td><strong>Cultivar</strong></td>
<td>2</td>
<td>0.3123</td>
<td>0.73128</td>
</tr>
<tr>
<td></td>
<td><strong>Soil Relative Water Content</strong></td>
<td>1</td>
<td>3.6601</td>
<td>0.05978</td>
</tr>
<tr>
<td>Leaf water potential (bagged canopy)</td>
<td><strong>Cultivar</strong></td>
<td>2</td>
<td>7.7725</td>
<td>0.0005766*</td>
</tr>
<tr>
<td></td>
<td><strong>Soil Relative Water Content</strong></td>
<td>1</td>
<td>0.2940</td>
<td>0.5888817</td>
</tr>
<tr>
<td>Leaf water potential (transpiring canopy)</td>
<td><strong>Cultivar</strong></td>
<td>2</td>
<td>3.8392</td>
<td>0.02329*</td>
</tr>
<tr>
<td></td>
<td><strong>Soil Relative Water Content</strong></td>
<td>1</td>
<td>22.9263</td>
<td>1.003e-05*</td>
</tr>
</tbody>
</table>
Appendix 12. Statistical comparison of mixed effects linear regressions among cultivars using a post-hoc Tukey’s analysis (corresponding to Appendix 11). Data shown are for soil relative water content corresponding to non-transpiring water potential data in phase I (i.e., range between 100% to $\Theta_{RWC}$). The corresponding mean estimate of the differences, standard error of means (SEM), t values, and p-values are given for each comparison. The asterisk (*) indicates $P < 0.05$.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Mean</th>
<th>SEM</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAC Viewfield – Stettler</td>
<td>0.280</td>
<td>0.0721</td>
<td>3.891</td>
<td>0.0004*</td>
</tr>
<tr>
<td>AAC Viewfield – Superb</td>
<td>0.142</td>
<td>0.0714</td>
<td>1.987</td>
<td>0.1182</td>
</tr>
<tr>
<td>Stettler – Superb</td>
<td>-0.138</td>
<td>0.0698</td>
<td>-1.983</td>
<td>0.1193</td>
</tr>
</tbody>
</table>
**Appendix 13.** Statistical comparison of mixed effects linear regressions among cultivars using a post-hoc Tukey’s analysis (corresponding to Appendix 11). Data shown are for soil relative water content corresponding to transpiring water potential data in phase I (i.e., range between 100% to $\Theta_{RWC}$). The corresponding mean estimate of the differences, standard error of means (SEM), t values, and p-values are given for each comparison. The asterisk (*) indicates $P < 0.05$.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Mean</th>
<th>SEM</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAC Viewfield – Stettler</td>
<td>0.1161</td>
<td>0.0561</td>
<td>2.069</td>
<td>0.0993</td>
</tr>
<tr>
<td>AAC Viewfield – Superb</td>
<td>0.1331</td>
<td>0.0559</td>
<td>2.380</td>
<td>0.0479*</td>
</tr>
<tr>
<td>Stettler – Superb</td>
<td>0.0169</td>
<td>0.0546</td>
<td>0.310</td>
<td>0.9483</td>
</tr>
</tbody>
</table>
**Appendix 14.** Statistical comparison of mixed effects linear regressions among cultivars using a post-hoc Tukey’s analysis (corresponding to Appendix 9). Data shown are for soil relative water content corresponding to flag leaf width data in phase I (i.e., range between 100% to $\Theta_{\text{RWC}}$). The corresponding mean estimate of the differences, standard error of means (SEM), t values, and p-values are given for each comparison. The asterisk (*) indicates $P < 0.05$.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Mean</th>
<th>SEM</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAC Viewfield – Stettler</td>
<td>-0.371</td>
<td>0.503</td>
<td>-0.738</td>
<td>0.7410</td>
</tr>
<tr>
<td>AAC Viewfield – Superb</td>
<td>-1.378</td>
<td>0.477</td>
<td>-2.887</td>
<td>0.0120*</td>
</tr>
<tr>
<td>Stettler – Superb</td>
<td>-1.007</td>
<td>0.497</td>
<td>-2.026</td>
<td>0.1088</td>
</tr>
</tbody>
</table>
**Appendix 15.** Statistical comparison of differences among cultivars for each epicuticular wax group using an ANOVA analysis (Type III). The corresponding degrees of freedom (df), F, and p-values are given below.

<table>
<thead>
<tr>
<th>Group</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>5</td>
<td>1.045</td>
<td>0.387</td>
</tr>
<tr>
<td>Aldehyde</td>
<td>5</td>
<td>0.34</td>
<td>0.72</td>
</tr>
<tr>
<td>Alkane</td>
<td>5</td>
<td>0.199</td>
<td>0.823</td>
</tr>
<tr>
<td>β-diketone</td>
<td>5</td>
<td>0.053</td>
<td>0.948</td>
</tr>
<tr>
<td>Ester</td>
<td>5</td>
<td>0.717</td>
<td>0.512</td>
</tr>
<tr>
<td>Fatty Acid</td>
<td>5</td>
<td>0.903</td>
<td>0.436</td>
</tr>
</tbody>
</table>
**Appendix 16.** Statistical comparison of differences among cultivars for each epicuticular wax group using an ANOVA analysis (Type III). The corresponding adjusted p-values are given below.

<table>
<thead>
<tr>
<th>Group</th>
<th>Adjusted P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Superb</em></td>
</tr>
<tr>
<td>Aldehyde – Alcohol</td>
<td>0.8887984</td>
</tr>
<tr>
<td>Alkane – Alcohol</td>
<td>0.0000657*</td>
</tr>
<tr>
<td>β-diketone – Alcohol</td>
<td>0.2267709</td>
</tr>
<tr>
<td>Ester – Alcohol</td>
<td>0.9995339</td>
</tr>
<tr>
<td>Fatty Acid – Alcohol</td>
<td>0.9999982</td>
</tr>
<tr>
<td>Alkane – Aldehyde</td>
<td>0.0009145*</td>
</tr>
<tr>
<td>β-diketone – Aldehyde</td>
<td>0.8099507</td>
</tr>
<tr>
<td>Ester – Aldehyde</td>
<td>0.7362698</td>
</tr>
<tr>
<td>Fatty Acid – Aldehyde</td>
<td>0.9240056</td>
</tr>
<tr>
<td>β-diketone – Alkane</td>
<td>0.0185542*</td>
</tr>
<tr>
<td>Ester – Alkane</td>
<td>0.0000307*</td>
</tr>
<tr>
<td>Fatty Acid – Alkane</td>
<td>0.0000843*</td>
</tr>
<tr>
<td>Ester – β-diketone</td>
<td>0.1284801</td>
</tr>
<tr>
<td>Fatty Acid – β-diketone</td>
<td>0.2685540</td>
</tr>
<tr>
<td>Fatty Acid – Ester</td>
<td>0.9981861</td>
</tr>
</tbody>
</table>
**Appendix 17.** Statistical comparison of thousand kernel weight among cultivars using ANOVA analyses (Type III). Values are the respective degrees of freedom (df), F, and p-values (Type III) for ‘cultivar’ effect, ‘treatment’ effect, and interaction effects. The asterisk (*) indicates $P < 0.05$.

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
<td>2</td>
<td>3.815</td>
<td>0.0364*</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>14.746</td>
<td>1.19e-05*</td>
</tr>
<tr>
<td>Cultivar x Treatment</td>
<td>2</td>
<td>1.916</td>
<td>0.1192</td>
</tr>
</tbody>
</table>
**Appendix 18.** Statistical comparison of thousand kernel weight among treatments using a post-hoc Tukey’s analysis. The corresponding p-values are given for each comparison. The asterisk (*) indicates $P < 0.05$.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>90% – 60%</td>
<td>0.5182762</td>
</tr>
<tr>
<td>90% – 45%</td>
<td>0.4149502</td>
</tr>
<tr>
<td>60%– 45%</td>
<td>0.0325110*</td>
</tr>
<tr>
<td>30% – 90%</td>
<td>0.0002775*</td>
</tr>
<tr>
<td>30% – 60%</td>
<td>0.0000090*</td>
</tr>
<tr>
<td>30% – 45%</td>
<td>0.0130782*</td>
</tr>
</tbody>
</table>
**Appendix 19.** Statistical comparison of thousand kernel weight among cultivars using a post-hoc Tukey’s analysis. The corresponding p-values are given for each comparison. The asterisk (*) indicates $P < 0.05$.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAC Viewfield – Stettler</td>
<td>0.8472570</td>
</tr>
<tr>
<td>AAC Viewfield – Superb</td>
<td>0.0385183*</td>
</tr>
<tr>
<td>Stettler – Superb</td>
<td>0.1177149</td>
</tr>
</tbody>
</table>
**Appendix 20.** Representative pictures of extracted root systems from three wheat cultivars (panel A ‘Superb’, panel B ‘Stettler’, panel C ‘AAC Viewfield’). The maximum length of the root system in pictures.
Appendix 21. Relationship of leaf solute potential and leaf relative water content (panel A ‘Superb’, panel B ‘Stettler’, panel C ‘AAC Viewfield’). The dark circular points represent the data gathered during the drydown. The red line represents a model which calculated leaf relative water content for each leaf solute potential point using the method by Babu et al. (1999). Data between 60% and 100% soil relative water content was used to create the linear model of leaf Ψs. A superimposed blue line represents 80% leaf relative water content where the difference between the gathered data and the model was determined. Each symbol represents a single measurement of an individual plant.