VARIATION IN CROP-LEVEL WATER-USE EFFICIENCY DUE TO CONTRASTING PATTERNS OF BIOMASS PARTITIONING AMONG POTATO CULTIVARS (*SOLANUM TUBEROSUM* L.)

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VARIATION IN CROP-LEVEL WATER-USE EFFICIENCY DUE TO CONTRASTING PATTERNS OF BIOMASS PARTITIONING AMONG POTATO CULTIVARS (SOLANUM TUBEROSUM L.)

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ABSTRACT

In arid southern Alberta, making efficient use of limited irrigation water is essential for increasing the production of crops like potato. The objective of this study was to test for variation in leaf-level and crop-level water-use efficiency (WUE) among eight cultivars of potato that are grown in southern Alberta. Measurements of the stable carbon and oxygen isotope composition, and the nitrogen content of plant leaf tissue were used to assess leaf- and crop-level WUE and total water-use by potato plants. In addition, aboveground plant biomass and tuber weight were determined for plants grown in a field experiment during May-August 2023. Results indicated that there were no significant differences among cultivars for leaf-level WUE. However, significant differences were observed among cultivars for crop-level WUE (ratio of tuber mass to seasonal cumulative plant water-use). Water-use by the different cultivars was strongly correlated with differences in aboveground biomass and total leaf area among cultivars. Crop-level WUE varied because of significant differences in cumulative water-use among cultivars, while tuber yield was very similar among the eight cultivars. Selection of the different cultivars has resulted in important differences in the partitioning of biomass to leaves, which controls total water-use, while leaf-level water-use and tuber yield was very similar among cultivars.

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DEDICATION

I would like to dedicate this achievement of mine to my beloved parents. I hope that this will be an important milestone towards the dream that you had dreamt of for me all those many years ago.

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LIST OF ABBREVIATIONS AND SYMBOLS

Analysis of variance (ANOVA)

Assimilation rate (A_c)

Carbon isotope composition of leaf organic matter ($\delta^{13}C_m$)

Coefficient of determination (R^2)

Concentrations of CO_2 in the air outside the leaf (c_a)

Concentrations of CO_2 in the air in the leaf intercellular air spaces (c_i)

Concentrations of water vapour in the air outside the leaf (w_a)

Concentrations of water vapour in the air in the leaf intercellular air spaces (w_i)

Degree of freedom (d.f.)

Evapotranspiration (E)

Model evapotranspiration (ET_c)

Oxygen isotope composition of leaf organic matter ($\delta^{18}O_m$)

Photosynthetically active photon flux density (PPFD)

Potato-days/Physiological days/Potato heat units (P-days)

Reference evapotranspiration (ET_r)

Scanning electron microscopy (SEM)

Stomatal conductance to CO_2 (g_c)

Stomatal conductance to water vapour (g_s)

Stable Isotope Ratio Facility for Environmental Research (SIRFER)

Transpiration rate (E)

Vapour pressure difference/deficit (VPD)

Water use efficiency (WUE

CHAPTER 1: INTRODUCTION

1.1 Background

Fresh water is an integral part of crop production for every variety of agricultural product, even though the amount of water required varies among them (Morison et al., 2008). The warmer temperatures and reduced soil moisture associated with climate change have significant effects on global crop production due to water shortages (Nasir and Toth, 2022), because water shortages directly or indirectly affect photosynthesis and plant growth (Zhu, 2002; Liu et al., 2018). Potato is one of the important agricultural products in the economy of southern Alberta (Larney et al., 2016). Being a drought-sensitive crop species, the growth and production of potato is severely affected by the deficiency of water (Plich et al., 2020). Thus, any characteristics that are linked to enhanced growth and production in potato crops (yield) with the lessened negative relationship with water shortages will be very beneficial by reducing water requirements, thereby leading to the more environmentally sustainable approach to crop production.

Different potato cultivars respond differently to water-deficit, and these varied responses are because of intrinsic differences among the cultivars in their physiological characteristics and associated variation in the timing of plant growth and potato production (Jefferies and Mackerron, 1997; Deblonde et al., 1999). Therefore, with the pressing need to maintain crop photosynthesis and productivity in water limited conditions, it is essential to develop cultivars with high water-use efficiency (Pathare et al., 2020).

1.2 Water-use efficiency on a broader scale

It is very crucial now to have knowledge of the factors influencing water-use efficiency in crops and a hope for the improvement in water-use efficiency has continued to be an important objective in many modern investigations (Sinclair et al., 1984). As water is the second most limiting factor for incremental increases of crop-production, behind land area, improvement in the water-use by plants must be given high research priority (Wittwer, 1975; Sinclair et al., 1984; Parry et al., 2005).

Water-use efficiency (WUE) is defined as the ratio of biomass accumulation, which can be expressed differently as carbon dioxide assimilation, total crop biomass or crop grain yield, to water consumed, expressed as transpiration, evapotranspiration, or total water input to the system, and at different timescales (instantaneous, daily or seasonal) (Sinclair et al., 1984). Further, water-use efficiency (WUE) can be used over a broad range of scales from the leaf-level up to crop-level (Morison et al., 2008; Boutraa, 2010). At the leaf-level, WUE is controlled by the ratio of leaf photosynthetic rate to transpiration rate (Pathare et al., 2020). Bierhuizen and Slatyer (1965) had identified the ratio of CO_2 assimilation and transpiration, or leaf transpirational water-use efficiency (WUE) to be driven by a concentration gradient between the leaf and air, and modulated by the resistances to diffusion resulting from stomata, leaf aerodynamic boundary layer, and leaf mesophyll resistance. At the crop-level, WUE can be defined as the ratio of crop yield to cumulative transpiration rate or total water used in production of yield during the growing season (Sinclair et al., 1984; Boutraa, 2010).

1.2.1 Leaf-level WUE

Leaves are primarily responsible for the transformation of solar energy to chemical energy, and they do so by fixing the atmospheric CO_2 through the process of photosynthesis (Sinclair et al., 1984). Stomata, the valves present in the epidermis of leaves, allow CO_2 to enter the plants for photosynthesis and at the same time, the open stomata allow water vapour to escape out of them from the plant's body (Sinclair et al., 1984). The rate of water loss is further proportional to the vapour pressure difference (VPD) between the atmosphere and the intercellular air spaces inside the leaf, which is the saturated vapour pressure of air at the leaf temperature (Sinclair et al., 1984). Gas exchange through the stomata is determined by the length, width, and depth of individual stoma and by stomatal density (Parlange and Waggoner, 1970). Stomatal size and guard cell length have been observed to be negatively correlated with stomatal density in all rainfall conditions (Franks et al., 2009). Changes in stomatal density may also influence maximum stomatal conductance to water vapour (g_s) (Maherali et al., 2002). At their widest apertures, the stomatal pores on the surface of leaves determine the maximum leaf diffusive conductance (stomatal conductance) to CO_2 and water vapour ($g_{c(max)}$ and $g_{w(max)}$, respectively) (Franks et al., 2009). When measures of change in stomatal density and size are reported together, there is often a negative relationship between the two (Franks et al., 2009), and it forms the basis of negative correlation between $g_{w(max)}$ and stomatal size and positive correlation between $g_{w(max)}$ and stomatal density. This overall pattern is a tendency for higher $g_{w(max)}$ to be achieved through a decrease in size and an increase in density of stomata (i.e. smaller, more numerous stomata) (Franks et al., 2009). Higher stomatal conductance is often characterized by smaller stomata but higher stomatal density (Franks et al., 2009). Smaller stomata will have shorter response times than the ones with larger area. Therefore, smaller stomata in combination with higher density may allow leaves to attain high stomatal conductance rapidly under favorable conditions (Hetherington and Woodward, 2003; Franks et al., 2009).

Leaves that matured under low rainfall typically have smaller stomata at any given density lowering the stomatal conductance and thereafter contributing to higher water-use efficiency (Franks et al., 2009). But there is considerable evidence for both short-term and long-term adjustments of size and density in response to environmental conditions during plant development and growth (Franks et al., 2009).

The differences in leaf-level water-use efficiency among the plant species or cultivars have also been associated with intrinsic differences in photosynthetic gas exchange characteristics (Farquhar et al., 1988). The major mechanisms contributing to modified WUE are variation in photosynthetic capacity and stomatal conductance (Farquhar et al., 1988; Farquhar et al., 1989; Condon, 2020). Plants might differ in WUE because of associated differences in stomatal conductance even if they have same biochemical capacity for photosynthesis, and if it happens to be so, the effect on transpiration will be more pronounced than on net photosynthesis (Farquhar and Sharkey, 1982). On the other hand, if the plants have the same stomatal conductance and transpiration rate, the differences in WUE will be because of associated variation in net photosynthesis rates, and this is associated with changes in the activity of major photosynthetic enzymes within leaf tissue, which influences photosynthetic capacity (Condon et al., 2002).

The measurement of carbon isotope composition ($\delta^{13}C_m$) of C_3 leaf tissue provides insights into differences in leaf physiological characteristics like changes in the ratio of net photosynthesis and stomatal conductance (Farquhar et al., 1989). Therefore, carbon isotope techniques have been important in studies of leaf-level WUE (Farquhar et al., 1988; Farquhar et al., 1989; Condon et al., 2004; Condon, 2020). The oxygen isotope composition ($\delta^{18}O_m$) of leaf organic matter, on the other hand, is independently controlled by variation in the leaf-air vapour pressure gradient and changes in stomatal conductance (Farquhar et al., 1988; Barbour, 2007). In addition, since $\delta^{18}O_m$ is affected by variation in stomatal conductance but is not affected by changes in photosynthetic capacity, leaf oxygen isotope measurements can be used to determine the major mechanism controlling variation in $\delta^{13}C_m$ (Barbour, 2007). When the primary cause of variation in $\delta^{13}C_m$ is stomatal conductance, there is a positive correlation between $\delta^{18}O_m$ and $\delta^{13}C_m$, however no correlation or a negative correlation means that the primary cause of variation in $\delta^{13}C_m$ is variation in photosynthetic capacity (Scheidegger et al., 2000; Flanagan and Farquhar, 2014). Therefore, simultaneous measurements of $\delta^{13}C_m$ and $\delta^{18}O_m$ provide powerful information about the factors that control leaf-level water-use efficiency. In this study I used measurements of the carbon and oxygen stable isotope composition of leaf tissue to make calculations and comparisons of leaf-scale WUE in eight potato cultivars typically grown in southern Alberta, Canada.

1.2.2 Crop-level WUE

Measurement of carbon isotope composition provides very strong insights into improvement of leaf-level WUE but improvement in leaf-level WUE may not always get translated into higher crop-level WUE or yield (Condon et al., 2004). Three important points need to be considered in leaf-level WUE for improvement of crop-level WUE: (i) more of the available water should move through the crop, (ii) more carbon (biomass) needs to be acquired by the crops for exchange of water transpired by them and (iii) they should partition more of the achieved biomass into the harvested product (Condon et al., 2004).

Crop-level WUE is an important attribute which describes the ability of crops to use the water available for them for carbon fixation (Farooq et al., 2019). Crop-level WUE and crop yield are very integrated as WUE at crop-level is measured as the ratio of crop yield to the crop water used (Farooq et al., 2019). Crop yield is positively correlated with crop-level WUE, but increased crop-level WUE does not always translate to increased yield (Farooq et al., 2019). The crops which are more water efficient are the ones which cope better with the water stress and produce better yield (Farooq et al., 2019). When studied among the crops which were planted in well-watered conditions, improvement in crop-level WUE led to reduced yield (Blum, 2009; Farooq et al., 2019), because the crops with higher crop-level WUE will have lower transpiration rate and thereby a reduced rate of carbon assimilation (Farooq et al., 2019). Hence, there are numerous factors that determine the plant water use, yield and therefore the overall crop-level WUE of the crops (Farooq et al., 2019).

Leaf morphology and anatomy is one of the important factors that determines leaf water use and therefore crop-level WUE (Farooq et al., 2019). Stomatal density of leaves plays an important role in determining plant water use because of its direct linkage with transpiration (Farooq et al., 2019). Manipulating stomatal density can improve WUE in some crops by the change in physiological processes like stomatal conductance, photosynthesis and transpiration (Franks et al., 2015; Farooq et al., 2019). Leaf thickness (thickness of palisade and spongy parenchyma) and presence of trichomes have been found to alter photosynthetic rates, transpiration rates, leaf conductance and WUE (Han et al., 2019; Farooq et al., 2019). Similarly, root architecture and anatomy play very important role in overall plant development because roots uptake water and nutrients for growth and development of shoot, and is sensitive to water availability, which further is regulated by hormones in plants (Farooq et al., 2019). Researches have shown that in order to adapt to water limited conditions, deeper roots had been reported in different susceptible crop species so that they would have limited access to water during earlier growth stages, whereas more as the crop would mature (Manschadi et al., 2006; Farooq et al., 2019).

The optimum plant density and crop canopy size determine crop yield by the shading effects and reduction of evapotranspiration and soil surface temperature which further reduce soil evaporation (Farooq et al., 2019). Plant density and canopy size also determine the rate of interception of solar radiation, a principle determinant of photosynthesis (Farooq et al., 2019). Under water limited conditions, moderate or smaller canopies help in reduction in water use and improvement in crop-level WUE (Farooq et al., 2019). Growth stage of the crop affects crop-level WUE and it further has an effect on yield (Tardieu et al., 2018, Farooq et al., 2019). Crops can withstand water stress up to a certain extent during the vegetative growth stages by modifications in the vegetative structures to reduce transpiration, but water stress during reproductive stages may lead to failure in reproduction and yield reduction (Farooq et al., 2019). High temperature and sunlight during the daytime also have an impact over crop-level WUE because the plants show variation in the overall leaf morphology, anatomy and canopy structure like rolling up of leaves to minimize the solar interception and reduce the transpiration rate (Tardieu et al., 2018; Farooq et al., 2019). However, there are some plants which increase the rate of transpiration during daytime and water-use to minimize the effects of high temperature. All these lead to differences in crop-level WUE and yield (Tardieu et al., 2018; Farooq et al., 2019).

As there are serious threats posed due to drought and heat stress in the current scenario of climate change, increases in crop-level WUE are vital for the sustainability of production. Scientists have been in search of techniques and ideas from all over, agronomic to genetic and transgenic prospects to improve crop-level WUE and crop yield (Du et al., 2010; Ul-Allah et al., 2018; Farooq et al., 2019).

1.3 Approaches to increase WUE at leaf-level and crop-level

Different approaches can be used to improve leaf-level WUE. Among many, one of the approaches is cultivar selection that may result in altered stomatal resistance through adjustment in leaf biochemistry as it includes the phenomenon like inhibition of formation of solute essential for increasing turgidity in guard cells in response to fluctuations in external stimuli like light, CO_2, humidity etc., and therefore results in adjustment of stomatal aperture to control transpirational water loss (Zelitch, 1965; Franks et al., 2009), or leaf morphology (eg. rolling up of leaves, reduction in leaf area, increase in the density of trichomes etc.) (Farooq et al., 2019), allowing plants to have high WUE. At the same time, there are different trade-offs in increasing stomatal resistance for sake of increasing WUE. Increased stomatal resistance is often associated with reduced CO_2 assimilation rate, which is again unsuitable from the agricultural perspective. For example, there are certain plants which take part in gaseous exchange only when the vapour pressure difference (VPD) is low and keep their stomata closed when VPD is high (mid-day) (Fischer and Turner, 1978; Sinclair et al., 1984). However, the mid-day closure of stomata comes with the reduced CO_2 assimilation (Sinclair et al., 1984). Another strategy can be growing the crops in humid climate and at the times of the year when the VPD would be lowest (cooler periods of the year) (Sinclair et al., 1984). However, associated challenge could be slow growth in plants to thrive the cooler temperatures (Sinclair et al., 1984).

At the crop-level, plants may not use all the water available for them in the field where they are grown, but instead the water is lost in the field by different processes like deep percolation and surface runoff (Sinclair et al., 1984). One of the approaches to improving water absorption in them is the development of crops with better root systems (Sinclair et al., 1984). Greater rooting density and deeper rooting depths would increase the access to the amount of soil water available for extraction (Taylor, 1983; Sinclair et al., 1984). Water reserves previously unavailable to the transpiring plant could be made available with an improved rooting system. However, the drawbacks associated with the deeper penetrating roots is that the greater root biomass would almost surely result in lowered harvest index, the ratio of marketable grain yield to total crop biomass as carbohydrates would be diverted for the construction and maintenance of a larger root mass (Sinclair et al., 1984). In a prolonged drought, rapid and more efficient use of stored soil water may be deleterious. Therefore, to survive through the drought, a less effective, slow-growing root system may be advantageous, as that would leave some water reserves partially available to the plants (Sinclair et al., 1984).

If the harvest index could be increased under water-limited condition, it would be an achievement in increasing crop-level WUE (Passioura, 1977; Fischer, 1979). The timing of planting certain crops can be modified and manipulated to improve crop-level WUE (Farooq et al., 2019). The plants which conserve water during vegetative growth can be prioritized so that there will be an adequate water supply for reproductive growth (Passioura, 1977; Fischer, 1979). The vegetative period of growth for some cereals can be shifted to cooler parts of the season (Fischer, 1979). Similarly, for water-limited environments, another approach could be the development of crops with roots with restricted water uptake rates (Passioura, 1977). Such alteration would conserve water during vegetative growth leaving more soil water for extraction during grain development, thereby increasing the total crop harvest.

Along with transpiration, soil evaporation causes water loss under field conditions. Rapid growth of leaves early in the season would reduce the amount of time the base soil is exposed to high levels of solar radiation and would thereby minimize soil evaporation (Passioura, 1977).

Many of the strategies that the agriculturists apply are focused on increased WUE and sustained reproductive growth, however, different crop varieties can be genetically improved for enhanced WUE (Krupnik et al., 2012; Farooq et al., 2019). Various breeding tools and techniques have been identified and developed to improve WUE in different crops by modifications in photosynthetic rates and carbon isotope ratio (Saranga et al., 1998; Farooq et al., 2019).

Similarly, seeding rate and plantation pattern are very important agronomic tools for the improvement in WUE. Seeding rate is essential to manage the production as a densely packed plant population will have to compete for nutrient uptake and space as well as water, thereby decreasing the potential of the crop for maximum yield and WUE (Hu et al., 2018; Ma et al., 2018; Farooq et al., 2019). Plantation pattern also has direct effects on plants' overall growth and productivity because they are directly responsible for interception of solar radiation, root growth, shading effects, etc. (Farooq et al., 2019). Compared to crops grown in the field, crops grown in raised beds showed improved crop-level WUE, because of reduced water consumption in wheat (Soomro et al., 2017; Farooq et al., 2019) and chickpea (Kumar et al., 2015; Farooq et al., 2019), and a better developed root system in maize (Khan et al., 2012; Farooq et al., 2019). Similarly, mulching, maintaining the levels of soil nutrients and weed management are alternative approaches as all of them influence yield, water use and therefore crop-level WUE (Farooq et al., 2019).

When overall WUE at global ecosystem level is considered, studies conducted in the later half of the twentieth century in C_3 as well as C_4 plants have revealed that the increase in CO_2 concentration associated with the changing climate has led to substantial increases in photosynthetic capacity/carbon assimilation (A), a response called as CO_2 fertilization (Chen et al., 2022) and associated decreases in stomatal conductance resulting in reduced evapotranspiration, and hence overall WUE had increased (Adams et al., 2020; Li et al., 2023). After CO_2 concentration, vapour pressure deficit (VPD) is also a very important atmospheric force in regulating WUE due to its role in imposing water stress in photosynthesis (Yuan et al., 2019; Li et al., 2023). However, recent studies have also revealed that with the start of 21st century, VPD has increased along with the increase in temperature, thereby reducing the CO_2 fertilization effect on WUE (Yuan et al., 2019; Li et al., 2023). At the same time, warming associated with climate change and increased leaf area index have been expected to down-regulate WUE (Li et al., 2023). Hence, due to the combined effects of all the explained factors at varied extent, there have been no changes in WUE of the global ecosystem since 2001 (Li et al., 2023). In other words, there has been saturation in ecosystem WUE.

1.4 Biomass Partitioning

Biomass partitioning can be defined as the differential distribution and deposition of assimilates among the plant tissues (Shrivastava and Gaiser, 2008). Crops grow by intercepting solar radiation, and during photosynthesis, they utilize the part of the absorbed energy to produce dry matter (Jenkins and Mahmood, 2003). It is not solely the total amount of biomass produced that determines the yield of the plants, yield depends also on the amount of biomass partitioned to the economically useful plant parts, like tubers in the case of potatoes (Ashok K et al., 2011; Jenkins and Mahmood, 2003). Therefore, those potato cultivars must be selected which will have the distribution of the dry matter in favor of yield formation. This distribution of dry matter is greatly controlled and modulated by the interaction of genetic and environmental factors, including the influence of available mineral nutrients (Cakmak and Engels, 1999; Jenkins and Mahmood, 2003).

Biomass accumulation and partitioning into different plant parts is a dynamic process influenced by interaction of climate conditions, cultural practices, genotype etc. (Ashok K et al., 2011). In potatoes, as the temperature exceeds 29 ºC, foliage growth, tuberization, net photosynthesis as well as biomass partitioning to tubers are reduced (Levy, 1992; Tolessa, 2019). However, deficiencies in water and nitrogen lead to increased biomass partitioning to tubers and large roots in potatoes (Belanger et al., 2001). Thus, in potato plants, tuber production depends on rate of plant development and subsequent allocation of assimilate from the aboveground portion of the plant into the tubers including interactions of climate conditions, cultural practices and genotype (Ashok K et al., 2011).

1.5 Objectives

The objectives of this study on eight established potato cultivars in field trials under irrigation at the Vauxhall experiment station include:

1. To analyze the variation in stomatal size and stomatal density using the images of leaflets collected via scanning electron microscopy (SEM).

This study aimed to analyze the differences in stomatal size and density so that the outcomes from the study can be used to test for variations in stomatal characteristics that occur among potato cultivars and may be associated with variation in water-use efficiency among them.

2. To study variations in leaf-level WUE using carbon and oxygen stable isotope techniques.
3. To study the variation in crop-level WUE by using measurements of yield and cumulative seasonal water use.
4. To identify the possible mechanisms that contribute to differences in crop-level WUE.

CHAPTER 2: MATERIALS AND METHODS

2.1 Plant Materials

There were a total of eight different potato cultivars used in the study, and these cultivars are grown regularly in southern Alberta. They were:

1. Atlantic (ATL)
2. Austin (AUS)
3. Russet Burbank (BUR)
4. Caribou Russet (CAR)
5. Ranger Russet (RAN)
6. Shepody (SHP)
7. Teton Russet (TET)
8. AC Vigor (VIG)

Of the cultivars used in this experiment, Shepody, Ranger Russet, Russet Burbank, Caribou Russet and Teton Russet are typically used for making French fries, and Atlantic, Austin and AC Vigor are typically used in making potato chips. Shepody has been identified as an early maturing variety with a determinate growth type, whereas Ranger Russet and Russet Burbank typically mature in the mid-season and late season, respectively, with Russet Burbank having an indeterminate growth type. Among the chip cultivars, Atlantic is an early-maturing cultivar with a determinate growth type, while Austin and Vigor have been identified as mid-to full season and late-season cultivars, respectively.

2.2 Experimental Field Set-up

An experimental field plot was set up at the Agriculture and Agri-Food Canada (AAFC) Vauxhall experiment station in Vauxhall, Alberta, Canada (Latitude: 50º 03’ 03.88” N; Longitude: 112º 07’ 23.36” W) during May-September 2023 (Fig. 1).

Figure 1. Aerial picture of the study area.

Technical support was provided to maintain the required level of fertilizer and irrigation water in the field to let the potato cultivars grow properly. The methods of field preparation and planting of potato cultivars in the experimental plot were done using the methods typically applied in southern Alberta (Harms and Konschuh, 2010). The experimental plot was divided into six different blocks surrounded by two guard rows and the experiment was set-up as a randomized, complete blocks design (Fig. 2). Each block had 24 rows with three adjacent rows of one cultivar, and for each set of three rows, one potato cultivar was chosen randomly to be planted. Ten tubers of a cultivar were planted in each row, rows were 3 m long, and there was a spacing of 0.9 m between the rows, the distance between hill peaks (Fig. 3). As the mechanical harvester for collecting potatoes would have to move down a row and collect all potatoes for a given row, a gap of 3 m was maintained between the block of rows so that potatoes collected from different cultivars were not combined. In the full experiment, there were a total of 144 rows, a set of three separate rows for each of 8 different cultivars replicated in 6 different blocks (3 x 8 x 6 =144 rows).

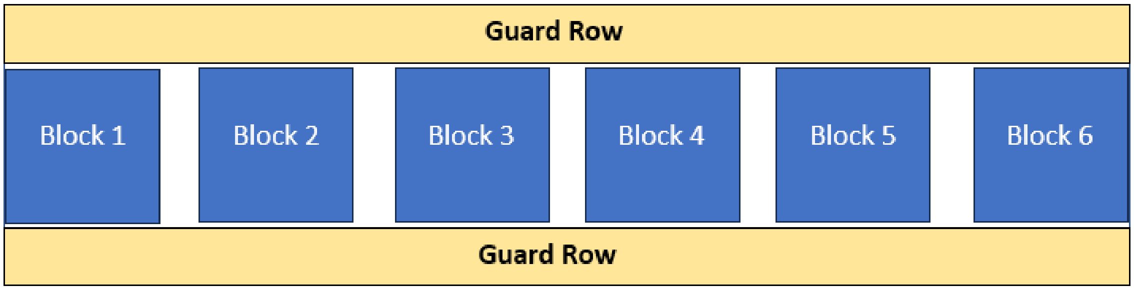

Figure 2. Schematic diagram of the study plot.

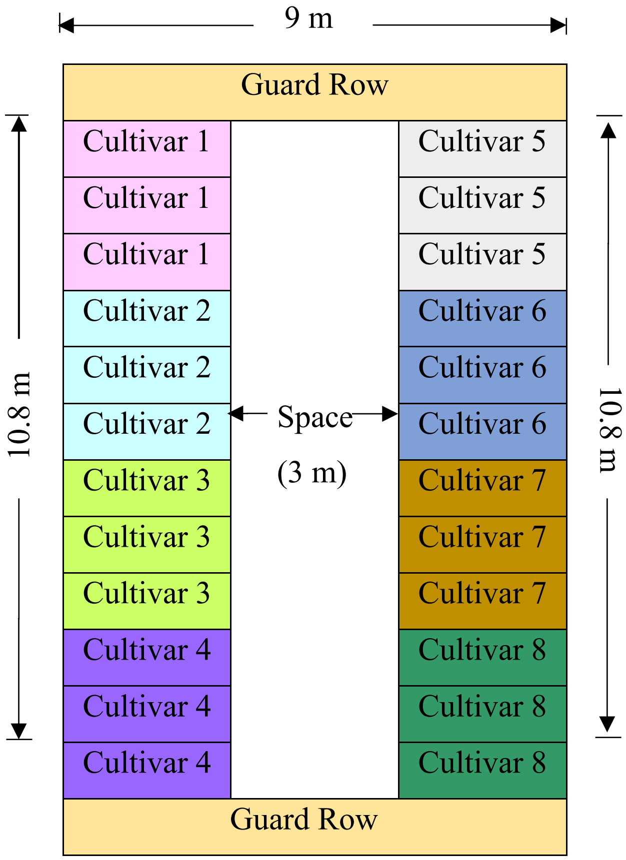

Figure 3. Schematic diagram of a single block (9 m × 10.8 m) with eight cultivars with three consecutive rows of the same cultivars with a distance of 3 m between the two sets of rows within a single block (each with 12 rows – 3 rows/per cultivar for each of 4 cultivars). This distance of 3 m was maintained to prevent intermixing of the harvested tubers.

2.3 Measurement of weather conditions

Measurement of different weather parameters is essential as plant growth and production are influenced by them, including water use and water use efficiency (Garcia y Gracia et al., 2009). The values for precipitation, incoming solar radiation, relative humidity, and air temperature (measured at 2 m above ground) at the Vauxhall CDA climate station (data available at the Alberta Climate Information Service; acis.alberta.ca/) were used to characterize the long-term climate for monthly average temperature and precipitation, in relation to weather that occurred during the 2023 growing season and to calculate P-days (also referred to as Potato Heat Units) (data available at the Alberta Climate Information Service; acis.alberta.ca/). Similarly, at the experimental site, several weather parameters were measured by instruments, installed at the experimental plot on May 24 and removed from the site on August 29. The complete set of measured weather parameters were as follows. Solar radiation intensity, air temperature and relative humidity, wind speed, precipitation were measured by an ATOMS41 weather station (METER GROUP), located at 1 m above ground. Soil temperature, soil volumetric water content and soil conductivity were measured in the experimental plot by using five TEROS12 soil sensors (METER GROUP). The above sensors were connected to, and measurements were stored by a data logger (ZL6 Advanced data logger, METER GROUP), located at 1m above ground. The soil sensors were installed at a depth of 20 cm below the hill-top, centered in the hill and installed in the space between plants. Average sensor readings were recorded at 30-minute intervals.

In addition, a modified rain-gauge was installed in the experimental plot, to collect the water both from irrigation and rain. These water samples were used to measure $\delta^{18}O$ values of water input to the soil.

Potato Heat Units, also called as "P-Days" were obtained from Vauxhall ACIS records (https://acis.alberta.ca/weather-data-viewer.jsp); these values were originally termed as “Physiological-days” (Sands et al., 1979). Among many factors essential for good growth and high productivity of crops, heat is particularly important. For potato, the minimum temperature essential for growth and development is 7 ºC (Praharaj et al., 2020). The most favorable temperature at which maximum growth and development is attained is 21 ºC, whereas it declines with further increase in temperature until it stops completely at 30 ºC (Praharaj et al., 2020).

2.4 Calculation of Greenness Index and Model Evapotranspiration

'Greenness' or the quantity of radiation in visible, green wavelength region reflected from an ecosystem is influenced by the amount of leaf area and photosynthetic capacity of that leaf area. The measurement of greenness, i.e. greenness index or the green chromatic coordinate (gcc) is the ratio of the green channel intensity to that of sum of red, green and blue channels. Green chromatic coordinate (gcc) is expressed as the given equation:

$$gcc = \frac{G}{(R+G+B)}$$

where, R, G and B are the digital numbers (intensity values ranging from 0-255) recorded by the camera for a given pixel within an image, for each of the three channels representing Red (R), Green (G), and Blue (B) waveband regions which are recorded in the image file.

In this study, gcc was calculated from photographs (jpeg images) taken with a drone (Model: DJI Mini 3 Pro). The drone had a camera mounted on it which was used to take the photographs of the agricultural field. These photographs can be used in studying plants' health, including their vegetative growth. Six digital photos were taken with the drone that moved across all six blocks and photos were stored as jpeg images during each visit to the study site at different times of the growing season of 2023 after plantation of tubers in the field. These images have three layers which record red, blue and green light respectively. The jpeg files were imported in MATLAB for quantitative analysis to calculate absolute gcc values.

The absolute gcc was normalized to scale from 0 to 1. The normalized greenness value gave an estimate of the crop coefficient, which is a measure of ratio of model evapotranspiration (ET_c) to reference evapotranspiration (ET_r) (Gonzalez et al., 2023). The crop coefficient changes over the course of time as the plants grow and their leaves start to develop. From this relation, ET_c was computed for the growing season. ET_r is calculated from the equation given by Allen et al. (2005) (Gonzalez et al., 2023). However, in this study, the values of ET_r were obtained from Alberta Climate Information Service (ACIS) (https://acis.alberta.ca/weather-data-viewer.jsp). ET_c was computed from the product of the crop-coefficient and ET_r, and it showed how much water (in mm) was evapotranspired by the crops throughout the whole growing season. It can be compared to the water input to the plants from rain and irrigation.

2.5 Leaf and total aboveground biomass collection

Leaflet samples for the measurements of the stable carbon and oxygen isotope ratio and total nitrogen content of the leaves were collected in mid-August (August 14 and 15). Six sets of three mature terminal leaflets were collected in a consistent pattern across the canopy of individual plants and were combined to provide an integrated sample for that individual plant. This was done for five plants per row, for each cultivar in a block across all six blocks. The leaflet samples were brought back to the university lab, dried in a forced air-oven at 60 ºC, and then ground to a fine powder using ball mill (MM200, Retsch, Haan, Germany).

Samples for measurement of total aboveground plant biomass were collected on August 29, 2023. To measure aboveground plant biomass, five plants were collected from a single row for each cultivar in every block. The five individual biomass samples were collected separately, dried and weighed. The weight of the five individual plant samples were averaged to represent a given sampled row.

2.6 Measurement of stomatal density

Leaflet samples for the stomatal measurements were collected from August 1-11. To measure the stomatal density, fully expanded terminal leaflets were chosen from two plants from a single row for all eight cultivars from all six blocks. Terminal branches with three leaflets were collected to ensure that I would have extra samples in case a leaflet was damaged, or a good quality picture could not be obtained from the terminal leaflet. Using a sharp cork borer, two small leaf sections from both left and right sides of a leaflet were cut from the terminal leaflet midway along both proximal-distal and medial-lateral axes. The diameter of the cork borer was chosen such that it cut out a section slightly smaller than the size of the stubs (12.7mm x 8mm) that were used for SEM. The abaxial/bottom of the leaf sections were observed in the SEM and clear images at 250 and 1000 magnifications were taken for stomatal count and stomatal size, respectively. The stomatal density and stomatal size were determined from the SEM images using ImageJ software. For stomatal density measurement, total number of stomata in the lower magnification were counted and divided by the area of the image. When counting the stomata, only those with more than 50% of the stomatal area falling within the image were considered. For stomatal length and width measurement, higher magnification (1000x) images with at least 3 complete stomata were chosen and all the stomata within the images were measured for length and width. Hence, I had at least three measurements of both stomatal length and width of each leaflet sample, which were averaged to get a single value each for stomatal length and width.

2.7 Measurement of leaf carbon isotope composition, oxygen isotope composition and total nitrogen content

To measure the carbon stable isotope ratio, dried leaf tissue was combusted in an elemental analyzer and the resulting carbon dioxide, purified via gas chromatography, was passed on to a gas isotope ratio mass spectrometer for analysis of its $\delta^{13}C$ value. To measure the oxygen isotope ratio, dried leaf tissue was subjected to pyrolysis in modified elemental analyzer, and the resulting carbon monoxide gas was purified using gas chromatography before being passed on to a gas isotope ratio mass spectrometer for measurement of its $\delta^{18}O$ value (Flanagan and Farquhar, 2014). Similarly, the total Nitrogen content of leaf tissue was also measured simultaneously when measuring $\delta^{13}C$ value. The significance of measuring total N content is that it can be used as a proxy to estimate the photosynthetic capacity of the plants (Evans, 1989). This complete analysis of the collected leaf samples was done in the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the Department of Biology, University of Utah (https://sirfer.utah.edu/).

2.8 Calculation of leaf-level water-use efficiency from leaf carbon and oxygen isotope compositions

Leaf-level water-use efficiency of different potato cultivars was calculated from the measurements of leaf $\delta^{13}C$ and $\delta^{18}O$ values. The $\delta^{13}C$ and $\delta^{18}O$ values for five plants in a row were averaged to represent one value for that cultivar in a single block. The row was the basic experimental unit representing one cultivar in each block. There were six true replicates based on the six blocks in the experimental plot.

Leaf-level water-use efficiency (WUE) is the ratio of leaf net CO_2 assimilation rate (A) and transpiration rate (E), which in turn are dependent on the gradients for CO_2 and H_2O diffusing into and out of leaves during photosynthetic gas exchange (Farquhar et al., 1988; Condon et al., 2002; Flanagan and Farquhar, 2014). They are represented as the equations:

$$A= g_c (c_a- c_i) \tag{1}$$

$$E= g_w (w_i -w_a) \tag{2}$$

$$WUE = \frac{A}{E} = \frac{g_c(c_a-c_i)}{g_w(w_i-w_a)} = \frac{c_a(1-\frac{c_i}{c_a})}{w_i\left(1-\frac{w_a}{w_i}\right)1.6} \tag{3}$$

where c and w are the concentrations of CO_2 (μmol mol^{-1}) and water vapour (mmol mol^{-1}), respectively, and subscripts refer to the air outside the leaf (a) and air in the leaf intercellular air spaces (i); g is total conductance (primarily controlled by stomata), and the subscripts refer to carbon dioxide (c) and water vapour (w); 1.6 is the ratio of the diffusion coefficients for H_2O and CO_2 in air (g_w/g_c=1.6; Farquhar et al., 1988). The carbon isotope composition of leaf ($\delta^{13}C_m$) is dependent on the isotope composition of atmospheric carbon dioxide ($\delta^{13}C_a$), as shown in the model describing fractionation of carbon isotopes during photosynthetic gas exchange in C_3 plants (Farquhar et al., 1982; Farquhar et al., 1989) :

$$\delta^{13}C_m=\delta^{13}C_a-a-(b'-a)(\frac{c_i}{c_a}) \tag{4}$$

where *a* is fractionation during diffusion of CO_2 into a leaf (4.4‰), *b′* is the net fractionation during carboxylation (27‰), and the extent that these two fractionations get expressed depends on leaf c_i/c_a. The two additional important processes that can influence the carbon isotope discrimination ($\Delta^{13}C_m$) during photosynthesis are transfer of carbon dioxide from the intercellular air spaces to the chloroplast (mesophyll conductance) and photorespiration (Farquhar et al., 1982; Farquhar et al., 1989; Flanagan and Farquhar, 2014), but these processes were not considered in this study because they have relatively small effects on the absolute values and would not likely affect the general patterns of variation observed among cultivars in this study (Flanagan and Farquhar, 2014). With an assumption that $\delta^{13}C_a$ and c_a are known, equation (4) can be rearranged to calculate leaf c_i once the measurement of $\delta^{13}C_m$ is done. It was an assumption that all potato plants were exposed during their summer growth period to c_a of 415 µmol mol^{-1} and $\delta^{13}C_a$ of -8.5‰, based on measurements made at similar latitudes as the Vauxhall site during the summer months by the National Oceanic and Atmospheric Administration Global Monitoring Laboratory (gml.noaa.gov/ccgg/about.html), and the University of Colorado, Institute of Arctic and Alpine Research Stable Isotope Lab (www.colorado.edu/instaar/research/labs-groups/stable-isotope-lab).

The oxygen isotope composition of leaf organic matter ($\delta^{18}O_m$) was controlled by the isotope composition of leaf water, which was further influenced by water taken up from the soil ($\delta^{18}O_{sw}$, source water), and isotope effects that occur during transpiration. The major drivers that contribute to the change in leaf water oxygen isotope composition during transpiration were variation in leaf temperature, air temperature and atmospheric humidity, and this would get recorded in the isotope composition of leaf biomass ($\delta^{18}O_m$). This study made use of the specific theory described in detail by Flanagan and Farquhar (2014), and so only the equations and calculations that were necessary to use $\delta^{18}O_m$ in calculations of w_a/w_i values have been presented in the text below.

Calculations of w_a/w_i were based on measurements of $\delta^{18}O_m$ and other information as noted below (Flanagan and Farquhar 2014):

$$\frac{w_a}{w_i} = \frac{\frac{(\delta^{18}O_m - \delta^{18}O_{sw} - \varepsilon_{wc} - \varepsilon_{cm})1.28)\wp}{1-e^{-\wp}} - \varepsilon^{+} - \varepsilon^{k}}{1-e^{-\wp}} \qquad (5)$$

where ε^+ is the equilibrium fractionation factor during the phase change from liquid water to water vapour (9.1‰ at 25 ºC, with temperature dependence described by $\varepsilon^+ = [2.644 - 3.206(10^3/T) + 1.534(10^3/T)^2]$‰ [where T is absolute temperature; Bottinga and Craig (1969)]; ε^k is the kinetic fractionation factor for water vapour diffusing through the stomatal pore (32‰, Cappa et al. 2003); ε_{wc} is the fractionation factor (27‰) for exchange of oxygen atoms between sucrose and leaf water before sucrose is incorporated in cellulose of leaf tissue (Cernusak et al., 2003); ε_{cm} is the difference in fractionation between cellulose and whole leaf organic matter. A few studies have shown that whole leaf tissue is less enriched in ^{18}O, compared to the exchanging water pool than is cellulose, a difference that is variable among species (Farquhar et al., 1997; Barbour and Farquhar, 2000, Cernusak et al., 2005). We assumed a value for ε_{cm} of -6‰, which was consistent with previous studies that showed ε_{cm} values ranged from -4 to -9‰ (Farquhar et al., 1997; Barbour and Farquhar, 2000; Cernusak et al., 2005). Water enriched in ^{18}O at the evaporative sites diffuses back into the leaf mesophyll, but this process is counteracted by advection of un-fractionated source water from the veins into the leaf mesophyll. The balance between these factors is described by a Péclet effect ($\wp$) (Farquhar and Lloyd, 1993; Ripullone et al., 2008), which depends on the transpiration rate, the effective path length for water movement through the leaf, the molar density of water, and the diffusivity of $H_2{}^{18}O$ in water. In the calculations involved in this study, the value for the Péclet ($\wp$) parameter was assumed to be 0.1 (dimensionless), which was consistent with other studies (Barbour and Farquhar, 2000; Ripullone et al., 2008; Kahmen et al., 2009; Flanagan and Farquhar, 2014). The $\delta^{18}O_{sw}$ and $\delta^{18}O_v$ are the oxygen isotopic compositions of source water taken up by plants and atmospheric water vapour, respectively. It was our assumption that all potato plants had identical source water available for them because the experimental plot was relatively small and was irrigated with the same water. The average value of $\delta^{18}O_{sw}$ was -14.9‰ based on measurement of water collected at intervals during May-August in the modified rain gauge that was installed in the experimental plot. In addition, we assumed that the $\delta^{18}O_v$ was in isotopic equilibrium with the $\delta^{18}O$ of local source water and had a value of -28‰. This simplifying assumption was supported by some previous studies (Lee et al., 2006; Helliker and Griffiths, 2007; Angert et al., 2008). In addition, the potential effect of $\delta^{18}O_v$ on leaf water $\delta^{18}O$ is minor in environments with relatively low atmospheric humidity during mid-day, as was the case in this study (see Appendix 1). The combined use of $\delta^{13}C$ measurements to estimate c_i/c_a (equation 4) and $\delta^{18}O$ measurements to estimate w_a/w_i (equation 5) allows leaf water-use efficiency to be calculated using equation (3), assuming that c_a and w_a are known from atmospheric measurements. We used measurements of local environmental conditions to calculate an integrated value of w_a that would be representative of the conditions that potato plants experienced during the summer growth period (May 24 to August 29), the time between when plants emerged aboveground and when the aboveground biomass samples were harvested. Total atmospheric pressure in Vauxhall, AB (elevation 779 m above sea level) is typically 92.3 kPa.

2.9 Measurement of crop-level water-use efficiency from tuber yield and cumulative seasonal water-use

For potato, crop-level water-use efficiency is defined as tuber yield produced per unit of water used by the crop (Hatfield and Dold, 2019):

$$\text{Crop-level WUE} = \frac{\text{Tuber yield (g)}}{\text{Cumulative seasonal water loss from evapotranspiration (kg)}} \quad (6)$$

(g kg^{-1})

Tuber yield was measured from the tubers harvested from the rows which were not disturbed by aboveground biomass and leaf sampling. A single-row digger was used to harvest the potato tubers. Tuber harvest was done on September 7. After the harvest, tubers were washed, and scanned in an optical grader which generated values for length, diameter, aspect-ratio (length/diameter), roundness (ratio of maximum diameter and minimum diameter), and volume. Total weight of all the tubers obtained from a single row was determined for all eight cultivars in all six blocks of the experiment.

Similarly, for the measurement of cumulative seasonal water use, the measurement of water lost by plants during evapotranspiration at leaf-level was estimated by integrating the instantaneous water loss for the whole season. The procedures used to calculate seasonal water loss in plants are explained through the series of steps as presented below:

1. Equations (1) and (2) give the instantaneous measurement of net photosynthesis or net CO_2 assimilation rate and evapotranspiration at the leaf-level. When these calculations

were made using stable isotopes measurements, they were then integrated during the growing season over the life of leaf. Overall, when the net CO_2 assimilation rate (A) and evapotranspiration (E) are integrated over the total growing season, equations (1) and (2) can also be expressed as:

$$\overline{A}= \overline{g_c}\ (\overline{c_a}- \overline{c_i}) \qquad (7)$$

$$\overline{E}= \overline{g_w}\ (\overline{w_i}- \overline{w_a}) \qquad (8)$$

where, $\overline{c}$ and $\overline{w}$ are the time-integrated concentrations of CO_2 (μmol mol^{-1}) and water vapour (mmol mol^{-1}), respectively, and subscripts refer to the air outside the leaf (a) and air in the leaf intercellular air spaces (i); $\overline{g}$ is time-integrated conductance over the growing season (primarily controlled by stomata), and the subscripts refer to carbon dioxide (c) and water vapour (w).

2. Maximum net CO_2 assimilation rate (A_{max}) can be estimated from the measurement of Nitrogen content expressed as unit of ‘g/m^2’. A_{max} was calculated from Nitrogen content values using the equation (Franks et al., 2009):

$$A_{max}=V_{cmax}[(\overline{c_i} -37)/(\overline{c_i}+622)]-0.01V_{cmax} \quad (9)$$

where V_{cmax} is the maximum velocity of ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco) for carboxylation. The value of V_{cmax} was determined from leaf nitrogen content values (g/m^2) of potato cultivars based on the equations published by Kattge et al. (2009). First, nitrogen content (in mg/g) was calculated from the nitrogen content of leaf tissue samples obtained from isotope analysis in units of percentage (%). The value of nitrogen content in the unit of g/m^2 was estimated from specific leaf area (180 cm^2/g) by multiplying the reciprocal of specific leaf area and leaf nitrogen content (mg/g). A single value of specific leaf area was used for all cultivars (a simplifying assumption). $\overline{c_i}$ values were obtained from δ^{13}C measurements. Once V_{cmax} was calculated, A_{max} was estimated. Once the value of A_{max} was determined from equation (9), the value of $\overline{g_c}$ could be estimated from equation (7). As equation (7) can further be written as:

$$\overline{g_c} = \overline{A}_{max} / (\overline{c_a} - \overline{c_i}) \qquad (10)$$

As ($\overline{c_a}$- $\overline{c_i}$) was calculated from δ^{13}C and ($\overline{w_i}$- $\overline{w_a}$) was calculated from δ^{18}O measurements, further calculations of $\overline{g_c}$ which were used to calculate $\overline{g_w}$ as $\overline{g_c}$ and $\overline{g_w}$ are related and the relationship is expressed as (Farquhar et al., 1988):

$$\frac{\overline{g_w}}{\overline{g_c}} = 1.6$$

In summary, I wanted to calculate evapotranspiration rate using equation 8 for a leaf using the isotopic measurements and leaf nitrogen content with some knowledge of specific leaf area and theory of leaf photosynthetic gas relationships.

3. The calculations done in step 2 were at the leaf-level. In order to convert transpiration values at the leaf-level to the whole-plant level, total transpiring leaf area for the plant was required. This total transpiring leaf area parameter was determined in a two-step process. First, an estimate of the ratio of total leaf area per plant aboveground biomass (0.01652 m^2 g^{-1}) was obtained based on data in Morrissette et al. (2006). This ratio was multiplied by plant aboveground biomass, averaged across all cultivars, to obtain an initial estimate of total transpiring leaf area per plant. I subsequently made calculations of plot-scale transpiration using the initial ratio noted above, but then adjusted or tuned

the initial ratio until the plot-scale calculations of transpiration closely matched calculated E_c values, which were in turn based on the product of E_r values and the crop coefficient (based on relative greenness). This tuning process resulted in a final value for the ratio of total leaf area per plant aboveground biomass (0.0080 m^2 g^{-1}) that was used in subsequent calculations. The total transpiring leaf area for each cultivar was then determined by multiplying the final value for the ratio of total leaf area per plant aboveground biomass by the average aboveground biomass for each cultivar. This was based on a simplifying assumption (but also conservative assumption) that all cultivars allocated aboveground biomass to leaf area in the same manner. Ultimately row-level transpiration or water flux was calculated by multiplying plant-level transpiration by the number of plants in a row.

4. To calculate the amount of water transpired by individual cultivars on a ground-area basis, values obtained from step 3 were multiplied by the total number of rows occupied by a cultivar in one block, and divided by the ground area occupied by the cultivar. Similarly, tuber yield was also calculated on a ground-area basis.
5. Evapotranspiration rate will change on a daily basis based on changes in solar radiation within the day. The prior calculated plant-level transpiration rates were assumed to be the peak value for a day and, therefore, they were scaled across the time of day by the normalized solar radiation measurement. Hence, solar radiation within the day (expressed on a 0-1 scale) was then multiplied by the peak transpiration rate to calculate daily variation in transpiration and subsequently daily-integrated transpiration rates.
6. The final step was the seasonal integration which involved calculation across all days within a growing season. I used relative greenness (0-1 scale) as a proxy for the

development of leaf area over time. The relative greenness value for a given day was multiplied by the daily-integrated transpiration rate for a given day and summed up over all growing season days to obtain a seasonal-integrated transpiration rate for the entire growing season. This was done separately for all 8 cultivars.

To conclude, all above mentioned series of steps were used to estimate evapotranspiration at leaf level, integrated to plant level for a given cultivar and further expressed at the ground level over time integration from the daily to seasonal scale.

2.10 Statistical analyses

A single row containing a single cultivar within each block was considered as the experimental unit of this study. Hence, stomatal density, stomatal size, stable isotope measurements, tuber yield and aboveground biomass measurements along with the calculated values of WUE, both at the leaf-level and crop-level and cumulative seasonal water-use were generated for plants within a row. All the obtained data sets were tested for normality and homogeneity of variances using Shapiro-Wilk and Bartlett tests, respectively. Data sets that passed those assumptions were tested by Analysis of Variance (ANOVA), based on a randomized, complete block experimental design. Obtained data sets were analyzed for block and cultivar effects using two-way ANOVA (parametric). If the assumptions of ANOVA were not met, non-parametric tests (Friedman tests) were used. Multiple comparison (Tukey-Kramer) tests were used for comparisons after a significant result was obtained with ANOVA or Friedman tests. Similarly, bivariate relationships were tested using least-squares linear regression and the significance of the associated slope of the linear regression were tested using ANOVA. Box and whisker plots were used for presenting several data sets in this study. The box plot format was: the horizontal line within the box represented the median; the bottom and top of the box represented the 25th percentile (first quartile) and 75th percentile (third quartile) for the data set, respectively. The smallest and largest values were represented by the whiskers. MATLAB software (R2022b, The MathWorks Inc., Natick, MA, USA) was used for plotting the data and conducting all statistical analyses.

CHAPTER 3: RESULTS

3.1 Environmental conditions during the growing season

3.1.1 Air Temperature and Precipitation:

Air temperature was higher, and precipitation was lower in 2023 than the climate normal (long-term average during 1961-2018, referred here as climate normal conditions). Average temperatures in May, June and August were greater than the normal by 4.32 ºC, 2.61 ºC and 3.17 ºC respectively, whereas July had an average temperature nearly 1.5 ºC above normal (Fig. 4a). In addition, July received slightly higher rainfall, approximately 10.7% increase above the long-term average, whereas May, June and August had lower than normal rainfall (79%, 82% and 49.3% respectively) (Fig. 4b).

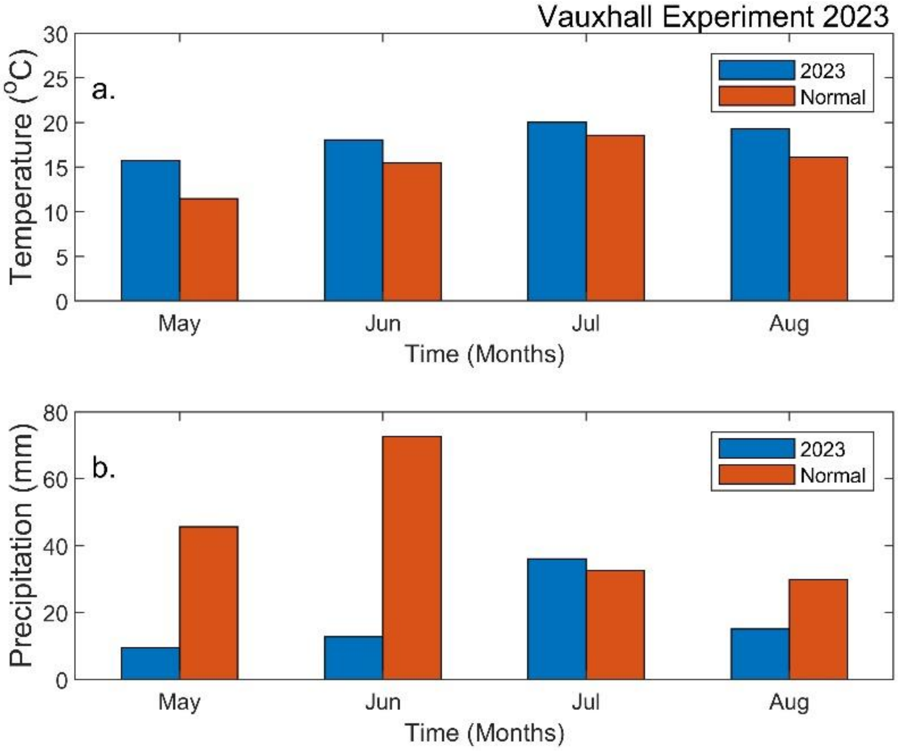

Figure 4. Comparison of (a) monthly average air temperature, and (b) monthly total precipitation for the growing season of 2023 with long term average values which were taken to be the representative of the local climate normal (1961-2018) recorded at ACIS climate station (Vauxhall Weather Station, Alberta) data.

3.1.2 Precipitation and Irrigation in the study plot

The amount of total water input through irrigation and precipitation was low in June (day 153 to day 177) as demonstrated in Fig. 5c. Higher amounts of irrigation water were supplied to the field starting at the end of June (day 178), the highest being at around mid-July to mid-August (day 193 to day 227) (Fig. 5a). Apart from irrigation water, the amount of water from precipitation also increased from mid-July with a major rainfall event occurring on July 30 (day 211) (Fig. 5b). Thus, total water input in field was increased after mid-July through to mid-August. Cumulative water input, the total amount of water that the potato plants in the field have access to, gradually increased up to middle of August (Fig. 5d) and after this time, amount of water supplied to the field was very minimal.

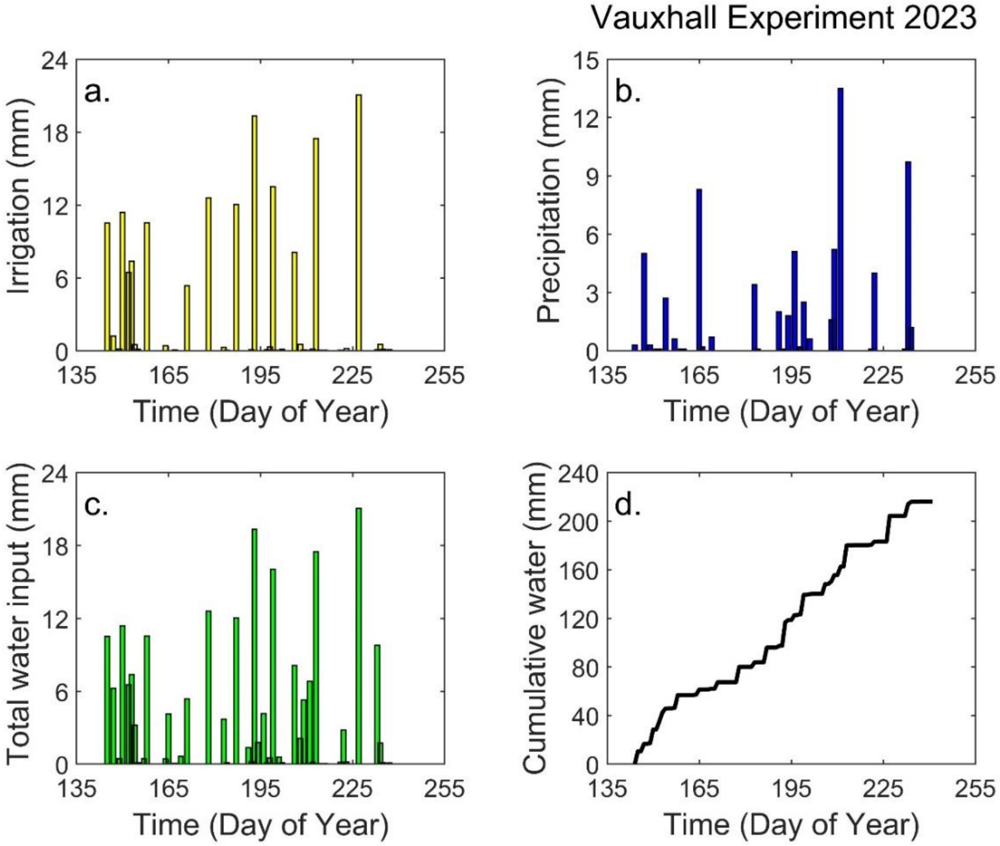

Figure 5. a Daily irrigation; b daily precipitation; c total water input: sum of precipitation and irrigation; d cumulative total water input in the field for the growing season of 2023.

Measurements of local environmental conditions were used to calculate an integrated value of w_a, that would be representative of the conditions that potato plants experienced between the time when plants emerged aboveground (approximately day 158) and when aboveground biomass samples were collected (day 241). To do this, the solar radiation-weighted average of w_a (1.475 kPa or 16.03 mmol mol^{-1}) was calculated from measurements of the mean diurnal pattern of solar radiation, air temperature and relative humidity (Appendix 1). Similarly, the seasonal pattern of important weather parameters: solar radiation, maximum and minimum air temperature and the maximum VPD were also measured in the study site (Fig. 6).

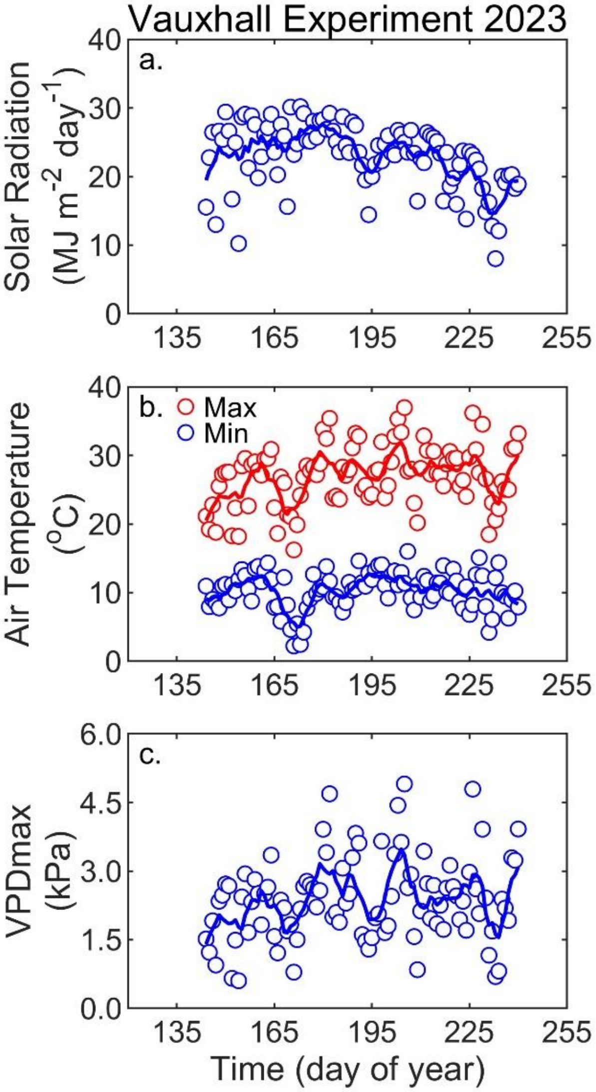

Figure 6. a Incoming solar radiation; b maximum and minimum air temperature (at 1 m above ground); c maximum vapour pressure difference between air and intercellular spaces of leaflet samples collected at Vauxhall Research Station, Alberta in the growing season of 2023. The lines represent 7-day moving averages fitted to the data.

3.1.3 Relative Canopy Greenness, Model Evapotranspiration, P-days and Soil Volumetric Water Content

Relative canopy greenness was the highest in the second week of August (day 219 to day 225) as shown in Fig. 7a, and leaflet samples for isotope analysis were collected during the same time, i.e. August 14-15. Only few days prior to this time, leaflet samples for stomatal analysis were collected.

In this study, during the course of time starting at day 135, there was a total of approximately 800 cumulative P-days (also called as Potato Heat Units) going up to the end of August (day 240) (Fig. 7c).

Average daily soil volumetric water content was the highest in June (day 153 to 180) (Fig. 7d), whereas average daily soil temperature was low in days between 166 to 173 (Appendix 1d). Soil volumetric water content in June was the highest among the other remaining months of summer of 2023, despite the amount of total water input through irrigation and precipitation being low (Fig. 5c). Volumetric soil water content started declining towards the end of June (nearly day 178) and this was the time when total water input in field was increased. Soil volumetric water content was maintained relatively constant after mid-August (day 222), until biomass was harvested when the precipitation input was low and air temperatures were warmer than normal (Fig. 4).

The seasonal variation in soil volumetric water content was explained by comparing model evapotranspiration (ET_c) computed from the product of the crop-coefficient and reference evapotranspiration (ET_r) and cumulative water input from rain and irrigation as shown in Fig. 7. ET_c and cumulative water input were generally very similar. But early in the season, before the crop was developed, there was more water input by rain and irrigation, but low ET$_c$, resulting in greater soil moisture. When plants started to grow, there was increase in ET$_c$, thereby decreasing the soil moisture. Now, at this point, the amount of water delivered to the field through rain and irrigation is matched to the amount of water lost through crop ET$_c$ for the rest of the season.

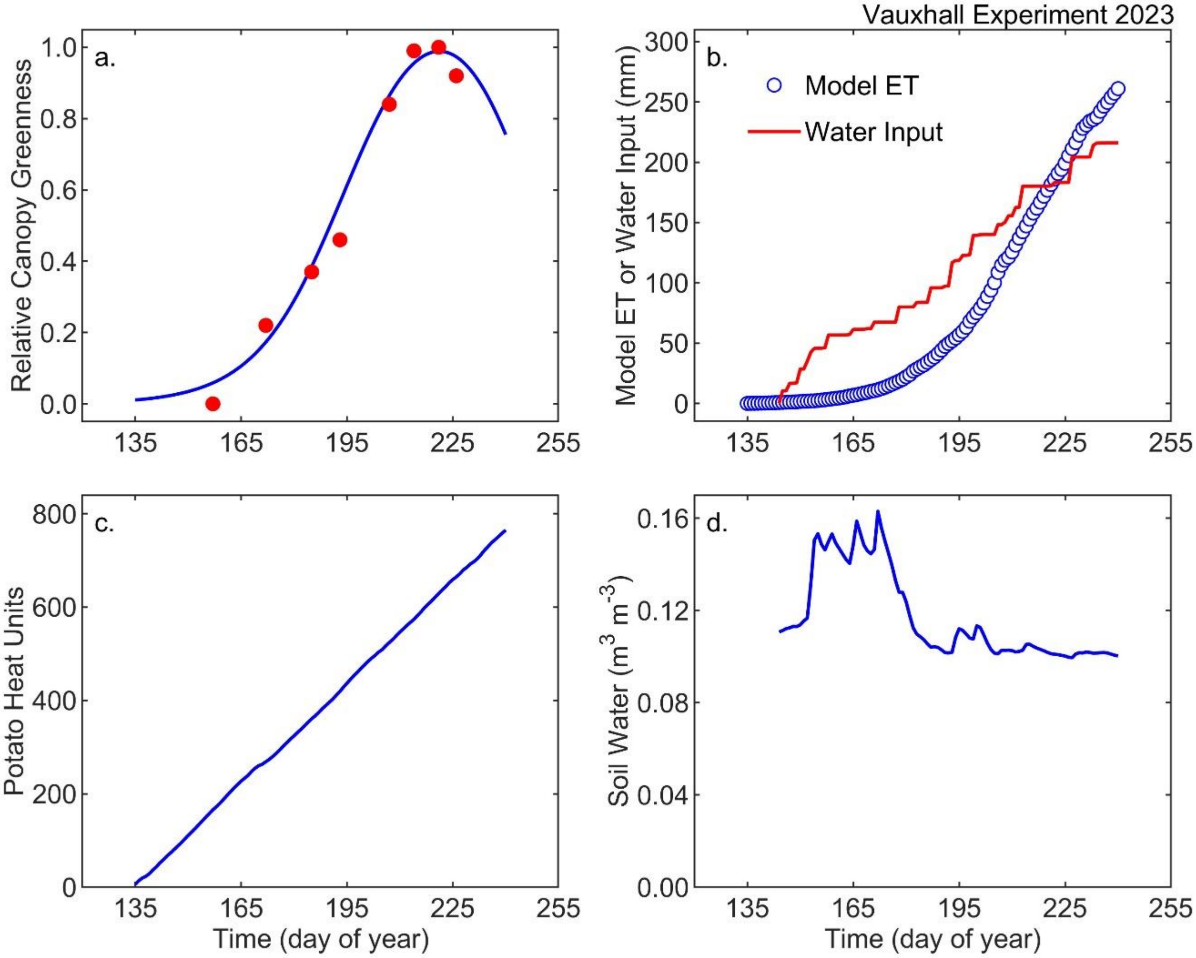

Figure 7. a Relative canopy greenness; b model evapotranspiration and measured water input from precipitation and irrigation; c Potato Heat Units (P-days) over the growing season of 2023 (from tuber plantation to tuber maturity); d soil volumetric water content.

3.2 Variation in stomatal density, stomatal length and stomatal width among cultivars

Significant differences were observed among the cultivars for stomatal density (Fig. 8; ANOVA, [Cultivar] F=6.82, d.f.=7, p-value<0.05) but no significant block effect occurred (Fig. 8; ANOVA, [Block] F=1.11, d.f.=5, p-value=0.37). Multiple comparison tests indicated that Austin (AUS) and Russet Burbank (BUR) were significantly different from Caribou Russet (CAR), Shepody (SHP) and AC Vigor (VIG).

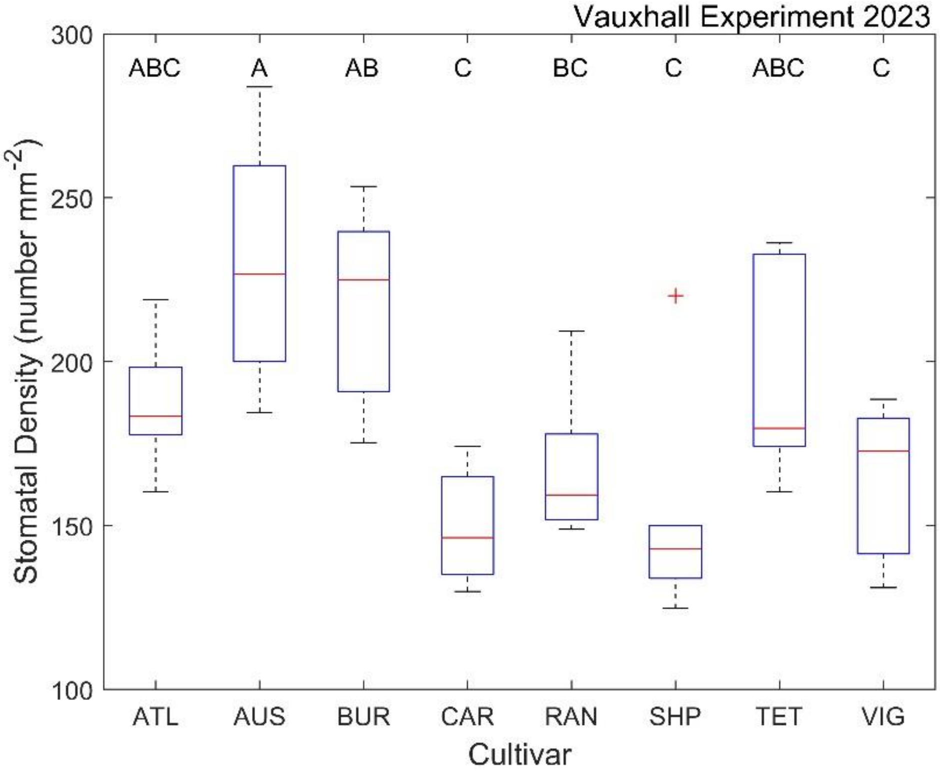

Figure 8. Comparison of stomatal density (number mm^{-2}) among eight potato cultivars. Statistical significance, as indicated by the different letters located inside the top edge of the graph box, was based on multiple comparison tests after ANOVA test [$F_{(7, 47)}$= 6.82, p-value<0.05].

In case of stomatal length, analysis of variance indicated that there were significant differences among the cultivars (Fig. 9; ANOVA, [Cultivar] F=4.38, d.f.=7, p-value=0.001), but no significant effect of block was observed (Fig. 9; ANOVA, [Block] F=1.55, d.f.=5, p-value=0.2). Subsequent multiple comparison tests showed that Russet Burbank (BUR) was significantly different than Atlantic (ATL), Ranger Russet (RAN), Shepody (SHP) and AC Vigor (VIG).

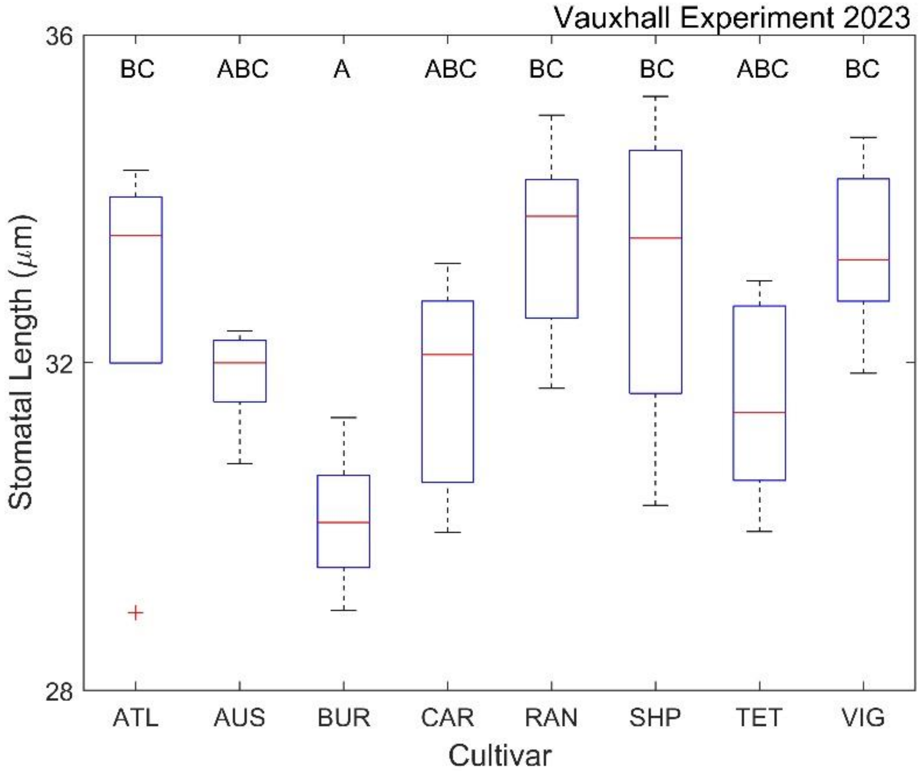

Figure 9. Comparison of stomatal length (μm) among eight potato cultivars. Statistical significance, as indicated by the different letters located inside the top edge of the graph box, was based on multiple comparison tests after ANOVA test [$F_{(7, 47)}$= 4.38, p-value=0.001].

There were significant differences among the cultivars for stomatal width (Fig. 10; ANOVA, [Cultivar] F=11.64, d.f.=7, p-value<0.05), but no significant block effect occurred (Fig. 10; ANOVA, [Block] F=1.72, d.f.=5, p-value=0.16). When multiple comparison test was performed, Austin (AUS) and Russet Burbank (BUR) were significantly different than Ranger Russet (RAN), Shepody (SHP), Teton Russet (TET) and AC Vigor (VIG).

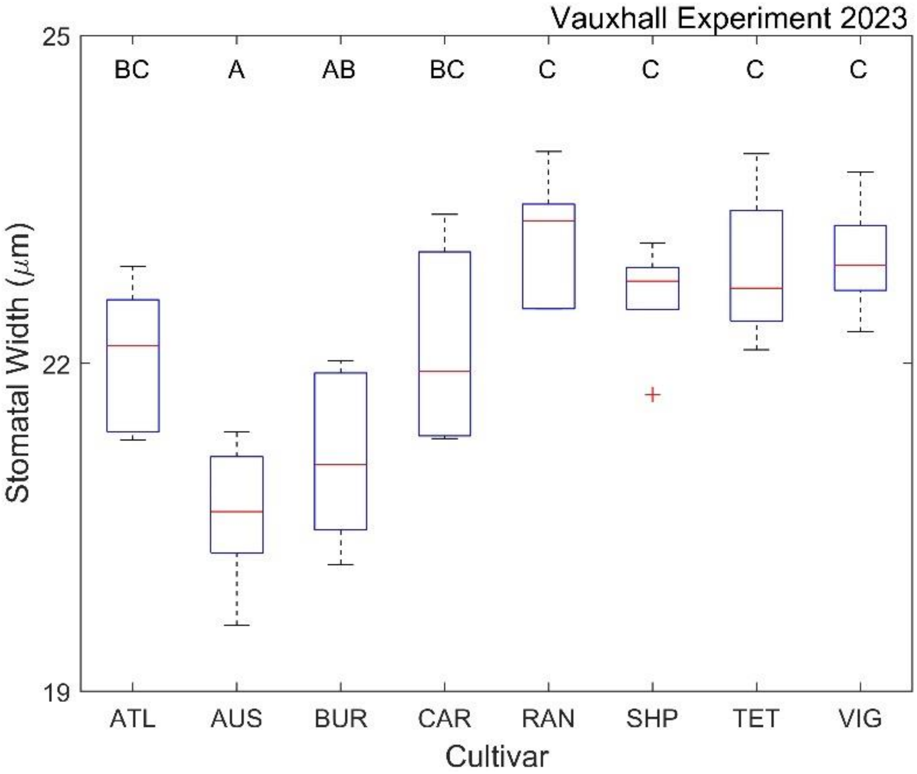

Figure 10. Comparison of stomatal width (μm) among eight potato cultivars. Statistical significance, as indicated by the different letters located inside the top edge of the graph box, was based on multiple comparison tests after ANOVA test [$F_{(7, 47)}$= 11.64, p-value<0.05].

3.3 Variation among cultivars for $\delta^{13}C_m$ and $\delta^{18}O_m$

Analysis of variance showed that there were significant differences among the potato cultivars for $\delta^{13}C$ but there was no significant effect of block (Fig. 11; ANOVA, [Cultivar] F=11.9, d.f.=7, p-value<0.05, [Block] F=2.27, d.f.=5, p-value=0.07). When the subsequent multiple comparison tests were performed, Russet Burbank (BUR) and Caribou Russet (CAR) were significantly different from Atlantic (ATL), Shepody (SHP) and Teton Russet (TET), whereas Ranger Russet (RAN) was significantly different from Shepody (SHP) and Teton Russet (TET). AC Vigor (VIG) was significantly different from only one cultivar, Shepody (SHP).

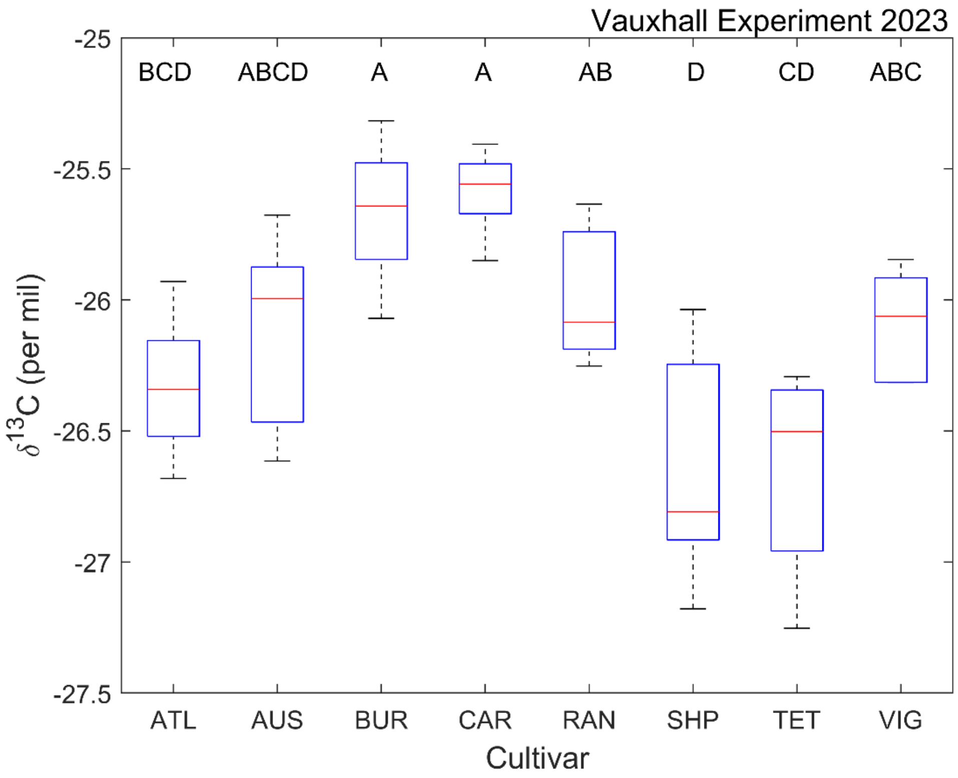

Figure 11. Comparison of leaf carbon isotope composition ($\delta^{13}C$ values, ‰) among potato cultivars. Statistical significance, as indicated by the different letters located inside the top edge of the graph box, was based on multiple comparison tests after ANOVA test [$F_{(7, 47)}$=11.9, p-value<0.05].

In case of $\delta^{18}O$, a Friedman non-parametric (2-way) test indicated a significant effect of cultivars (Fig. 12; Chi-square=16.33, d.f.=7, p-value=0.022) on $\delta^{18}O$, but there was no significant effect of block (Fig. 12; Chi-square=6.29, d.f.=5, p-value=0.28). However, multiple comparison tests did not indicate any significant differences among cultivars.

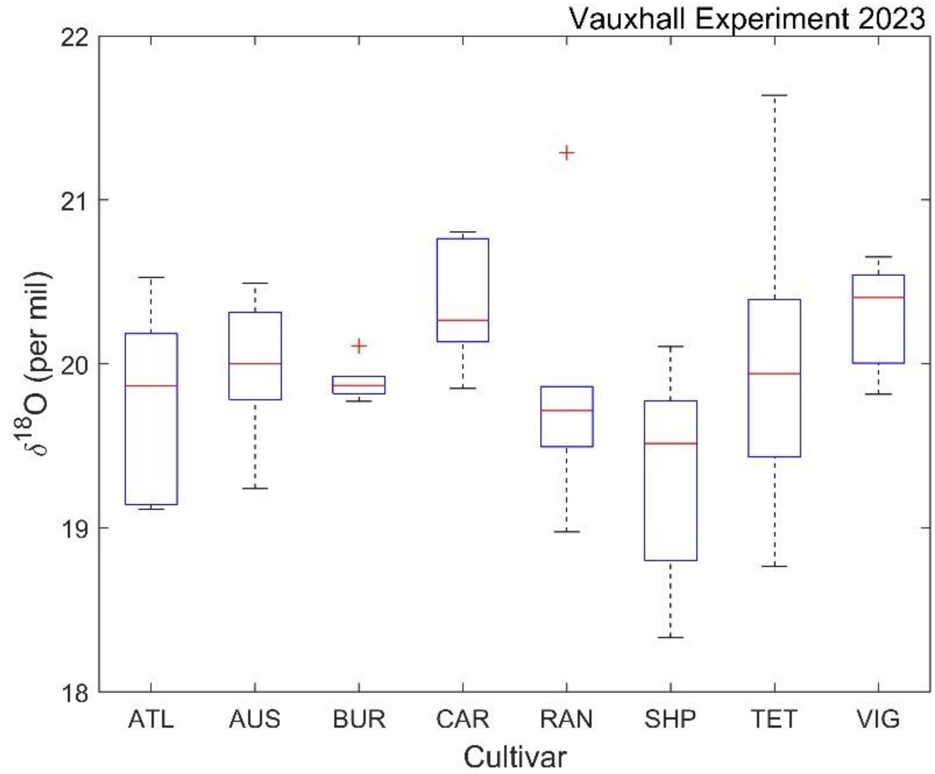

Figure 12. Comparison of leaf oxygen isotope composition ($\delta^{18}O$ values, ‰) among potato cultivars.

3.4 Variation among cultivars for leaf-level WUE

There was no significant difference among the cultivars for leaf-level WUE (Fig. 13; ANOVA, [Cultivar] F=2.05, d.f.=7, p-value=0.08). At the same time, there was no significant block effect among cultivars for leaf-level WUE (Fig. 13; ANOVA, [Block] F=1.13, d.f.=5, p-value=0.36).

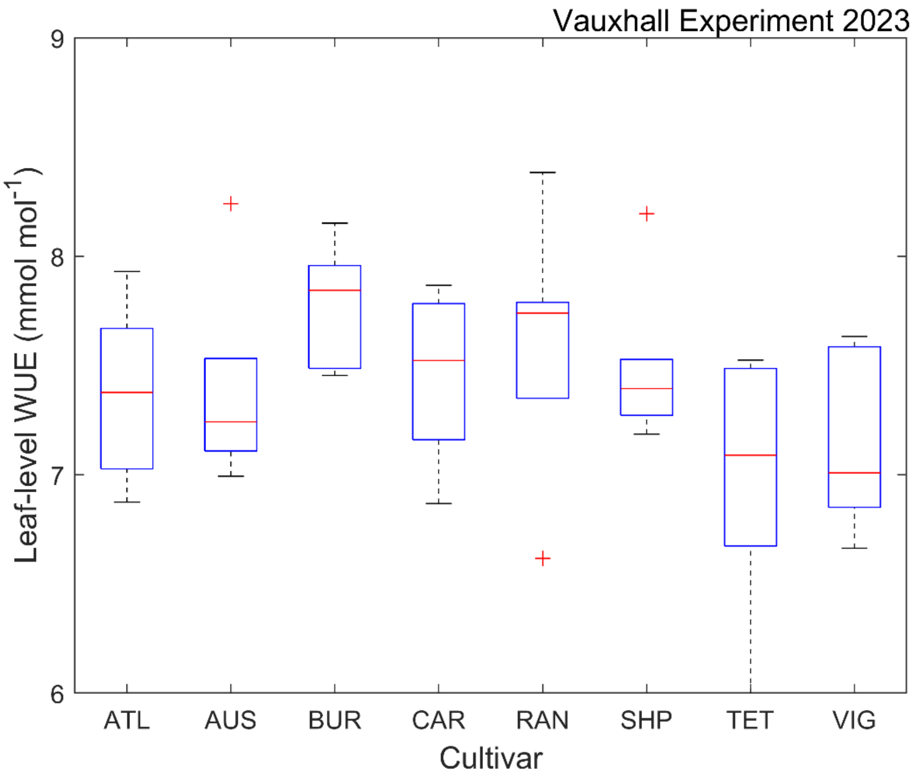

Figure 13. Comparison of leaf-level WUE (mmol mol^{-1}) among potato cultivars.

3.5 Variation among cultivars for leaf nitrogen content

There were significant differences among the cultivars for total leaf nitrogen content (Fig. 14; ANOVA, [Cultivar] F=3.55, d.f.=7, p-value < 0.05). The effect of block was not significant (Fig. 14; ANOVA, [Block] F=1.56, d.f.=5, p-value=0.20). After performing multiple comparison tests, the mean value of nitrogen content of Atlantic (ATL) and Austin (AUS) were significantly different than that of Russet Burbank (BUR), however, all remaining five studied cultivars had no significant differences in their mean value with any of the other cultivars. All cultivars had relatively high leaf nitrogen contents, with values typically ranging between 4-5% of leaf dry weight.

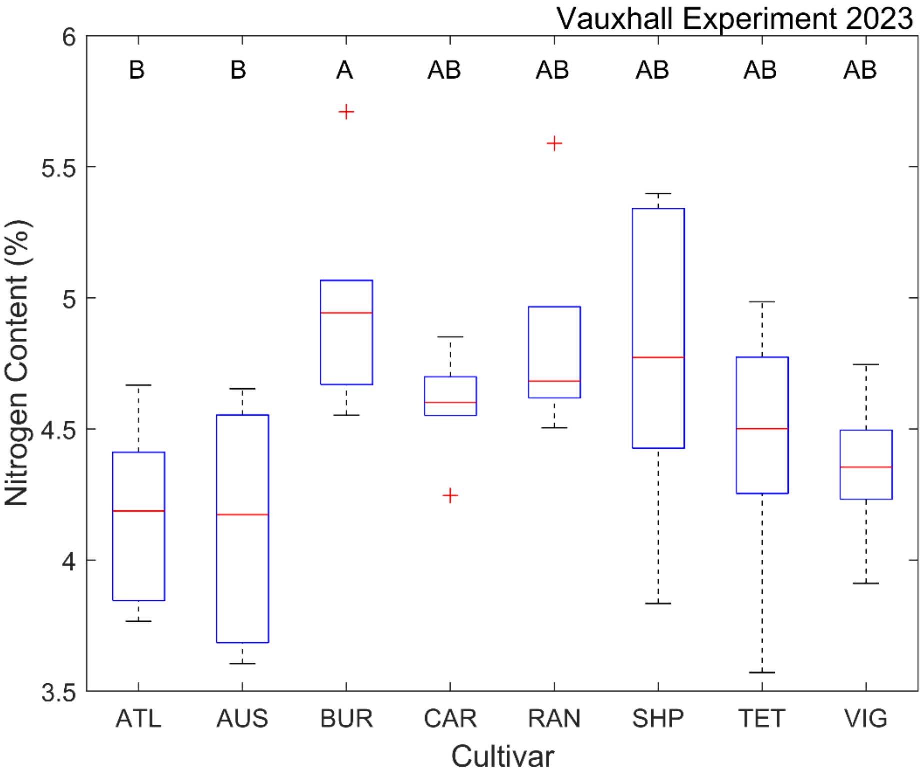

Figure 14. Comparison of leaf nitrogen content (%) among eight potato cultivars. Statistical significance, as indicated by the different letters located inside the top edge of the graph box, was based on multiple comparison tests after ANOVA test [$F_{(7,47)}$=3.55, p-value<0.05].

3.6 Variation among cultivars for aboveground biomass and tuber yield

In case of aboveground biomass, analysis of variance showed that there was a significant difference among the cultivars (Fig. 15; ANOVA, [Cultivar] F=12.04, d.f.=7, p-value<0.05), but no significant effect of block was observed (Fig. 15; ANOVA, [Block] F=0.44, d.f.=5, p-value=0.82). Based on multiple comparison tests, Atlantic (ATL), Caribou Russet (CAR) and Teton Russet (TET) were significantly different from Austin (AUS), Russet Burbank (BUR), Ranger Russet (RAN) and Shepody (SHP). AC Vigor (VIG) was significantly different than the later three, Russet Burbank (BUR), Ranger Russet (RAN) and Shepody (SHP).

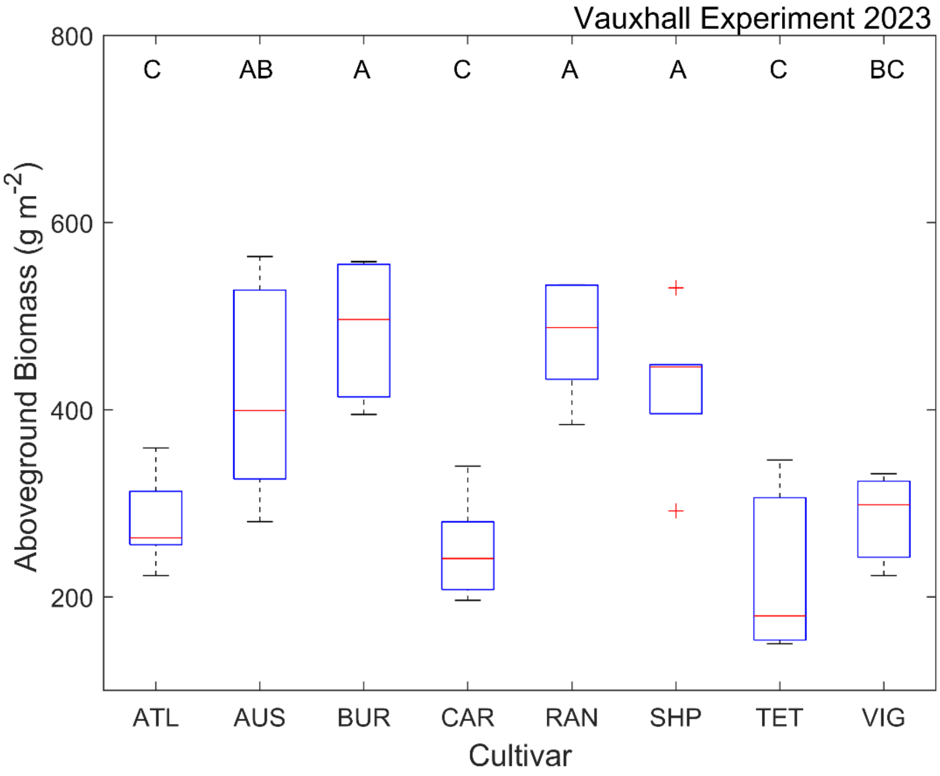

Figure 15. Comparison of aboveground biomass (g m^{-2}) among eight potato cultivars. Statistical significance, as indicated by the different letters located inside the top edge of the graph box, was based on multiple comparison tests after ANOVA test [$F_{(7, 47)}$=12.04, p-value<0.05].

There were statistically significant differences among cultivars for potato yield. Friedman non-parametric (2-way) test indicated a significant effect of cultivar (Fig. 16; Chi-square=14.28, d.f.=7, p-value=0.046) on yield, but there was no significant effect of block (Fig. 16; Chi-square=3.64, d.f.=5, p-value=0.60). When multiple comparison tests were done, only Ranger Russet (RAN) and Teton Russet (TET) were significantly different from each other, and rest of the six cultivars showed no significant differences.

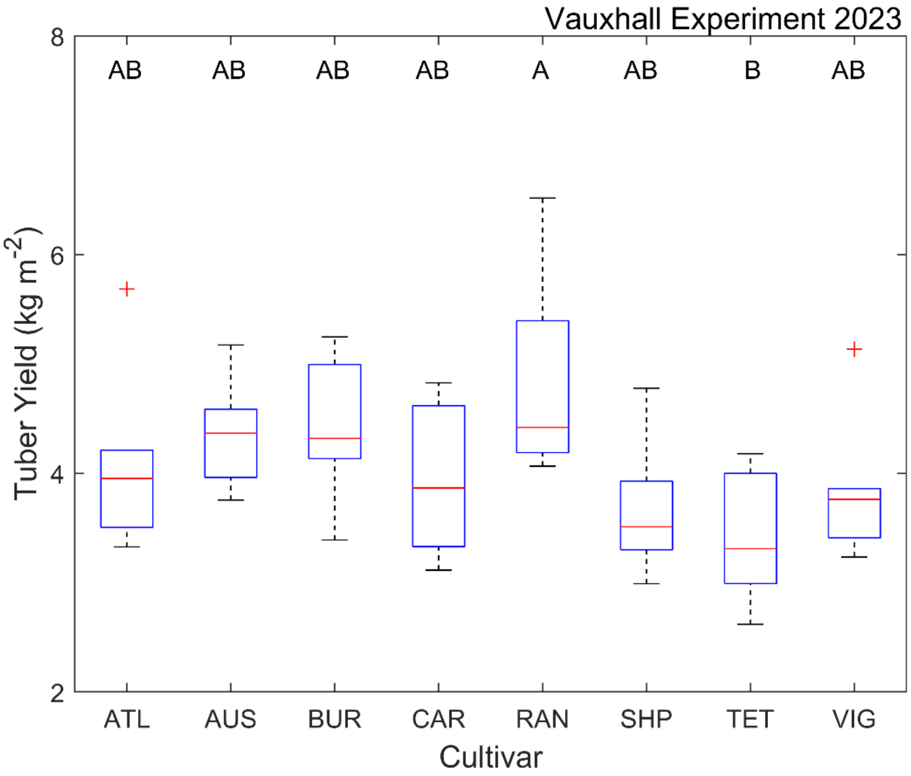

Figure 16. Comparison of tuber yield (kg m^{-2}) among eight potato cultivars. Statistical significance, as indicated by the different letters located inside the top edge of the graph box, was based on multiple comparison tests after Friedman test [Chi-square $_{(7, 47)}$ =14.28, p-value=0.046].

3.7 Variation among cultivars for cumulative plant water-use, and crop-level water-use efficiency

As there were no significant differences among the cultivars for leaf-level water-use efficiency, further calculations of integrated stomatal conductance, integrated leaf-air VPD and seasonal leaf area development were made so that cumulative seasonal water-use by potato plants could be calculated, and further calculation of crop-level WUE could be done.

Analysis of variance indicated that there were significant differences among the cultivars for cumulative seasonal water use (Fig. 17; ANOVA, [Cultivar] F=12.56, d.f.=7, p-value<0.05, [Block] F=0.28, d.f.=5, p-value=0.92).), but there was no significant effect of block for cumulative plant water use. Subsequent multiple comparison tests revealed that the water use of Atlantic (ATL), Caribou Russet (CAR) and Teton Russet (TET) were significantly different from Austin (AUS), Russet Burbank (BUR), Ranger Russet (RAN) and Shepody (SHP), whereas, AC Vigor (VIG) was different from the later three, Russet Burbank (BUR), Ranger Russet (RAN) and Shepody (SHP).

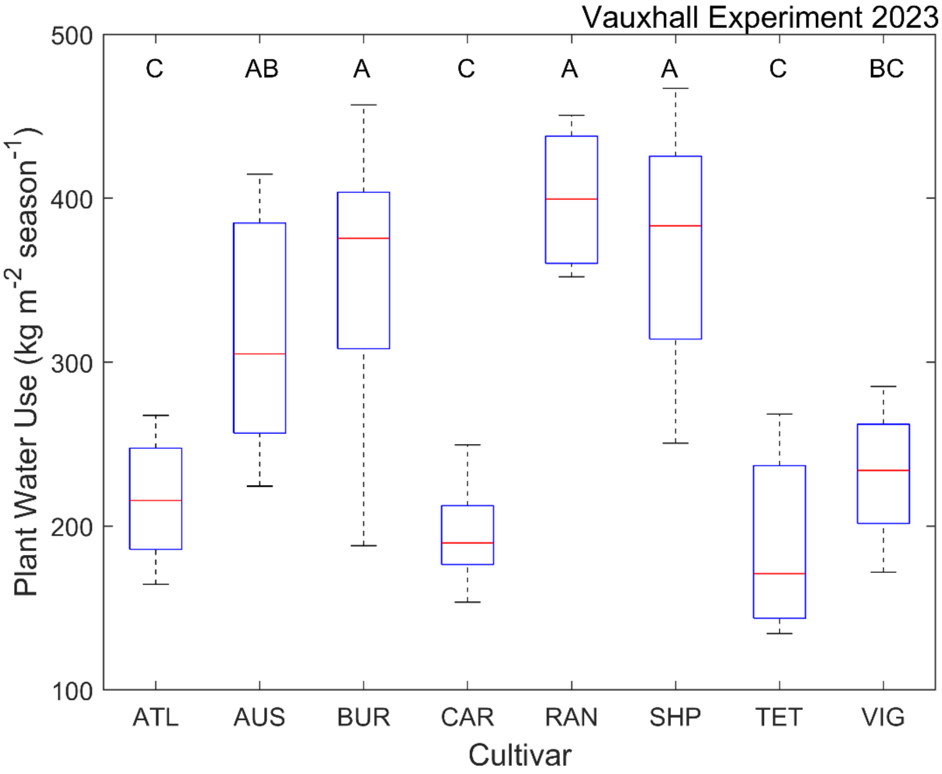

Figure 17. Comparison of plant water use among cultivars at ground level (kg m^{-2} season^{-1}). Statistical significance, as indicated by the different letters located inside the top edge of the graph box, was based on multiple comparison tests after ANOVA test [$F_{(7, 47)}$=12.56, p-value<0.05].

Analysis of variance indicated there were significant differences among the cultivars for crop-level WUE (Fig. 18; ANOVA, [Cultivar] F=7.3, d.f.=7, p-value<0.05, [Block] F=1.15, d.f.=5, p-value=0.35), but there was no significant effect of block for crop-level WUE. Subsequent multiple comparison tests revealed that crop-level WUE of Atlantic (ATL), Caribou Russet (CAR) and Teton Russet (TET) were significantly different from Russet Burbank (BUR), Ranger Russet (RAN) and Shepody (SHP).

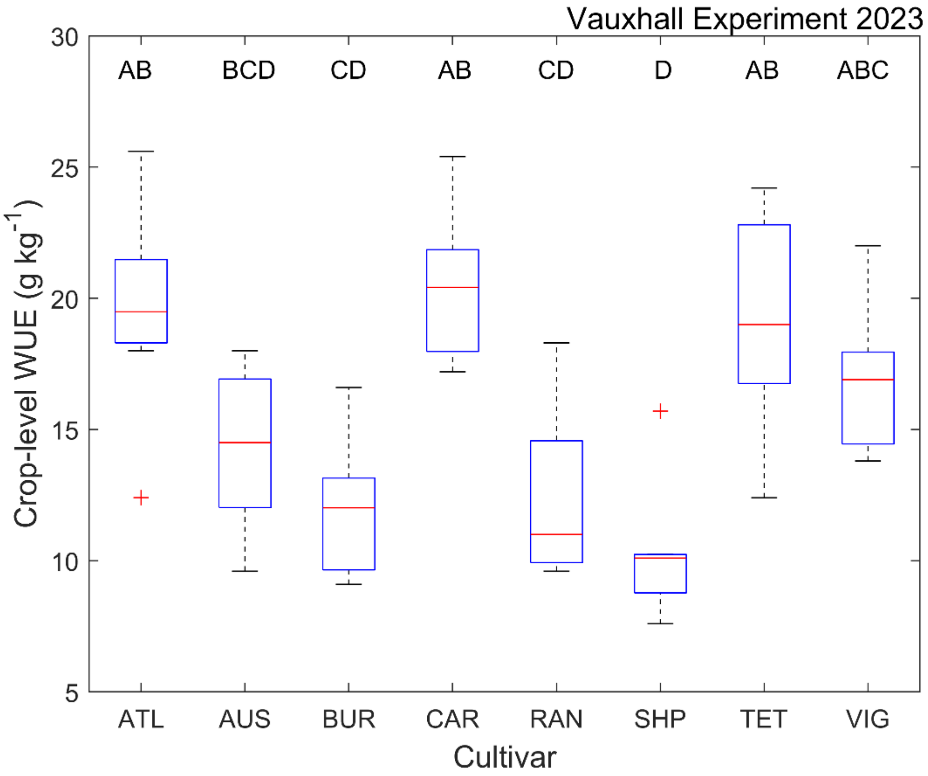

Figure 18. Comparison of crop-level WUE (g kg^{-1}) among cultivars. Statistical significance, as indicated by the different letters located inside the top edge of the graph box, was based on multiple comparison tests after ANOVA test [$F_{(7, 47)}$=7.3, p-value<0.05].

3.8 Relationship between aboveground biomass, water use, leaf-level and crop-level WUE

There was no statistically significant correlation between leaf-level and crop-level WUE (R^2=0.0342, p-value=0.21).

However, there was a strong linear relationship between above-ground biomass and total seasonal plant water use (Fig. 19; R^2=0.92, p-value<0.05).

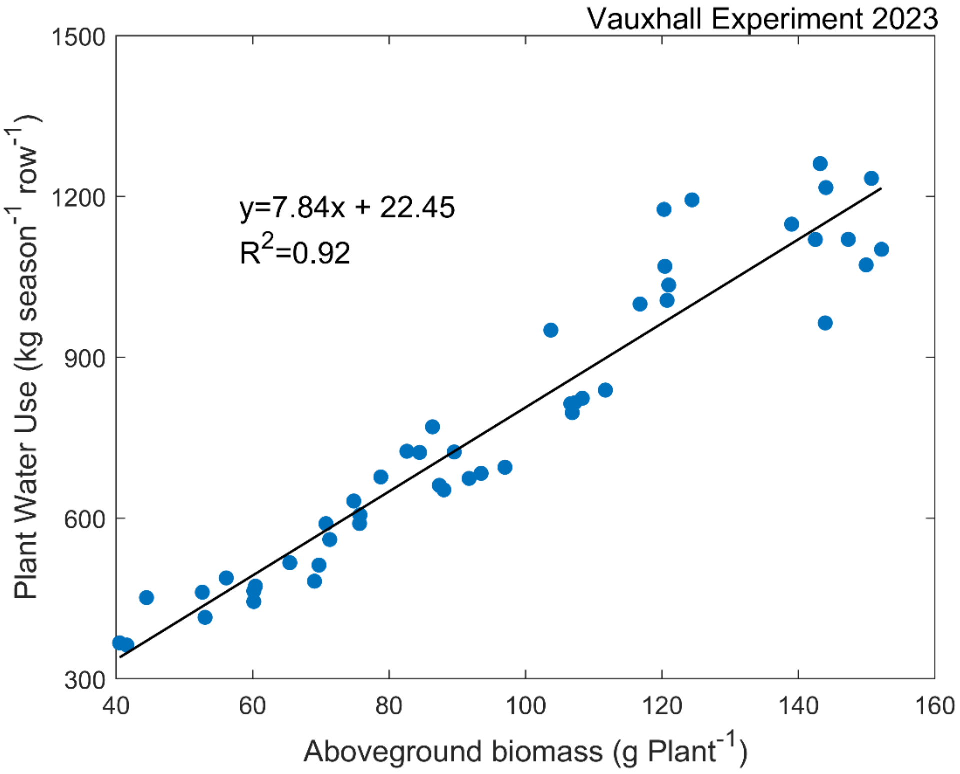

Figure 19. Relationship between aboveground biomass and cumulative plant water use.

In addition, a significant negative linear relationship was observed between seasonal cumulative water use and crop-level WUE (Fig. 20; R^2=0.72, p-value<0.05).

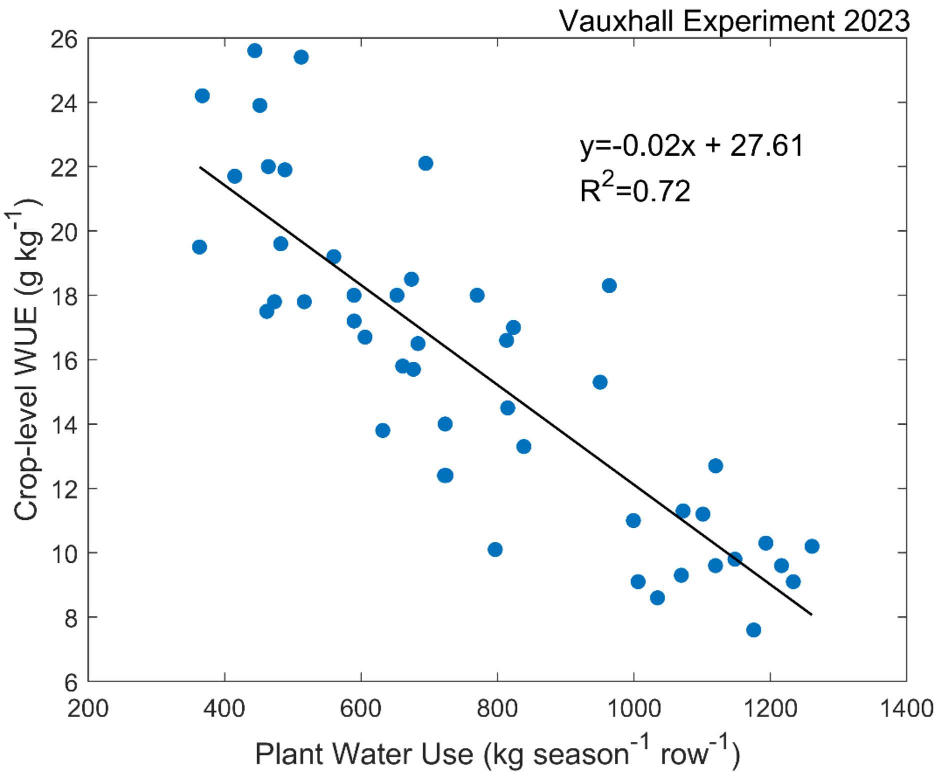

Figure 18. Relationship between cumulative seasonal plant water use and crop-level WUE.

CHAPTER 4: DISCUSSION

To provide perspective on the observed WUE values, I first discuss the environmental conditions for my experiment. The experiment was conducted in the growing season of 2023, and the year was hotter and drier than the normal (long-term average during 1961-2018, referred here as normal conditions). To supply the necessary amount of water for growth of potato plants after the tubers were planted, sufficient irrigation was done at different growth stages of potato plants. A minimal amount of water was required by the planted tubers during sprouting and emergence (Alberta Irrigation Management Manual, 2016). Hence, the total amount of water supplied through irrigation in the field was maintained at a minimum level during the month of May (approximately day 144 to day 150). Soil volumetric water content was also low during this time, and model evapotranspiration was also negligible as plants had not yet emerged, and hence there was no transpiration by the plants. The amount of irrigation water supplied to the field was gradually increased as the plants started to grow and, soil volumetric water content also started to rise from approximately day 153. Despite the low amount of water supplied to the experimental plot through precipitation and irrigation, soil volumetric water content in June was higher than the other remaining months of the growing season. This was because of lower demand of water by the potato plants during the initial vegetative growth (Alberta Irrigation Management Manual, 2016). Irrigation in the field was increased during the months of July and early August as water demand by the potato plants was highest during this time. But irrigation was decreased from mid-August because it was the time for full tuber bulking, tuber maturation and therefore the demand of water by the plants had declined (Alberta Irrigation Management Manual, 2016). Modelled ET showed steady increase only after day 180 (approximately) as plant leaf area developed and initially soil moisture declined in association with increases in plant leaf area. However, irrigation was provided to maintain soil moisture constant throughout the later portion of the growing season.

Heat is one of the very important factors for good growth and high productivity of potatoes with the minimum temperature essential for potato growth and development being 7 ºC (Praharaj et al., 2020). Based on the harvest date of the potatoes, it was found that Potato Heat Units had accumulated up to about 800 at the harvest date. However, it has been found in other studies that number of accumulated P-days differ among years and time of planting. For example, in one study by Miller et al. (2002), two cultivars (Russet Burbank and Ranger Russet) planted at three dates in a year were studied for three consecutive years and harvested towards the end of September. It was found that P-days ranged from 669 to 1135 with the lower P-day values being for the potatoes planted in June and highest for the ones planted in April. Models can be developed using P-days to predict the canopy cover in the potatoes (Connell et al., 1999). In my study, maximum range of canopy cover was observed between approximately day 209 to day 233 when the observed P-days values were between 540 to 712, whereas the highest canopy cover was observed on approximately day 221 with the accumulated P-days value of about 630.

Eight potato cultivars were planted in a systematic way to study WUE at leaf-level and crop-level, and the differences among them were studied and analyzed. I have further discussed the findings associated with leaf-level and crop-level WUE as follows:

4.1 From the study of isotopes and leaf-level WUE

Leaf-level WUE is determined by c_i/c_a (equation 4) and w_a/w_i (equation 5) ratio, and these two values can be obtained by the measurements of $\delta^{13}C_m$ and $\delta^{18}O_m$ in leaf tissue, respectively. The measurement of carbon isotope composition ($\delta^{13}C_m$) of C_3 leaf tissue help in studying and understanding differences in leaf physiological characteristics like changes in the ratio of net photosynthesis and stomatal conductance which are the major mechanisms contributing to WUE (Farquhar et al., 1988; Farquhar et al., 1989; Condon, 2020). Oxygen isotope composition ($\delta^{18}O_m$) of plant organic matter should reflect variation in the leaf-air vapour pressure gradient and changes in stomatal conductance (Farquhar et al., 1988; Barbour, 2007). Therefore, powerful information on the physiological and environmental factors which control leaf-level WUE can be gained through simultaneous measurements of $\delta^{13}C_m$ and $\delta^{18}O_m$ (Flanagan and Farquhar, 2014).

There were significant differences in $\delta^{13}C_m$ among the eight potato cultivars used in my study. In one study by Jefferies et al. (1997), there were differences in the values of carbon isotope discrimination ($\Delta^{13}C$) with the values ranging from approximately 17‰ to 22‰ in potato leaflets depending on the cultivars and water treatments (droughted or irrigated treatments), collected at different occasions starting from day 21 to 105 after 50% of plant emergence. Under irrigation, $\Delta^{13}C$ in leaflets increased significantly in leaflets as they matured and this was attributed to increase in stomatal conductance during leaf expansion whereas the reverse was the case under drought, with narrow range of $\Delta^{13}C$. These differences in $\Delta^{13}C$ suggest that there were differences in the values of $\delta^{13}C_m$ among the cultivars. The values of carbon isotope discrimination were low when leaflets were immature and young, and the values slowly started to increase with each harvest. Thus, differences in $\delta^{13}C_m$ values in my study might also be because of the differences in time periods when the leaflet samples were collected and several other factors which affected seasonal integration of $\delta^{13}C_m$ including amount of water supply in the field, stomatal conductance, plant genotypes, etc.

The range of variation of $\delta^{13}C_m$ values in my study was -27.25 to -25.32‰ which was lower than the observed $\delta^{13}C_m$ values seen in potato in previous studies by Kaminski et al. (2015), where the values of $\delta^{13}C$ ranged from -34.4 to -30.12‰. Apart from the changes due to plant's maturity, several factors like differences in the amount of water supplied to the plants during crop growth and variations in stomatal conductance could also be the reasons for such low values of $\delta^{13}C_m$ (Jefferies et al., 1997). Also, the genotypic differences among the plant cultivars might be responsible for these differences (Basu et al., 2021). Unlike my study where the cultivars were grown in field, the potato cultivars in the study of Kaminski et al. (2015) were grown in pots and in controlled environment (semi field condition in first year of study and in greenhouse in the second year), which also could have contributed to the large differences in $\delta^{13}C_m$ values between two studies.

The subtle differences in leaf physiological characteristics like changes in the ratio of net photosynthesis and stomatal conductance result in differences in the concentration of CO_2 in the leaf intercellular air spaces and in the chloroplast, which are represented by the differences in $\delta^{13}C_m$ among the potato cultivars (Farquhar et al., 1989; Flanagan and Farquhar, 2014). Further, the basis of the biochemical discrimination against ^{13}C in C_3 plants lies with the primary carboxylating enzyme, ribulose-1,5-bisphosphate carboxylase (Park and Epstein, 1960; Farquhar and Berry, 1982), which discriminates against ^{13}C because of the intrinsically lower reactivity of ^{13}C (Melander and Saunders, 1979; Farquhar et al., 1982). As the values of leaf $\delta^{13}C_m$ differ significantly among the plant species under a given set of environmental conditions, different plant developmental stages (Körner et al., 1986; Song et al., 2008) and plant genetic characteristics (Körner et al., 1988; Song et al., 2008), the variation in $\delta^{13}C_m$ among different cultivars of potato plants studied in this experiment can be attributed to the genetic differences among them because the experimental plot is of small scale with no differences between the blocks and therefore, the variations among the cultivars were not expected due to different environmental forces like precipitation, temperature, soil water content etc. (Song et al., 2008). Further, in my study, the leaves were collected at the same developmental stage, therefore, there are possibilities for some alterations in the values of isotopic measurements as these values get integrated over the period of development of the leaflets.

There was no significant variation in $\delta^{18}O_m$ observed among the eight cultivars in my study. Apart from variation in the leaf-air VPD and changes in stomatal conductance, other factors that affect the enrichment of $\delta^{18}O$ in leaf water during transpiration and variation in the $\delta^{18}O$ of source soil water taken up by plants can also affect $\delta^{18}O_m$ values in leaf tissue (Barbour, 2007; Flanagan and Farquhar, 2014).

The average values of leaf-level WUE in my study were found to be in range of 6.95 to 7.79 mmol mol^{-1}. In the study done by Vos and Groenwold (1989), there were significant differences in carbon isotope fractionation and WUE was positively related to it. Farquhar and Richard (1984) had studied carbon isotope composition and WUE in different wheat genotypes and found that water-use efficiencies ranged from 2.0 to 3.7 mmol mol^{-1} in a winter experiment and associated $\delta^{13}C_m$ composition was about -20.45‰. Similarly, when studied, isotopic WUE in different vegetations in northern Great Plains grassland was found at the range of 7.56 to 8.87 mmol mol^{-1}, when the range of $\delta^{13}C_m$ was -28.1 to -26.4‰ (Flanagan and Farquhar, 2014). Hence, different results in different experiments denote the genetic variability among them, and at the same time, gives an idea of genetic manipulation of the cultivars so that more water use efficient varieties can be developed.

Only a few studies of crop plant species have used both $\delta^{13}C_m$ and $\delta^{18}O_m$ for leaf-level WUE studies. The nature of relationships between $\delta^{13}C_m$ and $\delta^{18}O_m$ can be used to identify if the differences in $\delta^{13}C_m$ are primarily because of stomatal conductance or by photosynthetic capacity (Flanagan and Farquhar (2014). Positive correlation denotes that stomatal conductance is the primary cause of variation in $\delta^{13}C_m$ (Farquhar et al.,1998; Barbour and Farquhar, 2000; Barbour et al., 2000; Scheidegger et al., 2000; Flanagan and Farquhar, 2014), whereas negative or no correlation means photosynthetic capacity is responsible for the variation in $\delta^{13}C_m$ (Barbour, 2007). In the study conducted by Flanagan and Farquhar (2014) on vegetation of northern Great Plains grassland, significant positive correlation was observed between $\delta^{13}C_m$ and $\delta^{18}O_m$ over a course of time which indicated that variation in $\delta^{13}C_m$ was due to stomatal conductance and water stress-induced changes in the degree of stomatal limitation of net photosynthesis (Barbour, 2007; Farquhar et al., 2007). Similarly, in another study, there was strong significant positive correlation between $\delta^{13}C_m$ and $\delta^{18}O_m$ of the biomass from the field grown wheat cultivars in Mexico, and it was a result of variation in stomatal conductance (Barbour et al., 2000). To the contrary, the finding from the study of Brooks and Mitchell (2011) revealed that the higher photosynthetic capacity was responsible for the increase in A/g_s and growth in fertilized field plots of Douglas-Fir. Hence, the combined measurement of both ^{13}C and ^{18}O at the plant matter level may provide a time-integrated record of the photosynthetic and evaporative performance of the plant during crop growth (Cabrera-Bosquetz et al., 2009).

Many studies in important crop species have shown differences in leaf WUE based on $\delta^{13}C_m$. $\delta^{13}C_m$ is used as a proxy to WUE because it provides useful information on the integrated carbon and water balance of C_3 plants over longer period (Francey and Farquhar, 1982; Song et al., 2008). However, in my study, the differences in $\delta^{13}C_m$ among the cultivars were offset by the slight variation in $\delta^{18}O_m$, so there were no significant differences in leaf-WUE among the cultivars.

4.2 Aboveground biomass, water use and tuber yield for calculation of crop-level WUE

Crop-level WUE requires tuber yield and measurements or estimates of total cumulative potato water use by plants over the growing season.

In this study, there were significant effects of the cultivars for tuber yield. However, only Ranger Russet and Teton Russet were significantly different from each other, the difference being relatively small, and rest of the six cultivars showed no significant differences. Both cultivars were the French fry cultivars, and Ranger Russet has been identified as mid-season cultivar.

In my study, all the cultivars were harvested together even if some cultivars were late maturing (Russet Burbank and AC Vigor) and had indeterminate growth type (Russet

Burbank). There are possibilities that tuber yield could be higher in the ones which were late season and had indeterminate growth types if they had been kept in field for longer period. In a study conducted by Worthington and Hutchinson (2006) between two cultivars, one being Atlantic which was studied in my research as well, planting dates greatly determined the tuber yield. Therefore, the potato growers also must be mindful of planting and harvest dates as too early and too late can result in lower tuber yield due to several factors like water availability, temperature etc. (Worthington and Hutchinson, 2006).

In a study conducted by Deguchi et al. (2010), potato cultivars named Konyu and Konafubuki were studied for yield potential and aboveground characteristics. The values of aboveground biomass under irrigated treatments ranged from 435 to 845 g m^{-2}. Two cultivars which had lowest AGB were the ones with highest yield. The results indicated that the cultivars could have high harvest index due to lower dry weight partitioned to the shoots (Deguchi et al., 2010). Spitters (1987) also indicated that tuber yield was determined by the fraction of total biomass that was partitioned to the tuber. A different pattern was observed in my study where eight cultivars had very similar tuber yield despite large differences in AGB.

Biomass partitioning is very important in crop production (Shrivastava and Gaiser, 2008) because the farmers/producers/food companies are focused on the maximization of the crop component which has economic significance. In other words, improvement in crop yield demands higher harvest indices for the crops rather than total dry matter or biomass production (Shrivastava and Gaiser, 2008).

The total biomass production also directly depends upon the amount of light intercepted by the foliage (Cadersa and Govinden, 2001). The total amount of photosynthates available was directly proportional to the photosynthetic rate of the crop and its leaf area index (LAI) (Meyer and Green, 1980; Geremew et al., 2007), which was directly proportional to the canopy cover because greater canopy cover enables crops to intercept adequate photosynthetically active radiation (PAR), and leads to higher biomass production and accumulation (Spitters, 1987; Vos and Groenwold, 1989; Van Delden, 2001).

In case of potatoes, one of the major factors determining biomass production and its allocation to the sink, i.e. the tuber, is the length of the growth period, which varies greatly with the potato cultivars. Late cultivars can intercept large quantities of light while early cultivars are more efficient in using resources (Haverkort and Struik, 2015). Many researchers indicated that the allocation of assimilates within the plant may not be proportional among potato cultivars (Haverkort and Harris, 1987; Deblonde and Ledent, 2000). One of the reasons for such condition could be attributed to high temperatures. Some cultivars favour allocation of biomass to the foliage at the cost of tuber growth when temperature goes beyond 23°C (Haverkort and Harris, 1987; Jenkins and Mahmood, 2003). Also, the differences in assimilate allocation in potato is related to the growth pattern and maturity among cultivars, early or late maturing because differences in life span of the crop in the field allow them to have extended time to produce and allocate more dry matter/biomass to the sink (Van Heemst, 1986; Spitters, 1987). Drought and high temperature affect leaf area development and its persistence, and these in turn limit the photosynthetic activity of the crop and finally dry matter production and allocation. At the same time, rate of water loss/rate of water use will also be different. The growth rate of a potato crop that was well supplied with water and nutrients and free from pests and diseases was about proportional to its light absorption (Spitters, 1987; Van Delden, 2001). Thus, climate, cultivar, and crop management practices determine the growth and biomass production of a potato (*Solanum tuberosum*) crop (Geremew et al., 2007), and assimilate allocation was also the combined result of plant genotype, climate, growth and development which need to be studied all together for better understanding of the real mechanisms associated with water use efficiency at different levels (Meyling and Bodlaender, 1981).

It is expected that there should be large differences in plant cumulative water use among the cultivars in this study because plant cumulative water use will depend on leaf-level water use and the total leaf area of plants. While there were no differences in leaf water use, I did observe large (2-fold) variation in aboveground biomass, which implied large differences in total leaf area per plant among the cultivars, and similar results were obtained in the study of Liu et al. (2006), where the cultivar named Folva had highest total biomass along with the highest total leaf area per plant when fully irrigated, and the highest ET as well. Thus, the findings from my study implied that there were differences in total plant water use among the cultivars despite similar leaf-level water use. This type of relationship was also observed in the plant varieties used in a study of Mueller et al. (2005) where they had compared plants varieties including wheat, barley and maize, water-use measured as ET and aboveground biomass were found to have a positive correlation, however this relationship was entirely crop-specific. However, higher or lower aboveground biomass has implications for nutrient use in potatoes as well as other plant species. There are costs associated with higher biomass because larger plants require more resources including nutrients for maintenance and successful reproduction, and thus even temporary resource shortages may risk the existence of the plants and increase their mortality if sufficient resources cannot be stored (Falster et al., 2008; Goldberg et al., 2017).

4.3 Estimation of magnitude of differences in plant water use among the cultivars for calculation of crop-level WUE

I conducted a series of calculations to estimate the magnitude of differences in plant water use among the cultivars. Leaf-level water use in each cultivar was estimated from the transpiration rate of the sampled potato leaflets, which was based on $\delta^{13}C$ and $\delta^{18}O$, from which the respective values of g_w and VPD were determined using equation 2. These procedures were based on well established relationships from ecophysical theory.

Whole plant-level transpiration was then calculated by making estimates of the amount of transpiring leaf area per unit AGB so that total plot ET was consistent with modelled ET calculated based on Penman Monteith equation and meteorological data. These calculations indicated that cultivars with low AGB transpired less water and had higher crop-level WUE than the cultivars with higher AGB.

The significantly different cultivars in terms of cumulative seasonal water use (Fig. 17), had the values ranging from 188 to 400 kg m^{-2} $season^{-1}$. These differences in cumulative seasonal water use were correlated with the above-ground plant biomass as demonstrated by the strong linear relationship between the two (Fig. 19). To supply adequate amounts of water for all the growing stages for better tuber yield (Condon et al., 2002), supplemental water through irrigation must be supplied in regions like southern Alberta where precipitation is easily exceeded by evapotranspiration. Supplemental water supplied to the field to adjust the water loss due to evapotranspiration has been found to increase total yield of potatoes as presented by the study of Bélanger et al. (2000), and the study was conducted in New Brunswick where irrigation increased the total yield from 3.19 to 3.84 kg m^{-2} in two cultivars, namely Russet Burbank and Shepody, which were used in my study as well.

4.4 Crop-level WUE and its scope of improvement

In my study, the values of crop-level WUE ranged from an average of 10.3 to 20.4 g kg^{-1}, and Atlantic, Caribou Russet and Teton Russet were the most efficient varieties. These were the cultivars having low aboveground biomass and correspondingly low seasonal cumulative water use. A significant negative linear relationship was observed between seasonal cumulative water use and crop-level WUE (Fig. 20), which validates the relationship between increased crop-level WUE being associated with decreased water use, when yield was nearly constant for all the eight cultivars in my study.

From the study of 60 peer-reviewed ISI journals, Mbava et al. (2020) had deduced that different crops varied in terms of WUE at crop-level on different grounds like crop-type (C_3 vs. C_4 plants), growing season (summer vs. winter plants), climate class (desert/tropical/tropical/temperate), etc. Similarly, many of the summer grain crops were more water use efficient than the winter crops as summer season is characterized by warm temperature, soil moisture and long sunshine hours (Mbava et al., 2020).

Unlike the findings of my study, the study on ten hard red winter wheat genotypes in the Texas A&M AgriLife Research Station at Bushland, Texas revealed that there were no significant differences in wheat genotypes for crop-level WUE even if they were significantly different among each other in terms of yield (Xue et al., 2013). However, no significant differences were found in terms of evapotranspiration. The significant differences in their yield could be attributed to the varied amount of water supplied to the field, newer cultivars and genotypes with higher drought tolerance and genetic improvement. Differences in yield had been neutralized by the differences in the transpiration rates of the wheat varieties/genotypes as the genotypes with higher biomass might have higher transpiration and lower soil evaporation than those with lower biomass, even if there were no visible differences among them in terms of overall ET (Xue et al., 2013).

Research has shown that deficit irrigation, an optimization strategy in which irrigation is applied during drought-sensitive growth stages of a crop, and is limited outside these periods, or even is unnecessary if a minimum supply of water is provided by rainfall (Geerts et al., 2009), could help to improve both yield and crop-level WUE (Gebremariam et al., 2018). It has also been found that the growers/farmers could apply only 70-80% of crop water requirement/crop evapotranspiration to improve yield and WUE (Gebremariam et al., 2018). As discussed before, in the potato cultivar named Folva in the study of Liu et al. (2006), deficit irrigation had led to increased WUE at crop-level and it was greater than the WUE attained by the plants when fully irrigated in the study. At deficit irrigation, the amount of biomass partitioned to aboveground plant parts would be lower than root and tubers, as denoted by lower biomass and lower leaf area, whereas leaf area and aboveground biomass were reduced leading to lowered stomatal conductance and transpiration which could be the possible reason for enhanced WUE at crop-level (Liu et al., 2006). However, one should be mindful of probable poor tuber quality or reduced yield in some cultivars due to deficit irrigation as previous research showed that these qualities can get affected by water deficit even when briefly applied (Jefferies and MacKerron, 1993; Costa et al., 2007).

In the present-day context, there is a pressing need to develop and promote more water-use efficient crop varieties instead of higher water demanding varieties. In case of potatoes, the farmer/producer/food companies are focused on the tuber production rather than the above vegetative structures, therefore, the findings from this study can help to identify an effective way to select the varieties with higher crop-level WUE, as crops with lower aboveground biomass and subsequently lower water use and higher yield is what the present need is. Apart from this, there are other several ways for the improvement of water-use efficiency at crop-level, though they have some associated limitation in them. First one is cultivar selection for improved photosynthetic efficiencies or altered composition of plants biochemical components, however, the results might not be always as intended, and the alterations might lead to disappearance of some of the very essential characters from the plants like decreased oil and protein content as in the case of the seed of soyabean (Sinclair et al., 1984). Second way is the change in stomatal physiology which can also lead to improved WUE at crop-level (Sinclair et al., 1984; Franks et al., 2015). For evading mid-day water stress due to high VPD, mid-day stomatal closure in some plants is really advantageous, but there is lessened CO_2 assimilation, and thus there is potential reduction in crop yield (Sinclair et al., 1984). Improvement in harvest index is another direct approach to increase crop-level WUE (Sinclair et al., 1984; Morison et al., 2008), and one example to this is growing crop cultivars with short growth season so that they have high harvest index with their life cycles completed early before the available water is exhausted (Sinclair, 2018). Similarly, the growth conditions for the crop varieties could be altered to increase crop-level WUE like limiting VPD to minimum, or by shifting the period of plantation of the crops when VPD will be low, i.e. in cooler periods of year (Sinclair et al., 1984). However, the associated limitations would be special morphological or biochemical alterations are required that consume plant energy and/or lower harvest index potential. Also, shifting the growing period may not be feasible where the growing season is short and barely long enough for one crop as in case of Alberta. Further, minimization of surface runoff, soil evaporation, and deep percolation can help to allow more of the input water to be available to the plants for transpiration which will improve crop-level WUE (Sinclair et al., 1984; Morison et al., 2008). However, the associated limitations would be the exhaustion of soil water rapidly which would lower the harvest index (Sinclair et al., 1984).

CHAPTER 5: CONCLUSIONS

In this study, eight different potato cultivars grown in an experimental field plot, set up at the Agriculture and Agri-Food Canada (AAFC) Vauxhall experiment station in Vauxhall, Alberta, Canada were studied, and compared for their differences in leaf-level and crop-level WUE and cumulative seasonal water use calculated from measurement of stable carbon and oxygen isotopes, and the nitrogen content of the plant leaf tissue. Despite the significant differences in stomatal density, nitrogen content and $\delta^{13}C$, there were no significant differences in leaf-level WUE. However, in crops like potatoes, agriculturist/ farmers need to focus more on the maximization of tuber yield. Even if any of the potato cultivars were more water efficient at leaf-level, the plants might have to compensate through reduced tuber yield which is not preferred because reduced transpiration at leaf-level may also limit CO_2 assimilation and growth.

I further calculated crop-level WUE. Aboveground plant biomass and tuber weight were also determined from the plants grown during May-August of 2023 which were essential for the calculation of crop-level WUE. Aboveground plant biomass showed a strong linear relationship with cumulative seasonal plant water use. This is because of the increase in transpiration from the leaf surfaces: more aboveground biomass means more leaves and greater surfaces for loss of water through the process of transpiration. When measurements of tuber weight were done, the values were nearly equal for all the cultivars, except for Ranger Russet and Teton Russet, though that difference was very small, and quite large differences were observed in terms of seasonal water use due to significant differences in aboveground biomass. There were significant differences among cultivars for crop-level WUE, Atlantic, Caribou Russet and Teton Russet being the most efficient ones. Shepody, Ranger Russet and Russet Burbank were the ones with lower water-use efficiency. Hence, I conclude that partitioning of biomass to leaves varied among the studied potato cultivars which resulted in differences in total water-use and crop-level WUE as the tuber yield was very similar.

Globally, population growth and urbanization are resulting in higher demand of potato (Devaux et al., 2021). Potato is consumed in a number of ways by a large share of population (Hobbs and Kerr, 1997; Yari et al., 2021). Southern Alberta is a region of high potato harvest (Larney et al., 2016). Therefore, in the present context, if specific potato cultivars could be chosen such that, have minimum partitioning of biomass to the shoot, without compromising potato yield, that could be a sustainable approach to improve crop water-use efficiency. This study helps in identifying the varieties having low aboveground biomass which will reduce transpiration. Approximately 90% of water in plants is dissipated through transpiration (Liu et al., 2018), and water demand by the cultivars having reduced aboveground biomass will be low. At the same time, tuber yield was not apparently affected by lower aboveground biomass in this study. Hence, when choosing among the eight cultivars used in this study, the best choice will be the cultivars with lower above-ground plant biomass.

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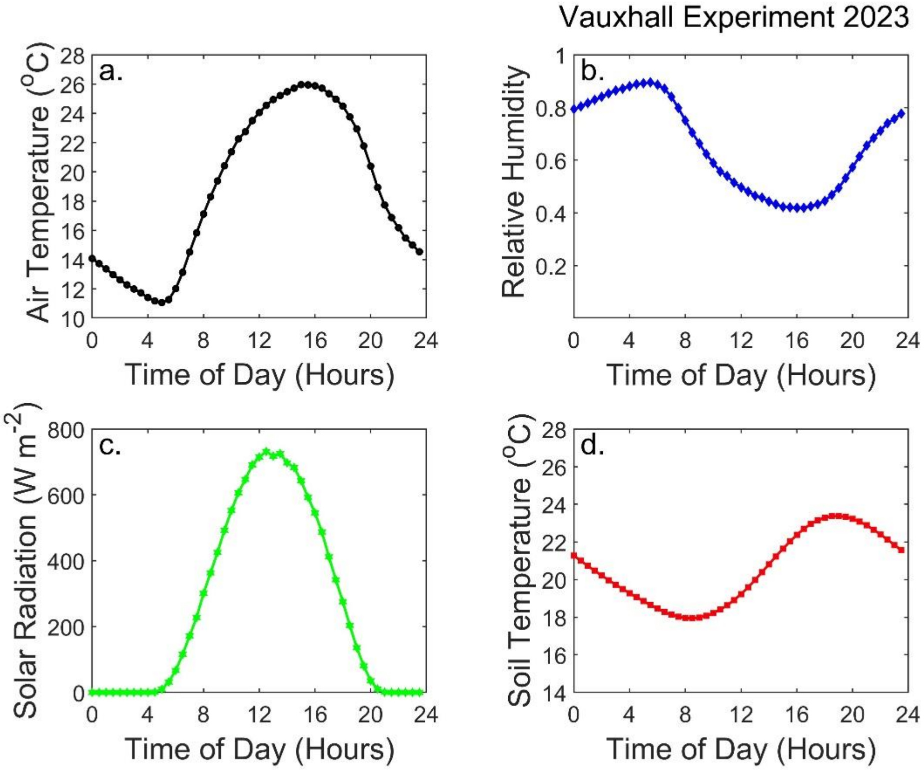
APPENDICES

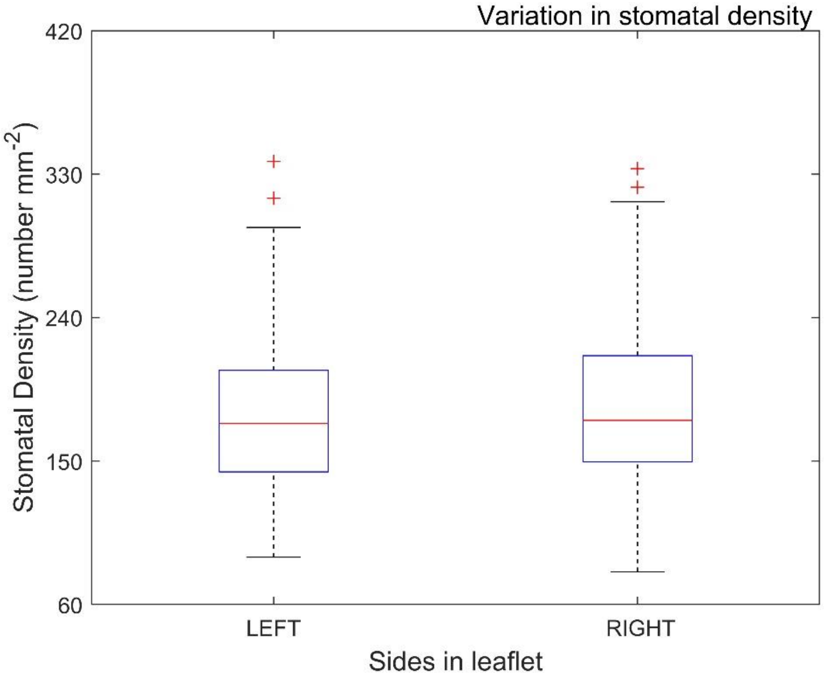
Appendix I



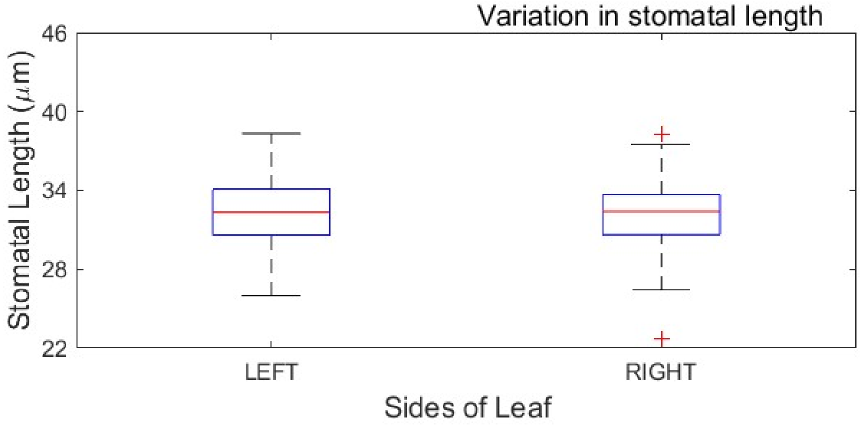
Mean diurnal patterns of variation in data collected between May 24 and August 29, 2023 at Vauxhall Research Station, Alberta. a Air temperature (at 2 m above ground); b Relative humidity (at 2 m above ground); c Incoming solar radiation; and d Soil temperature (20 cm depth, center of hill).

Appendix II

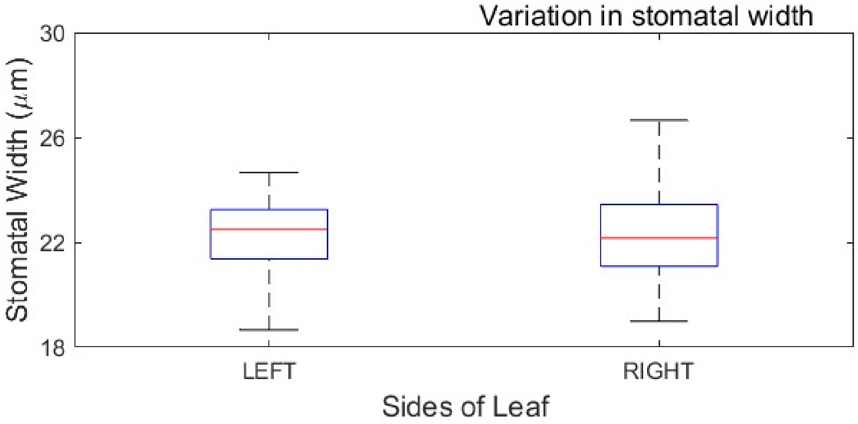
Comparison of stomatal density, stomatal length and stomatal width between two sides in potato leaflet was done in order to develop the sampling protocol for stomatal study, however no significant differences in stomatal density (Kruskal Wallis, F=0.94, d.f.=1, p-value=0.33), stomatal length (Kruskal Wallis, F=0.03, d.f.=1, p-value=0.87) and stomatal width (Kruskal Wallis, F=0.23, d.f.=1, p-value=0.63) were observed in between two sides.



Comparison of stomatal density (number mm^{-2}) between two sides of the leaflet experimented as background study.



Comparison of stomatal length between two sides of the leaflet experimented as background study.



Comparison of stomatal width between two sides of the leaflet experimented as background study.