Mapping of Quantitative Trait Loci (QTL) in *Brassica napus* L. for Tolerance to Water Stress

by

Bogaha Waththage Kaushika Samadhi Buddhini Jayarathna

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LIST OF ABBREVIATIONS

QTL – Quantitative trait loci

NADPH - Nicotinamide adenine dinucleotide phosphate

NCED - 9-cis-epoxy carotenoid dioxygenase

SNP – Single nucleotide polymorphism

DNA – Deoxyribonucleic acid

PEG – Polyethylene glycol

BLUPs - Best linear unbiased predictors

ABSTRACT

Jayarathna, Bogaha Waththage Kaushika Samadhi Buddhini. M.Sc., The University of Manitoba, September 2023. **Mapping of Quantitative Trait Loci (QTL) in *Brassica napus* L. for Tolerance to Water Stress**. Supervisors: Dr. Claudio Stasolla and Dr. Rob Duncan

Brassica napus L. plants are sensitive to water stress throughout their life cycle from seed germination to seed setting. This study aims to identify quantitative trait loci (QTL) linked to *B. napus* tolerance to water stress mimicked by application of 10 % polyethylene glycol-6000 (PEG-6000). Two doubled haploid populations (1901 and 1904), each consisting of 150 genotypes, were used for this research. Plants at the two-true leaf stage of development were grown in the absence (control) or presence (water stress) of PEG-6000 under controlled environmental conditions for 48 h, and the drought stress index (DSI) was calculated for the fresh weight and dry weight of whole plant, root, and shoot of each genotype. The 300 genotypes along with their parents were genotyped using the Brassica Infinium 90K SNP BeadChip Array. Inclusive Composite Interval Mapping was used to identify and confirm QTL. Eighteen QTL associated with water stress tolerance were identified across six chromosomes (A2, A3, A4, A9, C3, and C7). Collectively, 2,154 putative candidate genes for drought stress tolerance in *B. napus* were identified for all the identified QTL. Among them, 213 genes were chosen as directly associated with drought stress tolerance based on nine functional annotations. These results can be incorporated into future breeding initiatives to select plant material with the ability to effectively cope with water stress.

FOREWORD

This thesis adheres to the manuscript format specified by the Department of Plant Science and the Faculty of Graduate Studies at the University of Manitoba. The structure of the manuscript aligns with the guidelines outlined in the journal Genome. This thesis is presented as a single manuscript and contains an abstract, introduction, materials and methods, results, discussion, and conclusion. In lieu of the manuscript's introduction, a comprehensive literature review addresses the broader context of the research inquiry. Additionally, supplementary tables are appended following the main manuscript body.

1.0 LITERATURE REVIEW

1.1 Introduction

1.1.1 Effects of Water Stress on Agriculture

Modern agriculture faces numerous challenges (Gliessman,, 2020; Pereira, 2005; Sumberg & Giller, 2022). Among these, there is a significant demand to considerably enhance crop production to fulfill the requirements of the expanding global population. The effects of climate change and rapid changes in environmental conditions resulting in drought or flooding are posing significant difficulties to agricultural production (Bailey-Serres *et al*., 2012; Elferjani & Soolanayanahally, 2018; Koscielny *et al*., 2020; Lohani *et al*., 2020). Under the ongoing global climate-changing scenarios, climate extremes are expected to increase in the future, having detrimental effects on crop productivity (Troy *et al*., 2015). Starting from the time of the industrial revolution, the concentration of atmospheric CO_2 has risen from 280 ppm to over 410 ppm, and predictions suggest it could reach a range of 550 ppm to 1000 ppm by the end of this century (Cias *et al*., 2013; Dusenge *et al*., 2019). It is predicted that because of the global CO_2 concentration increase, the temperature of the higher latitudes may increase by 10 $^{\circ}$C by the end of 2100, while the temperature in the tropics may increase by 3-4 $^{\circ}$C (Cias *et al*., 2013). Consequently, predictions indicate that within the foreseeable future, plants on more than 90 % of the global cultivable land will experience one or multiple abiotic stresses (dos Reis *et al*., 2012; Trenberth *et al*., 2013).

Abiotic stresses stand as one of the major constraints that limit growth, development, and yield in crop plants (El-Badri *et al*., 2021; Wani *et al*., 2016; Zhao *et al*., 2010). Substantial decreases (up to 50 % - 70 %) in crop productivity due to abiotic stresses

have been observed on several occasions in the past (Mittler, 2006). Among abiotic challenges, water deficit is considered the most detrimental stress, as it impacts many crop species with effects that are often irreversible (Lambers *et al*., 2008). In general terms, water stress (or drought) is caused by absence of precipitation for a lengthy period or periods of below-normal precipitation that can cause serious imbalances in the hydrological cycle (Trenberth *et al*., 2014). Types of droughts are generally classified into four groups: meteorological, hydrological, socio-economical, and agricultural (Crocetti *et al*., 2020). Meteorological drought refers to the lack of precipitation for a longer period. Hydrological drought is related to a decrease in water levels in streams, lakes, and groundwater below the average normal levels (Trenberth *et al*., 2007). Socio-economic drought is referred to as increased demand for goods that exceeds the supply limits due to decreased availability of water (Crocetti *et al*., 2020). Agricultural drought is the most important term when relating drought to plant growth; it occurs when the available soil moisture (particularly in the uppermost layer of soil and within the root zone) decreases to an extent where it adversely affects crop yield and agroeconomic income (Maracchi, 2000). More than 40 % - 45 % of the global agricultural areas are experiencing continuous or recurring drought (Bot *et al*., 2000; Trenberth *et al*., 2014). Areas experiencing water limitations will increase from 15.4 % to 44 % by 2100, and consequently, the yield of major crop plants could be reduced by 90 % in 2100 if mitigating measures are not taken (Li *et al*., 2009). Drought events have already limited agricultural production, being responsible for substantial decreases (around 34 %) in crops and livestock production in the countries with minimal economic development and those categorized as having moderate to lower income levels. These losses have been estimated to be close to 37 billion USD from 2008 to 2018 (FAO, 2021).

While drought occurrences have been observed in numerous parts of Canada throughout the past years, the regions particularly susceptible to drought are the Canadian prairies and the interior valleys of British Columbia (Bonsal & Regier, 2007). This vulnerability is attributed to the significant fluctuations in both temporal and spatial patterns of precipitation (Environment Canada, 2004). Over the course of the previous two centuries, there have been more noticeable drought periods in the southern areas of Alberta, Saskatchewan, and Manitoba (Bonsal & Regier, 2007; Mishra & Singh, 2010). The worst drought seasons in Canada occurred in 1936-1937, 1961, 1984-1985, 1988, 2000-2002 (Bonsal & Regier, 2007), 2015 (Szeto *et al*., 2016), and 2021 (Statistics Canada, 2021). Different climate-predicting models have indicated that most of the regions of Canada will experience increased drought conditions as the 21st century draws to a close (Dibike *et al*., 2017; Masud *et al*., 2017). The consequences of drought have negative impacts on the Canadian economy. For instance, the drought experienced during 2000-2001 was categorized as the most financially challenging natural calamity in Canada, resulting in a decrease of $3.6 billion in agricultural output and a reduction of $5.8 billion in gross domestic product (Wheater & Gober, 2013). During this period, agricultural production losses in the Canadian prairie provinces alone were estimated at around $3 billion (Wheaton *et al*., 2008).

1.1.2 *Brassica napus* L., an Economically Important Crop Susceptible to Drought Stress

The international food market is mainly comprised of cereal, oil crops, and legumes (Lohani *et al*., 2020). Oilseed plants hold the second position in terms of global crop production, and within this category, *Brassica napus* L. (canola) stands as the second-

ranking contributor, generating an annual worth of 41 billion USD (Wu *et al*., 2018). *Brassica napus* is grown extensively in Europe, Canada, Asia, Australia, India, and the United States (McVetty & Duncan, 2015; Wan *et al*., 2009). It is grown on more than 8 Mha in Canada, valued at approximately 26 billion CAD (Elferjani & Soolanayakanahally, 2018; Koscielny *et al*., 2018; Kutcher *et al*., 2010). Saskatchewan, Alberta, and Manitoba are the major producers of canola in Canada (Canola Council of Canada, 2016). The total impact of the canola industry on the Canadian economy in 2018-2019 was approximately 27.7 billion CAD (Canola Council of Canada, 2020). Out of this amount, Saskatchewan accounts for 50 %, while Manitoba and Alberta account together for another 40 % (Canola Council of Canada, 2020).

Edible vegetable oils are composed of a collection of fatty acids, and the composition of fatty acids is the major determinant of whether the vegetable oil is suitable for consumables or industrial applications (Lu *et al*., 2011). The nutritional value of rapeseed oil was initially disputed as it contained a high amount of erucic acid (Russo *et al*., 2021; Wendlinger *et al*., 2013). At the same time, the high content of glucosinolates made it unsuitable for livestock feed as it reduced feed efficacy (Booth & Gunstone, 2004). Canola oil is different from conventional rapeseed oil as it contains low erucic acid content (<2 %) and low glucosinolate concentration (<30 pmol/g) in the meal (Lee *et al*., 2020). The first *B. napus* cultivar with low erucic acid and glucosinolate levels was developed by Dr. Baldur Stefansson of the University of Manitoba (Stefansson & Kondra, 1975).

Edible oil extracted from *B. napus* is in high demand globally due to the quality of the oil. Canola oil contains a very low percentage of saturated fatty acids (6 %), a higher percentage of monounsaturated fatty acids (e.g., oleic acid at 58 %), and moderate levels of polyunsaturated fatty acids (36 %), and linolenic acid (10 %) (Eskin & Mcdonald, 1991). Canola oil is a heart-healthy cooking oil with the lowest saturated fat content and highest polyunsaturated fatty acid content among all the other cooking oils (Rempel *et al*., 2014). The high protein content and a favorable balance of amino acids in the canola meal (a by-product of oil extraction) make it a premium source for livestock consumption (Wu *et al*., 2018). Overall, the exceptional quality of edible oil derived from *B. napus* solidifies canola oil's position as a highly sought-after and versatile product in both culinary and livestock industries (Raboanatahiry *et al*., 2021). Due to these properties, *B. napus* is an economically important crop for the Canadian economy.

Brassica napus is extremely susceptible to water shortage (Wan *et al*., 2009; Wu *et al*., 2018). Based on studies conducted in the northern Great Plains area, it was found that canola needs a minimum amount of seasonal water varying between 121 and 160 millimeters per seasonal cycle to sustain itself and ensure viable production (Johnston *et al*., 2002). The water demand of canola in its initial growth phases (from emergence to rosette formation) is approximately 1 mm per day. This demand progressively rises as the plant develops, ranging from 2 to 5 mm per day during the late rosette to bud development phases, and peaking at around 6 mm per day during crucial stages like flowering and pod establishment (Assefa *et al*., 2018). The need for water significantly decreases following seed development and until the harvesting period (Hergert *et al*., 2016). Several studies have demonstrated that supplying additional water beyond the

minimal water requirement leads to an improvement in canola yield, resulting in an increase ranging from 6.9 to 77 kg per hectare for each millimetre of water (Hergert *et al*., 2016). A 35.4 % reduction in canola yield was recorded in Canada during the 2021-2022 growing season as a result of insufficient rainfall (Agriculture Canada, 2022), and substantial losses during the same season were specifically reported in Saskatchewan (45.4%), Alberta (31.1%), and Manitoba (28.2%) (Manitoba Agriculture and Resource Development, 2021). Understanding the precise water requirements of *B. napus* is thus paramount, as a plant's vulnerability to water scarcity can have profound implications on its yield and overall productivity.

1.1.3 Water Stress Alters the Water Potential of the Soil and Plant Tissues

Water potential is defined as "the measure of the potential energy (per unit mass or volume) of water at a point in a system relative to the potential energy of pure free water" (Papendick & Campbell, 1981). The total water potential is composed of four components (osmotic potential, matric potential, pressure potential, and gravitational potential) (Zhang *et al*., 2022). Osmotic potential ($\psi\pi$) remains negative because of dissolved solutes in water. Matric potential (ψm) is likewise consistently negative and incorporates the solid phase's adsorption and capillary impacts. Gravitational potential (ψg) varies between negative and positive values based on elevation relative to the reference point. Pressure potential (ψp) represents the external gas or hydraulic pressure exerted on water (de Swaef *et al*., 2022; Novick *et al*., 2022; Papendick & Campbell, 1981).

Soil water potential is mainly governed by surface forces that maintain water in capillaries and on the surface (Pallardy, 2008). Water movement through the soil, into

roots, through the plant, and out into the air (Philip, 1966) depends on the soil water availability and is driven by the difference in water potential between the atmosphere and soil (Bouman & Tuong, 2001). Furthermore, soil water potential and leaf water potential show a positive linear relationship (Yang *et al*., 2007). The initial water uptake by the root is regulated by the water potential differences between the soil and the root cells (Bouman & Tuong, 2001). Many models of water uptake consider a threshold value of soil water potential where, below this point, water uptake by roots cannot occur (de Jong van Lier *et al*., 2008; Feddes *et al*., 1976). When water uptake by the roots is not possible, plant cells and tissues experience water stress, and numerous morpho-physiological growth parameters are affected (Lawlor & Cornic, 2002; Yamane *et al*., 2003; Zargar *et al*., 2017).

1.2 Effects of Drought on Plant Morphology and Physiology

Water deficit has negative consequences on plant growth and development by interfering with the growth of shoots and roots and gas exchange parameters, i.e. stomatal conductance, photosynthesis, and respiratory processes (Rodriguez-Dominguez *et al*., 2016). The following sections provide detailed information on how water deficit influences these processes.

1.2.1 Effects of Drought on Root and Shoot Growth

Like any organ experiencing water stress, root growth is generally limited during drought as cell elongation and division require water (Feng *et al*., 2016). Furthermore, the compaction of soil particles in water-depleted soils (Grzesiak *et al*., 2016) can further contribute to growth inhibition by causing root clumping and/or shrinkage (Beudez *et al*., 2013; Carminati *et al*., 2009; Tardieu *et al*., 1992). However, the degree

of root growth inhibition is often related to the level of stress experienced by the plant (Lipiec *et al*., 2013).

Despite the fact that roots are the first organs to perceive and respond to water shortage in the soil, shoot growth is also affected. Shoots are indeed very susceptible to water limitation and, even under mild drought stress conditions, can stop growing while roots continue to grow and elongate (Sharp *et al*., 2004; van der Weele *et al*., 2000). This is the result of hydromechanical limitations caused by the imbalance between the turgor pressure and the force required to enlarge the cells. Reduced cell division and cell expansion during drought stress contribute equally to the decrease in shoot growth (Arve *et al*., 2011). This was demonstrated in several crop plants, including *B. napus* (Channaoui *et al*., 2017; Li *et al*., 2018; Sharif *et al*., 2018; Yang *et al*., 2007), *Triticum aestivum* L. (Khan *et al*., 2010), and *Glycine max* L. (Creelman *et al*., 1990; Specht *et al*., 2001). Differential growth of organs in plants experiencing water stress depends on the allocation of energy and nutrients (Chaves *et al*., 2002). This concept is best explained by the "optimal partitioning theory," suggesting that plants allocate more resources to organs where there is limited access to necessary resources to optimize the normal function of plants (Bloom *et al*., 1985). Under mild drought-stressed conditions, plants often allocate more resources to the root and less to the shoot, as the root plays a major function in absorbing water and nutrients (Eziz *et al*., 2017; Poorter *et al*., 2012). However, this regulation of growth patterns is species dependent.

1.2.2 Water Stress Depresses Gas Exchange Parameters

One of the early responses to water stress is a depression in the photosynthetic rate (Flexas *et al*., 2009). This effect, observed in many plant species (Lawlor & Cornic, 2002), can be attributed to several causes, including a decrease in stomatal conductance, degradation of photosynthetic components, and reduced activity of photosynthetic enzymes (Parry *et al*., 2002).

Photosynthesis is a fundamental metabolic process in plants directly related to productivity. In C3 plants, photosynthesis is mainly limited by stomatal conductance, mesophyll diffusion conductance of CO_2, and biochemical photosynthetic capacity (Cano *et al*., 2013). Stomatal conductance is the diffusion of CO_2 from the external environment to the substomatal cavities of the leaf. Mesophyll conductance is the diffusion of CO_2 from the substomatal cavities to chloroplast stroma (Ouyang *et al*., 2017). When plants are exposed to drought stress conditions, the most immediate response is the closure of the stomata. This helps prevent the drastic reduction of leaf water potential due to evapotranspiration through leaf stomata, thereby ensuring that the water demand in the plant does not exceed the water supply (Scoffoni *et al*., 2017). The mechanism behind stomatal closure during drought stress conditions is poorly understood (Wang *et al*., 2018). The hormonal and leaf turgor signal hypotheses best describe the mechanisms responsible for the closure of the stomata. The hormonal hypothesis suggests that abscisic acid (ABA) synthesized in root cells following the perception of the water stress, is transported into the leaves where it induces the closure of the stomata (He *et al*., 2018). The leaf turgor signal hypothesis suggests that the decline of stomatal conductance during drought is the result of changes in the leaf turgor pressure (Rodriguez-Dominguez *et al*., 2016). Closure of the stomata, whether

through the hormonal hypothesis or the turgor signal hypothesis not only leads to reduced intake of CO_2, with the concomitant reduction in photosynthesis but also decreases evapotranspiration with consequences on mineral intake from the soil (Ainsworth & Rogers, 2007; Cao *et al*., 2022).

A reduction in photosynthesis during water stress is also the result of reactive oxygen species (ROS)-induced damage (Henmi *et al*., 2004). Like other forms of abiotic stresses, water deficit favors the production of ROS, which can damage several cellular components (Munne-Bosch & Alegre, 2004). "Hydrogen peroxide (H_2O_2), superoxide anions ($O_2^{\bullet-}$), hydroxyl radicles (OH•), and singlet oxygen ($^1O_2$)" are considered the most abundant ROS (You & Chan, 2015). Within the chloroplast, the reaction centers in PSI and PSII are the major generators of ROS, especially during water stress, when excitation energy/photon intensity exceeds the limited CO_2 fixation ability (Asada, 2006). Under these conditions, high energy electrons are released (Peltzer *et al*., 2002), and noxious levels of ROS are generated, as observed in photosynthetic tissue of water-stressed *T. aestivum* (Biehler & Focks 1996) and *Helianthus annuus* L. (Sgherri *et al*., 1996). The accumulation of ROS in water-deficient environments is not only the result of their increased production but also the consequence of a limited scavenging ability due to a depression of the antioxidant system, the activity of which requires water (Hernandez *et al*., 2012). The relevance of the antioxidant system in scavenging ROS will be discussed in the following sections.

While required for normal development at physiological levels, ROS can cause severe cellular damage when exceeding toxic thresholds. They can inactivate protein structures and alter protein function, as well as induce nucleic acid mutations and lipid

peroxidation, compromising the fluidity and function of membranes (Navari-Izzo *et al*., 2000). In the context of decreased photosynthetic activity in water-stressed plants, ROS can also degrade components required for the synthesis of chlorophyll pigments. This effect was observed in several species, including *T. aestivum* (Biehler *et al*., 1996), *H. annuus* (Reddy *et al*., 2004), *Cicera arietinum* L. (Mafakheri *et al*., 2010), and *Vaccinium myrtillus* L. (Tahkokorpi *et al*., 2007). Besides a reduction in chlorophyll pigment synthesis, the breakdown of existing chlorophyll molecules has been observed in dehydrating tissues following an induction in the activity of chlorophyllase. This enzyme plays a major role in chlorophyll catabolism by catalyzing the conversion of chlorophyll to chlorophyllide (Hu *et al*., 2021). Of note, chlorophyllase is also the rate-limiting enzyme in chlorophyll breakdown (Harpaz-Saad *et al*., 2007).

1.2.3 Direct Effects of Drought Stress on Photosynthetic Enzymes

Several independent studies suggest that water limitation can have a direct inhibitory effect on the function of photosynthetic enzymes. The initial reaction of CO_2 assimilation in C3 photosynthesis is catalyzed by the Ribulose-1,5-bisphosphate carboxylase (Rubisco) enzyme (Makino *et al*., 1983). In C4 plants, Phosphoenolpyruvate carboxylase is the initial carboxylation enzyme located in the outer mesophyll cells, while Rubisco resides in bundle sheath cells (Alonso-Cantabrana *et al*., 2018). It is widely accepted that the net rate of photosynthesis in higher plants is highly dependent on the activity of Rubisco and its regeneration rate (Jensen, 2000; Ku *et al*., 1979; Makino *et al*., 1983). The regeneration frequency of an average Rubisco molecule is between 1-10 $^{s-1}$, making it a limiting factor in photosynthetic CO_2 fixation under optimal conditions (Flamholz *et al*., 2019). Water stress reduces photosynthesis by decreasing/inhibiting the activity of photosynthetic

enzymes such as Rubisco and sucrose phosphate synthase (Chaves *et al*., 2009). Most studies in this area focus on the drought inhibition of Rubisco, which is apparent, especially during severe or long-term drought stress (Flexas & Medrano, 2002). This effect has been noted in several plant species (Parry *et al*., 2007; Zhou *et al*., 2007), although some discrepancies have been reported in which the enzyme is not deactivated by water limitations (Pelloux *et al*., 2001).

The water stress inhibition of Rubisco is linked to mechanisms required for the activation of the Rubisco enzyme (Wijewardene *et al*., 2021). For normal function, Rubisco needs to be activated by binding with CO_2 and Mg^{2+} before binding to its substrate Ribulose-1,5-bisphosphate (Miziorko & Lorimers 1983). A lysine residue in the active site of the enzyme needs to be carbamylated before it complexes with Mg^{2+} (Erb & Zarzycki, 2018). Several mechanisms disrupt the catalytic activity of the Rubisco enzyme. The most effective mechanism is the binding of inhibitors such as various sugar phosphate molecules, including carboxyarabinitol 1-phosphate (CA1P) and 3-ketoarabinitol bisphosphate (3KABP) to the carbamylated Rubisco, which prevents its catalytic activity (Wijewardene *et al*., 2021). Under these conditions, the binding of the substrate to the inactive Rubisco does not lead to the carboxylation step and CO_2 fixation. Removal of these inhibitors from the Rubisco molecule is mediated by Rubisco activase, an enzyme requiring ATP (Parry *et al*., 2002; Portis, 1995). Water stress elevates the level of the inhibitor CA1P, thus decreasing the activity of Rubisco. Increasing levels of CA1P have been observed in many water-stressed species, such as *T. aestivum* and *Nicotiana tabacum* L. (Parry *et al*., 2002). Water deficiency also reduces the level of ATP, which is required by rubisco activase to activate rubisco (Parry *et al*., 2002; Portis, 1995).

Additional events triggered by water stress and contributing to the inhibition of rubisco are linked to the decreased electron transport rate, decreased NADPH supply, reduced capacity for photophosphorylation, and decreased triose phosphate usage (Flexas & Medrano, 2002). Various rubisco-regenerating enzymes, including fructose-1,6-bisphosphatase, glyceraldehyde-3-phosphate dehydrogenase, and ribulose-5-phosphate kinase, require one or more of these events to function properly, and their activity declines in water depleted environments (Maroco *et al*., 2002). The acidification of the chloroplasts following water stress also contributes to reduced rubisco activity (Meyer & Genty, 1999).

1.2.4 Effects of Drought Stress on Respiration

Besides the reduction of photosynthesis, respiratory processes are also very susceptible to water shortage. Mitochondrial respiration provides the majority of the energy required for cell growth and development, while the Tricarboxylic acid cycle (TCA) intermediates provide the carbon skeleton essential for the synthesis of cellular components (Amthor, 2000; Wright *et al*., 2006). Several studies have shown that while mitochondrial respiration and the TCA cycle are generally inhibited by water stress, the extent of this inhibition is less pronounced than the one observed for photosynthesis. This has been demonstrated in several studies (Ghashghaie *et al*., 2001; Haupt-Herting & Fock, 2002; Ribas-Carbo *et al*., 2005). It must be noted, however, that in a few instances respiration increases (Gratani *et al*., 2007; Lawlor, 1976; Zagdanska, 1995) or remains unaltered (Galmes *et al*., 2007; Laboda, 2000) following the imposition of water stress. Flexas *et al*. (2005) pooled data from several studies, including a total of 14 plant species and observed a biphasic relationship

between relative water content in leaves and leaf respiration. Respiration decreased when the water content decreased to 70 %; it remained constant between 70-55 % and increased below 55 % water content. What is apparent from these studies is that the effects of drought stress on leaf respiration depend on the plant species, as well as by the degree and length of the stress (Flexas *et al*., 2005; Lawlor & Cornic, 2002). In those cases where drought depresses respiration, it has been suggested that this trend is attributable to a decline in photosynthesis, reducing the supply of substrates to mitochondria (Atkin & Macherel, 2009; Lawlor & Cornic, 2002). Conversely, increased respiration under drought stress conditions has been linked to elevated demand for respiratory energy to support metabolic adaptation, such as enhanced root growth (Atkin & Macherel, 2009).

In addition to respiratory reactions, water stress affects mitochondria electron partitioning (Gomez-Casanovas *et al*., 2007). The plant mitochondrial electron transfer chain splits into two parts, such that electrons in the ubiquinone pool are diverted between the cytochrome C oxidase pathway and the alternative oxidase pathway (Finnegan *et al*., 2004; Millar *et al*., 2011). The non-phosphorylating alternative oxidase pathway in mitochondria plays a major role in dissipating redox equivalents from the chloroplast (Raghavendra & Padmasree, 2003). Under severe drought stress conditions, electron portioning toward the alternative oxidase pathway increases, thus reducing the amount of ATP (energy) produced in mitochondria (Ribas-Carbo *et al*., 2005).

1.3 Strategies to Cope with Water Stress

1.3.1 Escape vs. Avoidance

Plants have developed various strategies to manage water stress, encompassing morphological, physiological, and molecular adaptations (Khan *et al*., 2018; Martignago *et al*., 2020). It is generally accepted that these mechanisms follow two general strategies: escape and avoidance. Drought escape refers to the capacity of plants to expedite their lifecycle by reducing the growth duration, augmenting growth speed, and accumulating additional reserves in certain organs for subsequent mobilization during seed production (Chaves *et al*., 2003). This is common in many cereal crops, such as *T. aestivum* (Shavrukov *et al*. 2017). This adaptation involves an "early maturation," as observed in *T. aestivum* and *Secale cereale* L. (Brisson & Casals, 2005; Kottmann *et al*., 2016; Li *et al*., 2011). Early flowering as a strategy to evade the negative impact of drought stress was also observed in *Brassica rapa* L. (Franks, 2011). Plants that exhibit early flowering normally have fewer leaf nodes, suggesting that flowering occurs at an earlier life cycle stage (Franks *et al*., 2007). It has been demonstrated that plants that show early flowering maintain a high stomatal conductance. This sustains the plants with a rapid gain of carbon, supporting their fast growth and development (Wu *et al*., 2018).

Drought avoidance is the ability of plants to withstand water deficit by developing strategies to mitigate its effects. These strategies include the development of an extensive root system to explore deeper regions of soil (de Dorlodot *et al*., 2007; Kavar *et al*., 2008; Wu *et al*., 2018). In some plants, soil water deficit can increase the depth of the root system, with a reduction in the number of lateral roots, and the overall root mass (van der Weele *et al*., 2000). In water-depleted environments, in fact, roots can

reach water retained within lower soil strata while the uppermost layer experiences drying (Koevoets *et al*., 2016). To reach deeper soil, plants allocate more energy to the growth of axile roots by inhibiting lateral root growth. This was also demonstrated *in vitro*, where *Arabidopsis thaliana* L. seedlings grown on agar media supplemented with osmoticum agents (PEG, sorbitol, or mannitol) demonstrated significant suppression of lateral root development and elongation (Deak & Malamy, 2005; Xiong *et al*., 2006). This inhibition is caused by ABA produced by water-stressed root tips, which move apically and repress the initiation of lateral roots (Xiong *et al*., 2006). An increase in the depth of the root system in water-depleted soils has been observed in *B. napus* (Johnston *et al*., 2002), *H. annuus* (Killi *et al*., 2017), and *G. max* (Fenta *et al*., 2014). In addition to a deeper root system, plant species such as *Phaseolus vulgaris* L. (Polania *et al*., 201 6), and *Zea maize* L. (Lynch, 2013) tend to develop fine roots in the topsoil to take advantage of intermittent rains. Thus, the effects of water stress on root morphology are often species-specific.

1.3.2 Cellular Osmotic Adjustments to Mitigate Water Stress

Water movement is dictated by the water potential, as water moves from regions of high potential to regions of low potential (Bleby *et al*., 2010; Caldwell *et al*., 1998). Water depletion in the soil reduces the water potential, and when the water potential of the soil becomes more negative than the potential of the root cells, plants are unable to take up water. Plant cells can lower their water potential (Girma & Krieg, 1992) through osmotic adjustments involving the accumulation of metabolites such as proline, glycine, betaine, soluble sugars, and inorganic ions, which promote water retention without interfering with metabolic processes (Chen & Jiang, 2010; Takahashi *et al*., 2020).

Osmotic adjustment sustains the metabolic activities of plant tissues and supports regrowth at the end of drought stress (Morgan, 1984). Both organic compounds and inorganic ions have a vital function in facilitating osmotic adjustments (Farooq *et al*., 2012). Organic osmolytes found in higher plants usually encompass highly soluble substances with low molecular weights, such as sugars and their derivatives (sucrose, polyol), and methylated tertiary nitrogen compounds, including glycine, betaine, and homarine, and amino acids (proline and glutamate) (Chen & Jiang, 2010). Organic osmolytes protect plants against water stress through various means: by contributing to cellular osmotic adjustment, detoxifying ROS, maintaining membrane integrity, and stabilizing proteins (Ashraf & Foolad, 2007). Increased content of free proline was observed in drought stress *Pisum sativum* L, *T. aestivum* (Alexieva *et al*., 2001), and *B. napus* (Ma *et al*., 2004). In Brassica, osmotic adjustment increased turgor recovery following the resupply of water after a drought period (Levy *et al*., 2006; Norouzi *et al*., 2005).

The accumulation of organic osmolytes to attenuate water stress is an energy-consuming process. In some instances, this type of accumulation is more expensive than absorbing a sufficient amount of ions directly from the soil (Fang & Xiong, 2015; Per *et al*., 2017). Therefore, when possible, roots prefer to actively intake ions in order to lower the cellular osmotic potential. It is well known that Na^+, K^+, and Ca^{2+} are the main inorganic ions that contribute to osmotic adjustment in many plant species, including halophytic plants adapted to survive in extreme conditions of water stress (Chen & Jiang, 2010). Potassium ion is the most abundant cation in the cytosol and is suitable for osmotic adjustment without being toxic (Blum, 2017; Shabala & Lew,

2002). An elevation in the level of K^+ has been reported in water-stressed *Vitis vinifera* L. (Patakas *et al*., 2002) and *B. napus* (Ma *et al*., 2004).

1.3.3 Plant Responses Mediated by Growth Regulators

Plant growth regulators play a crucial role in mediating drought responses in plants (Mehrotra *et al*., 2014; Tuteja, 2007; Zhu, 2016). Drought stress alters the endogenous synthesis of hormones such as abscisic acid (ABA), auxin, gibberellin, and cytokinin (Farooq *et al*., 2012; Korver *et al*., 2018; Seo *et al*., 2010). The role of abscisic acid will be examined in more detail, given its major role played during water deficit.

Abscisic acid (ABA) is considered the major stress-mediating hormone in plants as it regulates most of the abiotic stress responses (Mehrotra *et al*., 2014; Tuteja, 2007). It is considered the potential candidate in signal transduction between roots and shoots under stress conditions, and it plays a vital role, especially during drought (Vishwakarma *et al*., 2017; Zhu, 2016). Increases in the levels of ABA through activity of ABA biosynthetic enzymes have been observed in many water-stressed species (Seki *et al*., 2007; Wang et al., 2021). Among the ABA biosynthetic enzymes, 9-cis-epoxy carotenoid dioxygenase (NCED), which catalyzes the rate-limiting step in the ABA biosynthetic pathway, has received the most attention (Ali *et al*., 2020). Under water deficit, a significant increase of NCED transcripts, protein, and activity was detected in leaves, and these changes were associated with a rise in ABA. This was observed in crop plants, including *Solanum lycopersicum* L. (Burbidge *et al*., 1999), and *Z. mays* (Tan *et al*., 1997).

Abscisic acid also has a role in the closure of the stomata to prevent water loss in water-deficient environments (Kim *et al*., 2010). Despite being produced in many organs and tissues, water-stressed roots are the major source of ABA, which is translocated apically to the guard cells, where it induces the closure of the stomata (Zhang & Davies, 1987). Stomatal conductance is controlled by ion fluxes in guard cells through Ca^{2+}-dependent and Ca^{2+}-independent pathways, both mediated by ABA (Murata *et al*., 2001). Abscisic acid induces an increase in cytosolic Ca^{2+} in guard cells, which activates slow (S-type) ion channels and downregulates inward K^{+} channels. As a result, turgor pressure decreases, leading to stomatal closure (Schroeder & Hagiawara, 1989). Foliar applications of ABA also contribute to the closure of the stomata and enhance tolerance to water stress (Zhu *et al*., 2010).

Besides influencing morphogenesis, such as the preferential growth of the root relative to the shoot to explore deeper regions of the soil for water (Koevoets *et al*., 2016), ABA contributes to a rise in proline content needed to lower the water potential of root cells. Under drought conditions, increased ABA content modulates proline synthesis both at transcriptional and post-transcriptional levels (Hare *et al*., 1999). This was observed in many plant species, including *Hordeum vulgare* L. (Bandurska *et al*., 2017), *T. aestivum* (Saeedipour, 2013), and *Vigna unguiculata* L. (da Costa *et al*., 2011).

1.3.4 Activation of the Antioxidant System to Remove Noxious Levels of ROS

Reactive oxygen species are required for normal growth and development, and their level is regulated by their synthesis and degradation (Mittler, 2017). As indicated above, during water stress, the amount of ROS increases, causing damaging effects on cellular components (Hasanuzzaman *et al*., 2012). Plants have effective antioxidant machinery

composed of several enzymes including “superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidase (GPX), and molecules possessing antioxidant properties such as flavonoids, carotenoids, alkaloids, phenolic acids, and α tocopherol” (Hasanuzzaman *et al*., 2020). Activation of the antioxidant machinery in an effort to reduce the levels of ROS during water deficit contributes to drought tolerance (Zhu, 2016).

Superoxide dismutase (SOD) and CAT cooperate in ROS detoxification. Superoxide dismutase (SOD) catalyzes the reaction converting superoxide anion ($^{\bullet}O_2^-$) to H_2O_2 (Bowler *et al*., 1992), and CAT subsequently converts H_2O_2 to H_2O (Noctor & Foyer, 1998; Willekens *et al*., 1997). Enhanced activity of superoxide dismutase in water stress tissue was observed in crop plants, including *P. vulgaris* (Zlatev *et al*., 2006), *H. vulgare* (Acar *et al*., 2001), and *Oryza sativa* L. (Wang *et al*., 2019). A rise in CAT activity was also observed in water-stressed *O. sativa* (Sharma & Dubey, 2005), *C. arietinum* (Mafakheri et al., 2011), *B. napus* (Abedi & Pakniyat, 2010). Ascorbate peroxidase contributes to the scavenging of H_2O_2 using ascorbate as a substrate, and this action is also favored by additional plant peroxidases, which oxidize phenolic compounds (Zhang *et al*., 2023). Recycling of ascorbate is then mediated by the glutathione-ascorbate cycle through the regulation of several redox enzymes (Caverzan *et al*., 2012). In this intricate network of ROS detoxification, the coordinated action of these enzymatic pathways not only mitigates the negative impact of oxidative damage but also showcases the multifaceted strategies employed by plants to counter the challenges posed by water stress and maintain cellular homeostasis.

1.3.5 Overview of Molecular Changes Underpinning Plant Responses to Water Stress

All the plant responses described above are controlled by complex genetic networks (Lie *et al*., 2014; Gad *et al*., 2021). Upregulation and downregulation of many genes at the transcriptional level, and the accumulation of stress proteins are important adaptive mechanisms observed under water stress conditions (Kavar *et al*., 2008). Drought stress signaling pathways are categorized as ABA-dependent and ABA-independent pathways (Shinozaki & Yamaguchi-Shinozaki, 2007). Several studies have identified specific clusters of genes induced by drought that confer tolerance. Most of the key genes within these pathways are categorized as "DRE binding protein (DREB) / C repeat binding factor (ABF), MYC, and MYB" (Bartels & Sunkar, 2005). The Abscisic acid-responsive element (ABRE) and a dehydration-responsive element/ C repeat (DRE/CRT) are both present in the promoter regions of these genes. These elements are activated by the presence of ABA produced under water deficit (Seki *et al*., 2003; Yamaguchi-Shinozakiaib & Shinozaki, 1994). While some of the genes encode transcription factors activating downstream responses, others encode proteins, contributing to the protection of cellular components (Liu *et al*., 1998).

A well-described example of these genes is *CLE25*, which is expressed in vascular tissues of roots as a result of increased synthesis of ABA. The CLE25 peptide is known to transmit water deficiency signals from roots to leaves (Takahashi *et al*., 2020), and affects ABA accumulation in guard cells and thus stomatal conductance in leaves (Takahashi *et al*., 2018). Other examples of ABA-regulated genes include the transcriptional modulator known as *OBF Binding Protein4 (OBP4)*, which interacts with the promoter region of *Root Hair Defective6-Like2 (RSL2)*, resulting in the

inhibition of *RSL2* expression, thereby suppressing root hair growth (Rymen *et al*., 2017), and Pyrroline-5-Carboxylate Synthetase (P5CS), an important enzyme in the proline biosynthetic pathway (Hare *et al*., 1999).

1.4 Introduction to *Brassica napus* L. Genome

Three Brassica species: *B. rapa* L. (A genome, 2n = 20), *Brassica nigra* L. (B genomes 2n = 16), and *Brassica oleracea* L. (C genomes 2n = 18) underwent a spontaneous hybridization and formed three species. *Brassica napus* L. (AACC, 2n = 38) is an allopolyploid species formed by the interspecific hybridization between *B. rapa* (A genome, 2n = 20) and *B. oleracea* (C genome, 2n 18) (Mason *et al*., 2017). *Brassica juncea* L. was generated by *B. rapa* and *B. nigra* (AABB genomes 2n = 36), while *Brassica carinata* L. (BBCC genome, 2n = 34) was formed by the hybridization between *B. nigra* and *B. oleracea* (Trick *et al*., 2009). The relationship between these six major Brassica species is known as the "Triangle of U" (Figure 1) (Nagaharu, 1935).

In *B. napus*, it is observed that the assembled C sub-genome, spanning 525.8 Mb, exceeds the size of the assembled A sub-genome, which covers 314.2 Mb (Chalhoub *et al*., 2014). These A and C sub-genomes originate from ancestral polyploidization events and naturally exist in duplicated or triplicated states (Durstewitz *et al*., 2010; Schranz *et al*., 2006). The segregation pattern in allotetraploids is notably more complex than in diploid species, as allotetraploids can segregate into five distinct clusters: AAAA, AAAB, AABB, ABBB, and BBBB (Akhunov *et al*., 2009). Consequently, the process of SNP marker identification and SNP analysis in *B. napus* becomes considerably more complicated than in diploid species (Durstewitz *et al*., 2010).

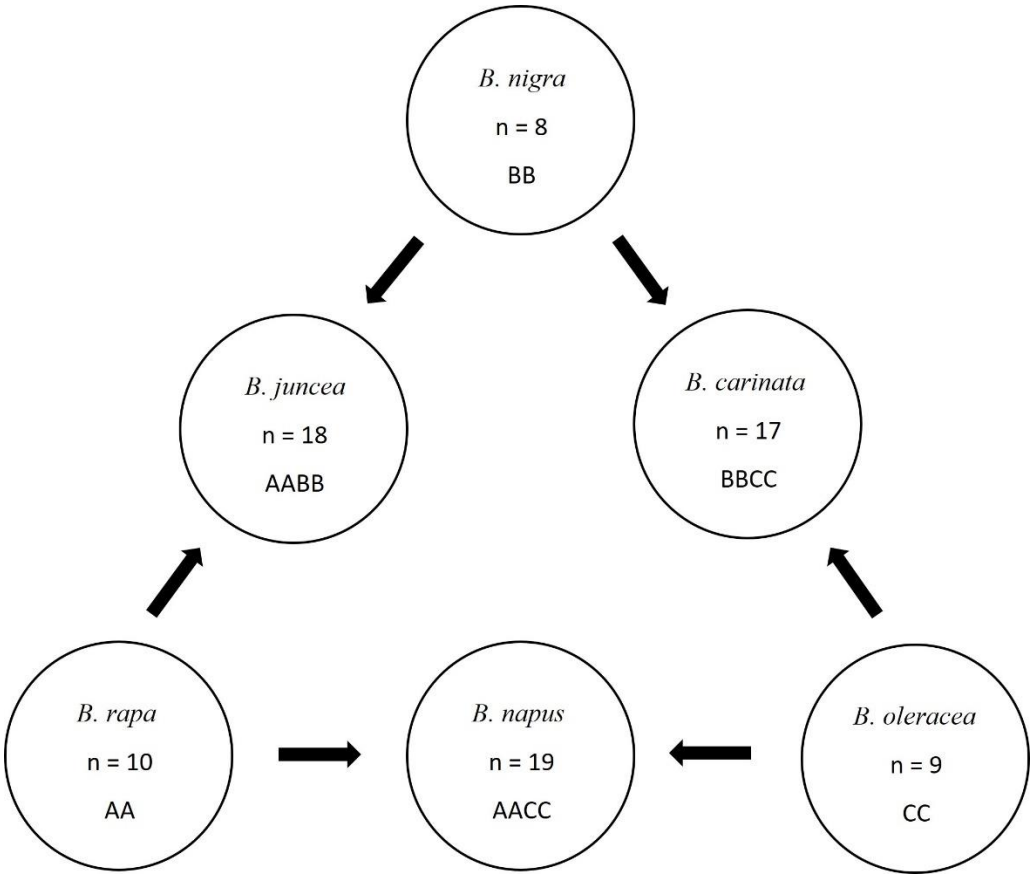

Figure 1: Triangle of U (Genetic relationship between the major six *Brassica* species) adapted from Nagaharu (1935)

1.5 Genetic Markers for Drought Tolerance

1.5.1 Introduction

Information about genetic variation within plant species and between plant populations plays a valuable role in the effective selection of plant materials for breeding programs (Cole, 2003). Genetic markers are one of the major developments in the field of plant breeding (Kebriyaee *et al*., 2012). There are three primary categories of genetic markers: morphological markers (phenotypic traits), biochemical markers (isoenzymes), and DNA markers (van Bueren *et al*., 2010; Winter & Kahl, 1995). Among them, DNA markers are the most preferred markers in many applications due to their prevalence and especially their independence from the developmental stage of the plant (Winter & Kahl, 1995). DNA markers are a DNA sequence/fragment with a known location on the chromosome near the target gene or tightly linked to that particular gene, acting as a sign or a flag (Collard *et al*., 2005) to detect polymorphism between genotypes (Jiang, 2013). Molecular markers are classified into three groups based on their mode of action, transmission, and detection (Semagn *et al*., 2006). Depending on the method of DNA marker detection, they can be mainly categorized into three classes: hybridization-based, polymerase chain reaction-based, and DNA sequence-based (Jiang, 2013; Semagn *et al*., 2006).

Among all the molecular marker methods, “Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), and Single Nucleotide Polymorphisms (SNP)” are some of the most discussed molecular marker techniques (Collard *et al*., 2005). No single molecular marker technique is suited for all applications; therefore, it is more important to carefully select which marker method

is more useful to achieve the objectives of the research (Hasan *et al*., 2021; Nadeem *et al.,* 2018). According to Jiang *et al*. (2013), an optimal DNA marker for effective utilization in marker-assisted breeding initiatives should possess a substantial degree of polymorphism, a uniform dispersion throughout the entire genome, genome specificity, and distinct and easily discernible allelic characteristics. Single nucleotide polymorphic (SNP) arrays are the most preferred method for *Brassica napus* L. due to their robust and efficient features (Clarke *et al*., 2016). Plant breeders use molecular markers for different purposes including germplasm conservation (Igwe *et al*., 2021; Kim *et al*., 2021), assessment of heterosis in breeding (Nie, 2019; Tomkowiak *et al*., 2020), creating genetic maps (Chesnokov *et al*., 2020), QTL mapping and characterization, association mapping (Kim *et al*. 2021), and marker-assisted backcross breeding (Batieno *et al*., 2016; Cheng *et al*., 2017). The judicious selection of molecular marker techniques tailored to the specific research goals is pivotal, as it allows scientists and breeders to harness the full potential of these tools in diverse applications ranging from germplasm conservation to advanced breeding strategies in *B. napus* and other crop species.

1.5.2 Single Nucleotide Polymorphisms (SNP)

Single nucleotide polymorphisms (SNP) occur as a result of the difference in a single nucleotide between two different DNA sequences (Mason *et al*., 2017). The establishment of techniques with enhanced capabilities to discover the insertion and deletion of a single nucleotide (indels variation) has led to the development of SNPs as molecular markers (Batley *et al*., 2007; Batley & Edwards, 2007). Single nucleotide polymorphisms can be classified into transition (C/T or G/A) or transversion (C/G, A/T, C/A, or T/G), based on the way that the nucleotide substitution occurs (Mason *et al*.,

2017). Single nucleotide polymorphic markers are the currently preferred molecular markers for high throughput genotyping in the study of complex plant genetic traits due to their abundance in the genome, and low mutation rate (Deschamps *et al*., 2012; Syvanen, 2001; van Bueren *et al*., 2010), and their potential to have a strong linkage with the trait of interest (Hayward *et al*., 2012). These SNPs could generally be found in all regions of the genome, and a single gene could have multiple SNPs (Rafalski, 2002). In plant genomes, the occurrence of SNPs is typically found at a rate of approximately one SNP per every 100 to 300 base pairs (Edwards *et al*., 2007). In previous studies, two SNPs have been recognized within approximately 400 bp in the DNA sequence of *G. max* (Coryell *et al*., 1999), and approximately one SNP for every 48 bp in *Z. mays* (Rafalski, 2002; Tenaillon *et al*., 2001). High throughput SNP discovery could be achieved either by genome-by-sequencing or high-density SNP arrays (Clarke *et al*., 2016). Although genome-by-sequencing analysis does not require the knowledge of existing SNPs within the species, it significantly relies on the computational capabilities and the knowledge of the person involved in the analysis (Deschamps *et al*., 2012). When compared with genome-by-sequencing, high-density SNP arrays require limited computational knowledge. However, it is very important to use a sufficient amount of sequencing data from a comparatively large number of genotypes to access the polymorphism level accurately (Ganal *et al*., 2012).

Besides all the advantages of the SNP array marker system, there are some disadvantages as well. It requires great effort for the array establishment, which can be expensive (Vos *et al*., 2015). This method is cost-effective only when used for a complete genome or larger sets of genotypes (Wang *et al*., 2014). Sometimes, it is difficult to determine the exact physical position of markers, and there might be

ascertainment bias due to the inadequacy of the available reference genome sequences (Moragues *et al*., 2010).

Single nucleotide polymorphic arrays have been successfully utilized in genotyping many plant species. Maize 600K SNP array (Unterseer *et al*., 2014), Barley 50K iSelect SNP array (Bayer *et al*., 2017), and Rice700K SNP array (McCouch *et al*., 2016) are some of the available SNP arrays for diploid plant species. Illumina alfalfa SNP array (Li *et al*., 2014), 8K Infinium Potato SNP array (Felcher *et al*., 2012), Axiom_Arachis 58K SNP array (Pandey *et al*., 2017), Wheat 660K SNP array (Sun *et al*., 2016), 60K *Brassica napus* SNP array (Clarke *et al*., 2016) and 90K *Brassica napus* SNP array (Scheben *et al*., 2019) are some examples of SNP arrays for polyploid plant species.

1.5.3 Single Nucleotide Polymorphic (SNP) Arrays for *Brassica napus* L.

Single nucleotide polymorphic markers proved to be a valuable source in constructing genetic linkage maps (Parkin *et al*., 2005). A linkage map is a list of linkage groups ordered according to their co-segregation patterns (Cheema & Dicks, 2009; Rhonda *et al*., 1993). These maps represent the distances between each locus and their order on a chromosome (Cheema & Dicks, 2009). Numerous genetic linkage maps have been developed for a diverse array of crop species, including *B. napus* (Foisset *et al*., 1996; Parkin *et al*., 2005; Raman *et al*., 2013). Genetic linkage maps prove valuable in the investigation of the inheritance patterns of both qualitative and quantitative characteristics (Morgante & Salamini, 2003). In genetic linkage mapping, a vast collection of SNPs covering the whole genome is an essential factor in determining a mutation, SNPs flagged with a mutation, or SNPs responsible for quantitative trait loci

(Aranzana *et al*., 2005). Therefore, it is necessary to genotype a substantial quantity of progeny at multiple loci to create an accurate genetic map (Troggio *et al*., 2007).

To date, several attempts have been made to identify SNPs in the *B. napus* genome. Trick *et al*. (2009) identified 23 330 - 41 593 putative SNPs in two *B. napus* cultivars: Tapidor and Ningyou 7 by using Solexa transcriptome sequencing from Illumina. However, the majority (87.5 % - 91.2 %) of the identified SNPs were 'hemi-SNPs'. In another study, 604 SNPs were identified in nine commercially available *B. napus* varieties along with *B. rapa* and *B. oleracea* varieties, and three synthetic *B. napus* varieties (Durstewitz *et al*., 2010). One hundred amplicons derived from Expressed Sequence Tags (EST) from the NCBI EST database were used to identify SNPs (Durstewitz *et al*., 2010). In the study, the authors observed approximately one SNP for every 42 bp. For the validation of the identified SNPs for their applicability in genetic mapping, the Illumina GoldenGate assay was created (Durstewitz *et al*. 2010). Later high-density 6K Infinium^{TM} array for *B. napus* with 5306 SNPs was produced by using four *B. napus* cultivars (Dalton-Morgan *et al*., 2014). The authors screened 1070 samples achieving a high success rate: 95 % of SNPs were validated as true SNPs and 94.5 % of the SNPs in the 6K array were validated as polymorphic. Effective SNPs from this 6K Infinium array were further used to develop the 60K Infinium array (6.5 % of this) (Dalton-Morgan *et al*., 2014).

The 60K high-density SNP Illumina Infinium array for *B. napus* has been one of the most advanced SNP arrays with 52,157 markers (Mason *et al*., 2017). It also can be applied to *B. rapa* and *B. oleracea*. Clarke *et al*. (2016) used 24 528 374 SNP loci encompassing SNPs that had been previously verified. They used several criteria to

filter them to identify high-confidence SNP loci. Those criteria were: " (1) SNP positions without suitable flanking sequence (60 bp on at least one side of the SNP with no variation); (2) SNP positions with more than two variations within the surveyed genotypes; (3) SNP positions with high levels of heterozygous calls biased allele ratio, or missing data; (4) Illumina Assay Design Tool (ADT) score less than 0.6, and (5) SNP positions where the variation was the result of a transversion" (Clarke *et al*., 2016). This array offers comprehensive representation across the *B. napus* genome, enabling the identification of a single SNP marker for every 15 kb interval. However, the main drawback is that it is more biased toward the sub-genome A when compared with the sub-genome C (Clarke *et al*., 2016). The latest SNP array utilized for *B. napus* is the Brassica 90K Illumina Infinium SNP array, an upgraded version of the 60K high-density SNP Illumina Infinium array. It has been developed by integrating an extra 30,000 markers from the B genome of Brassica, broadening its applicability to all Brassica species (Tesfaye *et al*., 2023).

1.6 Introduction to Quantitative Trait Loci (QTL)

1.6.1 Population Development

The primary objective of QTL (Quantitative Trait Loci) mapping is to identify the chromosomal region that has a significant effect on a variation of a quantitative trait in a population by combining phenotypic data with genotypic data (Miles & Wayne, 2008; Morrell *et al*., 2012). Quantitative trait Loci mapping is also helpful for understanding how many QTL significant effects on a particular trait have, as well as how many QTL have additive effects and dominant effects on a trait, and also epistasis (Tanksley & Nelson, 1996). The crucial stages in QTL mapping involve the choice of two distinct parents, developing mapping populations, phenotyping, genotyping, construction of the

genetic map, QTL identification, and QTL validation (Collard *et al*., 2005; Nadeem *et al*., 2018).

Two or more segregating populations are required for QTL analysis (Crepieux *et al*., 2004). Parents selected to create these populations should differ from each other at least for one or more traits of interest. Different types of mapping populations could be used for QTL mapping, each with its advantages and disadvantages. Biparental populations, including F_2 populations, backcrosses (BC), doubled haploids (DH), or recombinant inbred lines (RIL), could be used as the experimental population for QTL mapping (Mathew *et al*., 2021; Young, 1994). Populations that are derived by crossing the F_1 hybrid to one of their parents are referred to as backcross populations. The main advantage of using this population in QTL mapping is that population creation requires only a short period of time (Li *et al*., 2019). The main disadvantage of using this population is related to the instability of genetic and phenotypic data due to the high level of heterozygosity (Seymoura *et al*., 2012). Recombinant inbred lines are produced by inbreeding F_2 individuals. However, the generation of this population is time-consuming (Balasubramanian *et al*., 2009). Doubled haploid populations are generated by inducing chromosome doubling in haploid cells such as ovaries, unfertilized ovules, and immature pollen grains (microspores) (Zargar *et al*., 2022). Generating a DH population can be achieved solely in plant species that can be readily cultured through tissue culture methods (Seymoura *et al*., 2012). The major advantage of using DH populations and recombinant inbred lines for plant breeding purposes is the ability to produce homozygous genotypes with fixed genetic traits at each locus (Brauner *et al*., 2019). The size of the population is a very important factor to consider in genetic mapping. Usually, 50 - 250 individuals are required for a study. Nonetheless, opting for

a larger population size results in finer resolution in genetic mapping (Mohan *et al*., 1997).

1.6.2 Quantitative Trait Loci Calculation

The linkage between the markers and the trait is determined by the logarithm of the ratios, which is commonly known as the logarithm of odds (LOD) (Rice *et al*., 2008). It is a statistical estimate used to determine whether two genetic loci in a particular chromosome are close enough to be inherited together (Morton, 1996). Generally, linkages with a LOD value greater than three are used to construct the genetic map (Collard *et al*., 2005). Depending on the situation, the LOD value could be lowered (Collard *et al*., 2005). The Kosambi mapping and the Haldane mapping functions are routinely used for this conversion (de Tan & Fornage, 2008; Huehn, 2011). The Haldane mapping function was introduced without considering the interference, while the Kosambi mapping function was introduced considering the interference (Akond *et al*., 2019). However, because the Kosambi mapping function fits with experimental data, it has been widely used over the past years (Peleman *et al*., 2005).

1.6.3 Quantitative Trait Loci Detection

The most widely used methods to detect QTL are simple interval mapping and composite interval mapping. The simple interval mapping method uses the likelihood ratio at every position between adjacent markers to detect existing QTL in a particular interval along chromosomes simultaneously (Lander & Botstein, 1989). During this process, QTL that do not exist might appear due to errors in the method (Haley & Knott, 1992). The composite interval mapping technique is regarded as more precise, as it employs multiple marker regression analyses, integrating an extra subset of markers

into the statistical model alongside the adjacent marker pair (Jansen, 1993). The power of detection, the location of the QTL and the effects of the QTL are more accurate when using composite interval mapping (Li *et al*., 2007)**.**

1.6.4 Quantitative Trait Loci for Drought Stress Tolerance Traits in Crop Plants

The integration of QTL in breeding efforts has become successful in increasing productivity in many crop species (Hu & Xiong, 2014; Kushanov *et al*., 2021; Raboanatahiry *et al*., 2018). Numerous previous studies deal with water stress. By using a back cross inbred population developed from a cross between *O. sativa* variety Japonica and variety Nipponbare, Ishimaru *et al*. (2001) identified two QTL on chromosome 1 responsible for adaxial stomatal frequency, two QTL on chromosome 3 responsible for abaxial stomatal frequency, and one QTL on chromosome 8 responsible for leaf rolling due to dehydration under drought stress conditions. In the same study, the authors demonstrated that QTL for adaxial and abaxial stomatal frequency overlapped, suggesting that the same QTL controls stomatal frequency on both sides of the leaves (Ishimaru *et al*., 2001). Another study using *O. sativa* DH populations of 154 genotypes derived from the varieties Japonica and Indica, identified putative candidate QTL for osmotic adjustment, total root dry weights, and root length under drought stress (Zhang *et al*., 2001). There were five putative QTLs for osmotic adjustment (chromosomes 1, 2, 3, 8, and 9), five candidate QTLs for total root dry weight (Chromosome 1, 2, 4, 6, 10), and one QTL on chromosome 1 for penetrated root length (Zhang *et al*., 2001). Besides *O. sativa*, QTL analyses related to water stress have been documented in other species, including *T. aestivum* and *Z. mays*. Ren *et al*. (2021) used 309 genotypes from a population of recombinant inbred lines developed from a cross between Berkut and Worrakatta varieties of *T. aestivum* to identify eighteen QTL

related to drought tolerance traits. These traits included germination potential, germination rate, germination index, and coleoptile length. Among the detected QTL there were fifteen newly discovered QTL (Ren *et al*., 2021). In the same species, twelve additional QTL (from 1B, 2A, 2B, 2D, 3A, 4A, 4B, 5D, and 6B) were linked to leaf rolling during water stress (Verma *et al*., 2020). In *Z. mays*, a total of eight QTL for grain yield from chromosomes 1, 3, 5, 7, 9, and 10, and ten QTL for anthesis silking intervals from chromosomes 1, 2, 3, 5, 8, 9, and 10, were identified during water deficit (Almeida *et al*., 2013).

Despite the impact water stress has on canola's productivity (Wan *et al*., 2009), few studies document the response mechanisms to this stress or the identification of QTL linked to *B. napus* tolerance to water shortage. In this context, Li *et al*. (2014) identified several QTL linked to plant height (rph1.3, rph3.3, rph2.4, and rph5.3), root length (rrl1a.3 and rrl15.3), shoot dry weight (rsdw1.4, rsdw13.3, rsdw15.4, rsdw16.3, and rsdw7.3), and root dry weight (rrdw1.33 rrdw15.4, rrdw20.4) in *B. napus.* By using a field irrigation system, Fletcher *et al*. (2015) detected QTL for root morphology, flowering time, and yield in *B. napus* under drought stress and were able to identify twenty additive QTL co-localized to certain regions on linkage groups A3, A10, and C2. Based on these results, it was concluded that QTL for flowering time overlapped with those responsible for root architecture and that the two regions on A10 and C2 accounted for a significant amount of variation in each trait, and their effects were consistently greater than other QTL detected in other linkage groups (Fletcher *et al*., 2015). Several QTL for *B. napus* germination-related traits, located in chromosomes A2 and C1, have been recently identified in a study done by using 15 % PEG (Gad *et al*., 2021). Among them, seven QTL associated with the root-to-shoot length ratio under

drought stress were located on A2, A6, and C3. For root length, three QTL were detected in chromosomes C1 (qRL-11-1, qRL-11-2, and qRL-11-3) and qRL-9-1 and qRL-19-1 in chromosomes A9 and C9. These studies collectively contribute to a deeper understanding of the genetic factors influencing responses of *B. napus* to water stress and provide valuable insights into the complex interactions between various traits and QTL in the face of challenging environmental conditions.

1.7 Patent Information Related to Drought Stress Tolerance in *Brassica napus* L.

In recent years, there has been a rise in the number of patent applications related to drought stress tolerance (Nuccio *et al*., 2018). Pioneer® Optimum® AQUAmax® is an example of one such commercialized drought-tolerant corn hybrid under the patent number US-5491295-A (DuPont Pioneer, Johnston, IA, USA). This variety achieved drought tolerance by traditional breeding methods. Monsanto's Genuity™ DroughtGard™ (Monsanto Co., St. Louis, MO, USA) corn variety protected by one or more patents (US-7786353-B2, US 8450561-B2, US-8735659-B2, US-9228197-B2, US-10100328-B2 US-10428345-B2, and US-10851385-B2) exhibited drought tolerance through introduction of a cold shock protein B. Drought tolerant soybean variety HB4® was introduced by Verdeca, the alliance of Arcadia Biosciences and Bioceres under the patent number US 2022/0090114-A1 (Bioceres LLC, Wilmington, DE, USA). This variety showed enhanced drought tolerance through the expression of *HaHB4* gene, without detrimental effects on other agronomic traits. The patent with publication number US-10687492B1, issued to Monsanto Technology LLC (St. Louis, MO, US), is an example of a canola variety that has been patented, listing abiotic stress tolerance (including drought stress tolerance) in its description.

As illustrated above, drought tolerance involves a complex interplay of physiological, biochemical, and morphological traits. Quantitative trait loci analysis helps dissect these traits, allowing breeders to precisely target specific genetic regions that confer tolerance. This breeding approach leads to more predictable and reliable results, ensuring that the desired traits are effectively incorporated into new cultivars.

Based upon these premises, the objective of this thesis is to identify quantitative trait loci (QTL) linked to *B. napus* tolerance to water stress mimicked by applications of 10 % polyethylene glycol-6000 (PEG-6000).

2.0 MATERIALS AND METHODS

2.1 Plant Materials

Two *Brassica napus* L. doubled haploid (DH) populations (1901 and 1904) were used in this study. The 1901 *B. napus* DH population was produced from a cross between two parents: RRHR7705 (drought-sensitive) x Sentry (drought-tolerant) (Rimmer *et al*., 1998). Parents for the 1904 population were Red River 1826 (drought-sensitive) (McVetty *et al*., 2006) x Reston (drought-tolerant). The two populations were developed through microspore culture (Fletcher et al. 1998). A total of 150 genotypes from each population (300 in total) were selected based on the availability of seeds produced. All genotypes, including the parents, were phenotyped and genotyped as described below.

2.2 Phenotypic Characterization of the Two Populations

The 300 genotypes and the parents of the two populations were evaluated for their survival under water stress (Gad *et al*., 2021; Zhang *et al*., 2015). Seeds were germinated in pipette tip boxes on cheesecloth soaked with deionized water and incubated in the dark at 25 °C. When radicles were 3-5 mm long, seedlings from each genotype were transferred to hydroponic boxes filled with ½ strength Hoagland's solution (Arnon & Hoagland, 1939; Zhang *et al*., 2015). The seedlings were grown under controlled environmental conditions with 18 °C-22 °C temperature with a 16 h photoperiod. After 10 days, half of the seedlings of each genotype were exposed to ½ strength Hoagland's solution supplemented with 10 % polyethylene glycol (PEG) 6000 to induce water stress (Guo *et al*., 2017; Michel & Kaufmann, 1973; Michel, 1983; Zhang *et al*., 2015) for 48 hours (Zhou *et al*., 2007). The concentration of PEG and the

duration of water stress were selected empirically based on preliminary experiments. Under these conditions, water stress symptoms were uniform among plants and easily identifiable. The other half of the seedlings (control) were exposed to the exact same conditions but with the exclusion of PEG. Hydroponic boxes were supplied with continuous aeration by bubbling ambient air. The experiment was arranged in a randomized complete block design (RCBD) with three biological replicates (each with four seedlings) for each genotype in both treatments. Experiments for each biological replicate were conducted at different times. This experiment was conducted in six cycles. Within one cycle all three biological replicates of 50 genotypes were completed. After 48 hours, control (-PEG) and water-stressed (+PEG) plants were harvested, and fresh weight (FW), and dry weight (DW) were recorded. The drought stress index (DSI) was calculated for each genotype using the following formula: $\mathrm{DSI} = \frac{\text{Weight of the treated seedlings (+PEG)}}{\text{Weight of the controlled seedlings (−PEG)}} \times 100\%$ (Kpoghomou *et al*., 1990; Zhang *et al*., 2015).

2.3 Genotypic Characterization of the Two Populations

Genomic DNA was extracted from 2-week-old leaf tissue using the DNeasy® Plant Mini Kit, exactly as recommended in the instructions. DNA quality was evaluated by using the Nanodrop 2000 (Thermo Scientific, Massachusetts, USA) with criteria of >1.8 for 260/280 and 260/230 ratios. The Brassica Infinium 90K SNP BeadChip Array (Illumina Inc., San Diego, CA, USA) was used to genotype all 300 genotypes along with the parental genotypes (Whalen *et al*., 2018). Purified DNA samples were hybridized to the array following the manufacturer's standard protocol (Illumina Inc., San Diego, CA, USA). Genotyping was conducted in the lab of Dr. Isobel Parkin at

Agriculture and Agi-Food Canada, Saskatoon, Canada. The default clustering parameters were used to analyze the raw data using the genotyping module of Illumina® GenomeStudio 2.0.5 (Illumina Inc., San Diego, CA, USA).

2.4 Construction of the Genetic Linkage Maps

A total of 150 genotypes from each population were used to create the genetic linkage map. Before constructing the genetic maps, SNPs were filtered by excluding SNPs with poor-quality data (Ren *et al*., 2016). All SNPs that were monomorphic between the two parents and those with > 0.2 missing data were excluded from the linkage analysis (Clarke *et al*., 2016). Furthermore, SNPs that deviate from the expected segregation ratio were filtered out. Finally, 5174 SNPs from the 1901 population and 8792 SNPs from the 1904 population were used to create the final linkage maps. The genetic linkage map was created using MSTMap (Wu *et al*., 2008).

2.5 Identification of Quantitative Trait Loci (QTL) Associated with Water Stress Tolerance

The QTL linkage analysis was carried out using Qgene v.4.4.0 (Joehanes & Nelson, 2008). The genetic map, along with the phenotypic BLUPs (adjusted mean values of raw data and DSI values) (Thambugala *et al*., 2021), was used to identify QTL through inclusive composite interval mapping (Li *et al*., 2007), and only QTL having minimum LOD threshold value above 2 were reported. The method outlined by Wang *et al*. (2016) was used for the QTL nomenclature. The consensus QTL were named with the 'q' followed by the name of traits (DSI or RD for raw data), hyphen (-), FWP, FR, and FS (fresh weight of whole plant, root, and shoot, respectively) or DWP, DR, and DS (dry weight of whole plant, root, and shoot respectively), and the linkage group. A number

followed by the linkage group was added if more than one QTL was located on the same linkage group.

To identify the candidate genes for water stress tolerance, probe sequences of SNPs flanking these QTL were blasted to the *B. napus* reference genome assemblies cultivar Darmor-*bzh* version 10 (Rousseau-Gueutin *et al*., 2020) using BLASTN (Wright *et al*., 2020) standalone tool. From the pool of putative candidate genes, genes that are directly associated with water stress tolerance were selected, based on previous reports (Alexieva *et al*., 2001; Chen & Jiang, 2010; Hasanuzzaman *et al*., 2020; Ma *et al*., 2004; Vishwakarma *et al*., 2017; Zhu, 2016).

2.6 Statistical Analysis

The software Multi Environment Trial Analysis with R for Windows (META-R) version 6.0 was used to calculate the best linear unbiased predictors (BLUPs) for all the considered traits (Alvarado *et al*., 2020). The PROC GLIMMIX procedure in SAS version 9.4 was used for the Analysis of variance (ANOVA) (Ruiz *et al*., 2023). Replicate nested within the cycle was considered a random effect, while genotype was considered a fixed effect. Histograms were generated by using RStudio version 2021.09.2.

3.0 RESULTS

3.1 Phenotypic Data Analysis

3.1.1 Fresh Weight

Within the 1901 population, the drought stress index (DSI) values of the susceptible parent (RRHR 7705) were 18.45 % (whole plant), 30.87 % (root), and 15.59 (shoot) (Figure 2). The values of the tolerant parent (Sentry) were 78.70 % (whole plant), 76.08 % (root), and 79.73 % (shoot). A total of seven genotypes (whole plant), forty genotypes (root), and five genotypes (shoot) demonstrated possible transgressive segregation. The susceptible parent (Red River 1826) used for the 1904 population exhibited DSI values of 17.82 % (whole plant), 27.23 % (root), and 15.91 % (shoot), compared to respective values of the tolerant parent (Reston) of 73.02 % (whole plant), 74.98 % (root), and 76.19 % (shoot) (Figure 2). Possible transgressive segregation was observed in a total of eight genotypes at the whole plant level, one genotype at the root level, and seven genotypes at the shoot level. Analysis of variance showed significant differences ($p < 0.001$) among the genotypes for all the fresh weight traits in the 1901 population (Table 1) and in the 1904 population (Table 2).

3.1.2 Dry Weight

For the dry weight data in the 1901 population, the DSI values of the susceptible parent (RRHR 7705) were 20.99 % (whole plant), 23.81 % (root) and 20.11 % (shoot), while those of the tolerant parent (Sentry) were 72.31 % (whole plant), 72.40 % (root) and 74.49 % (shoot) (Figure 3). A total of three genotypes (whole plant), and five genotypes (root and shoot) demonstrated possible transgressive segregation. Within the 1904 population, the susceptible parent (Red River 1826) yielded a DSI value of 32.10 % for

the whole plant, 39.72 % for root, and 29.41 % for shoot. This was in contrast to the tolerant parent (Reston) with DSI values of 60.31 % (whole plant), 64.32 % (root), and 62.89 % (shoot) (Figure 3). Possible transgressive segregation was observed in a total of eight genotypes at the whole plant level, forty three genotypes at the root level, and six genotypes at the shoot level. Analysis of variance showed significant differences ($p < 0.001$) among the genotypes for all the dry weight-related traits in the 1901 population (Table 1) and the 1904 population (Table 2).

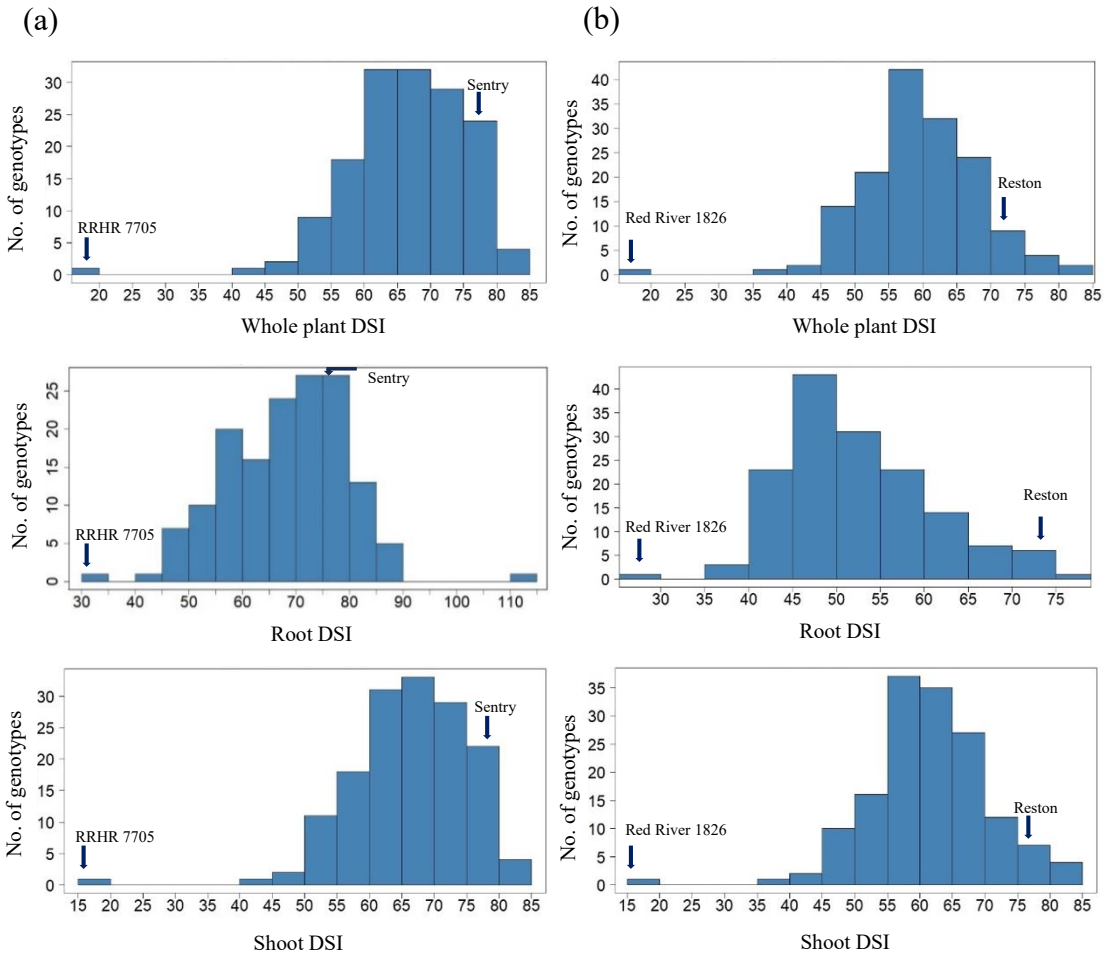

Figure 2: Frequency distribution of best linear unbiased predictors (BLUPs) for whole plant DSI, root DSI, and shoot DSI of (a) 1901 population (Sentry-tolerant parent, RRHR 7705- sensitive parent) and (b) 1904 population (Reston-tolerant parent, Red River 1826- sensitive parent) calculated from fresh weight.

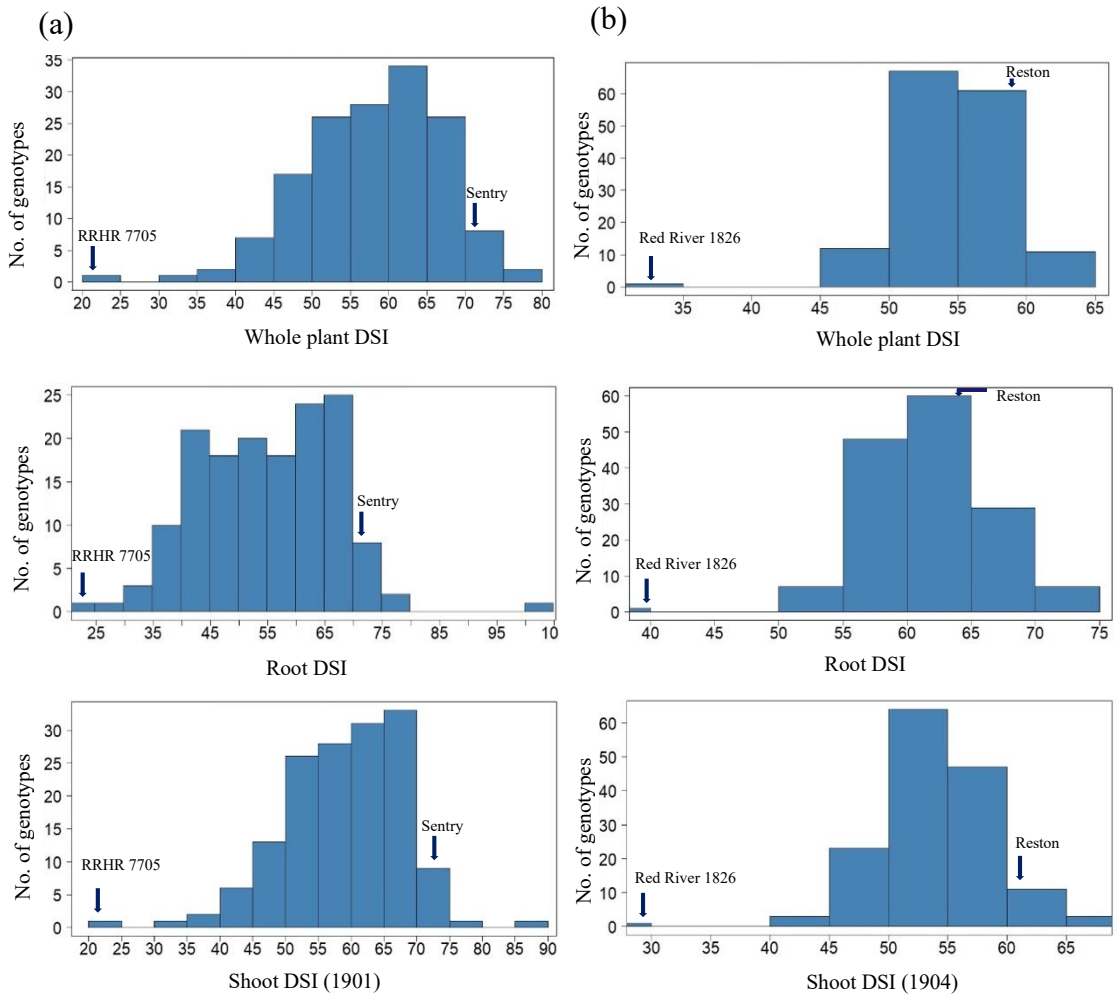

Figure 3: Frequency distribution of best linear unbiased predictors (BLUPs) for whole plant DSI, root DSI, and shoot DSI of (a) 1901 population (Sentry-tolerant parent, RRHR 7705- sensitive parent) and (b) 1904 population (Reston-tolerant parent, Red River 1826- sensitive parent) calculated from dry weight.

Table 1: Analysis of variance for BLUPs of whole plant DSI, root DSI, and shoot DSI of the fresh weight and the dry weight of the 1901 population. F values for genotype and replicate (cycle) are shown.

Source of variation	Fresh weight DSI			Dry weight DSI		
	Whole plant DSI	Root DSI	Shoot DSI	Whole plant DSI	Root DSI	Shoot DSI
Genotype	5.09***	5.01***	4.69***	3.11***	3.24***	2.87***
Replicate (Cycle)	0.019	0.012	0.007	0.046	0.039	0.04
Grand mean of DSI	66.64	68.53	66.55	58.44	59.22	52.02
LSD[a]	0.066114	0.08601	0.06812	0.082207	0.11216	0.086185

***p < 0.001; ** p < 0.01; * p < 0.05
[a]Least significant difference

Table 2: Analysis of variance for BLUPs of whole plant DSI, root DSI, and shoot DSI of the fresh weight and the dry weight of the 1904 population. F values for genotype and replicate (cycle) are shown.

Source of variation	Fresh weight DSI			Dry weight DSI		
	Whole plant DSI	Root DSI	Shoot DSI	Whole plant DSI	Root DSI	Shoot DSI
Genotype	3.11***	3.24***	2.87***	2.38***	1.96***	2.35***
Replicate (Cycle)	0.014	0.095	0.015	0.01	0.075	0.03
Grand mean of DSI	58.44	59.22	52.02	54.84	61.95	53.90
LSD[a]	0.082207	0.11216	0.086185	0.11824	0.13492	0.1249

***p < 0.001; ** p < 0.01; * p < 0.05
[a]Least significant difference

3.2 Genetic Linkage Map

A linkage map was developed for each of the 1901 and 1904 populations. The linkage map of the 1901 population consisted of 26 linkage groups. The total map length was 1195.852 cM. The linkage map of the 1904 population consisted of 24 linkage groups. The total map length was 2680.252 cM. In some instances, a chromosome consists of more than one linkage group. There were three linkage groups in A1 and two linkage groups in A2, A6, A10, C3, C5, and C7 in the linkage map of the 1901 population. There were two linkage groups in A5, A6, A7, C3, C4, and C5 in the linkage map of the 1904 population. In the linkage map of the 1901 population, linkage group 4 is shared between A1 and C1 chromosomes. In the linkage map of the 1904 population, linkage group 6 is shared between A7 and C6 chromosomes. This might be a result of a possible homeologous translocation between the two chromosomes in the parental genotypes.

3.3 QTL Analysis

Eighteen QTL linked to drought stress tolerance in *B. napus* seedlings were identified. They were located on 6 different chromosomes (Table 3). A single QTL was located on the A9 chromosome (in the A9_5 linkage group), while the C3 chromosome contained two QTL (in the C3_11 linkage group). Additionally, three QTL were identified on the A2 (one in A2_5 and two in A2_7 linkage groups), A4 (in A4_10 linkage group), and C7 chromosomes (in C7_22 linkage group), while a total of six QTL were identified on the A3 chromosome (in A3_2 linkage group) (Figure 4). The majority of the QTL were identified on the A3 chromosome. The range of phenotypic variation (PVE) among the identified QTL was 6.0 to 10.4 (Table 3).

Table 3: Detailed information of identified QTL for water stress tolerance in *B. napus* L.

Trait	QTL designation	LG[a]	Population	QTL region in cM	LOD[b]	PVE[c]	Add[d]
Whole plant DSI	qDSI-FWP-A4-10	A4-10	1901	0 - 12.204 - 12.737	2.30	6.8	-2.118
Root DSI	qDSI-FR-A4-10	A4-10	1901	17.682 -18.016 - 19.003	2.11	6.3	-0.038
	qDSI-FR-C7-22-1	C7-22	1904	102.625 - 110.644 - 111.593	2.23	6.7	2.192
	qDSI-FR-C7-22-2	C7-22	1904	111.593.114.446 - 119.784	2.09	6.3	2.094
	qDSI-DR-C7-22	C7-22	1904	100.958 - 104.293 - 119.784	2.44	7.3	1.242
Shoot DSI	qDSI-FS-A4-10	A4-10	1901	0 - 12.204 - 12.737	2.196	6.5	-2.045
	qDSI-DS-A9-5	A9-5	1904	186.764 - 187.72 - 188.515	2.024	6.1	1.156
Raw data – whole plant	qRD-FWP-A2-7	A2-7	1904	85.748 -96.48 -96.803	3.00	8.9	0.07
	qRD-FWP-A3-2	A3-2	1904	30.267 - 42.834 - 54.022	3.48	10.3	0.076
	qRD-DWP-A3-2	A3-2	1904	30.267 - 42.834 - 54.022	3.262	9.7	0.004
	qRD-DWP-A2-5	A2-5	1901	0 - 0 - 1.09	2.206	6	-0.002
Raw data – root	qRD-FR-A3-2-1	A3-2	1904	37.846 - 42.834 - 44.647	2.25	6.8	0.009
	qRD-FR-A3-2-2	A3-2	1904	175.399 - 177.201 - 179.005	2.11	6.2	-0.008
	qRD-FR-C3-11	C3-11	1904	78.655 - 81.658 - 81.992	2.13	6.4	0.009
	qRD-DR-C3-11	C3-11	1904	60.428 - 61.429 - 72.826	2.348	7	0.001
Raw data - shoot	qRD-FS-A2-7	A2-7	1904	85.748 - 96.48 - 96.803	3.079	9.1	0.061
	qRD-FS-A3-2	A3-2	1904	30.267 - 42.834 - 54.022	3.512	10.4	0.066
	qRD-DS-A3-2	A3-2	1904	30.267 - 42.834 - 54.022	3.321	9.8	0.004

[a]LG, linkage group

[b]LOD, peak LOD score; LOD significance threshold = 2,

[c]PVE, phenotypic variation explained (R^2; %)

[d]Add, additive effect of allele substitution

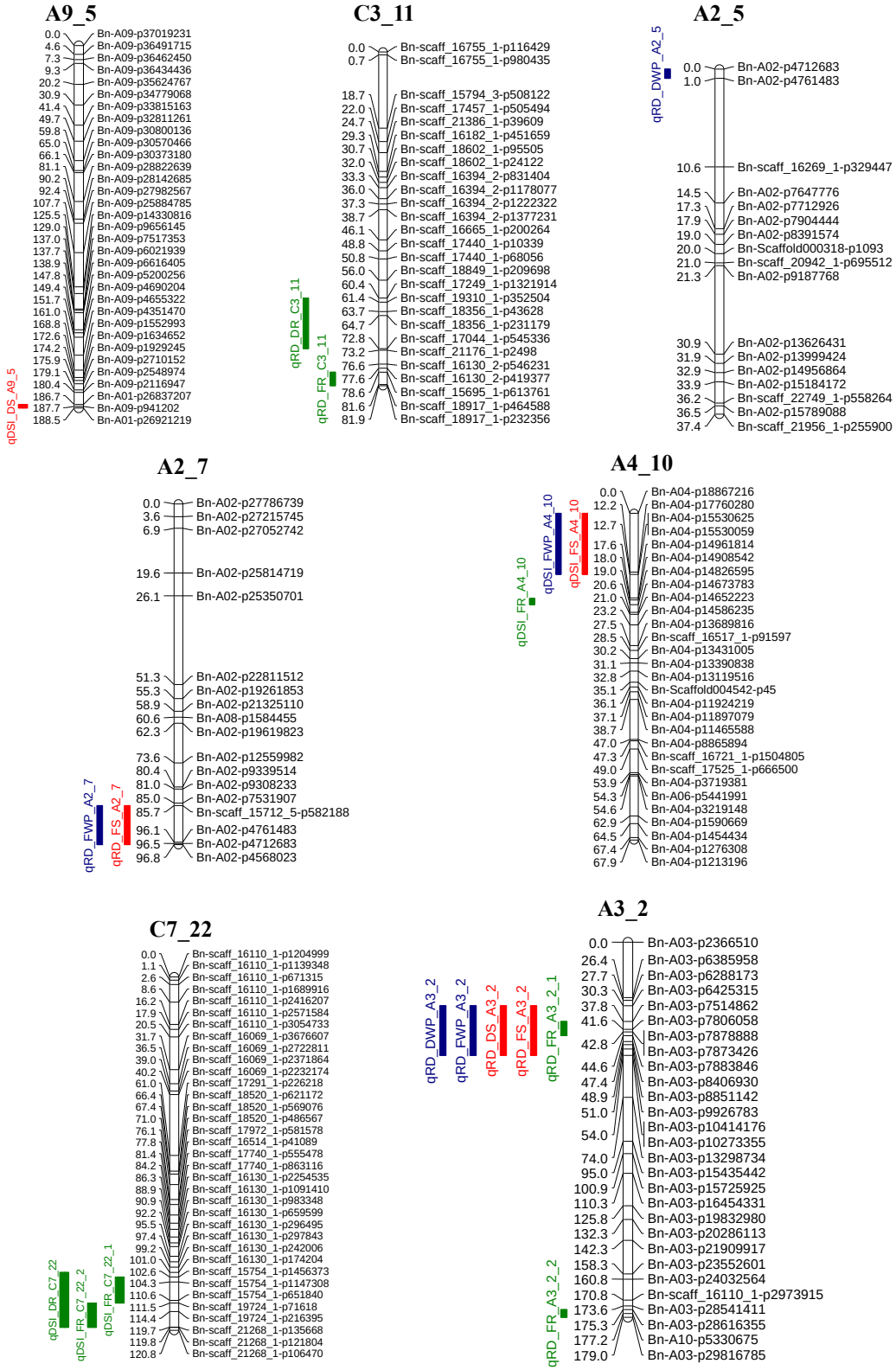

Figure 4: The location of QTL for water stress tolerance. The linkage groups are shown at the top, the genetic distance (cM) is on the left, and markers are shown on the right. The QTL associated with whole plant weight, root, and shoot are indicated in blue, green, and red respectively.

3.4 Identification of Putative Candidate Genes

Collectively, 2154 putative candidate genes for drought stress tolerance in *B. napus* were identified for all the listed QTL (Table 4). Among them, 213 genes (Supplementary Table S1) were chosen as being directly associated with drought stress tolerance based on nine functional categories known to be affected by the stress (antioxidants, phytohormones, osmotic adjustments, late embryogenesis abundant (LEA) proteins, photosynthesis, respiration, signal transduction, hydraulic conductance, and growth and development). The A3 chromosome harbored the highest number of potential candidate genes and the highest count of genes that are intrinsically correlated with water stress tolerance, whereas the least quantity of putative candidate genes were observed on the A9 chromosome (Table 4).

Table 4: Summary of the numbers of putative candidate genes in each QTL

QTL designation	Chromosome	Total number of putative candidate genes	Number of genes directly related to drought stress tolerance
qRD-DWP-A2-5	A2	9	1
qRD-FS-A2-7	A2	22	0
qRD-FWP-A2-7	A2		
qRD-FR-A3-2-1	A3	74	8
qRD-FR-A3-2-2	A3	579	77
qRD-FS-A3-2	A3	286	14
qRD-DS-A3-2	A3		
qRD-FWP-A3-2	A3		
qRD-DWP-A3-2	A3		
qDSI-FR-A4-10	A4	19	2
qDSI-FS-A4-10	A4	730	62
qDSI-FWP-A4-10	A4		
qDSI-DS-A9-5	A9	14	4
qRD-FR-C3-11	C3	152	19
qRD-DR-C3-11	C3		
qDSI-FR-C7-22-1	C7	110	15
qDSI-FR-C7-22-2	C7	159	11
qDSI-DR-C7-22	C7		

4.0 DISCUSSION

Understanding the genetic foundation of drought stress tolerance in *Brassica napus* L. is a key approach in breeding high-yielding *B. napus* cultivars that can withstand water stress conditions. Plants' responses to water stress are governed by a complicated genetic network that involves many interacting factors together (Osakabe *et al*., 2014). However, only a few studies were previously conducted on drought tolerance in *B. napus* (Li *et al*., 2014; Wang *et al*., 2017; Zhu *et al*., 2016). Therefore, the identification of QTL and potential candidate genes involved in water stress tolerance is an important step toward the development of drought-tolerant germplasm (Hu & Xiong, 2014; Kushanov *et al*., 2021; Raboanatahiry *et al*., 2018).

Plants experiencing water scarcity prioritize their survival in challenging environments over their growth and maturation (Kasuga *et al*., 1999; Zhang *et al*., 2020). They invest a greater amount of their energy into mechanisms that aid in adapting to these conditions, with a consequential decrease in overall growth (Liu *et al*., 2004). As a result, the overall biomass of plants under drought stress decreases. Nevertheless, this occurrence is contingent on the plant species and depends on the duration and severity of the stress (Eziz *et al*., 2017). In the case of Brassica, imposition of the water stress depresses the whole plant's fresh and dry weight (Figures 1 and 2). While roots are the initial organs to detect and respond to water scarcity, shoot development is also impacted (Pooter *et al*., 2012), as clearly revealed in our study, showing an even higher susceptibility of the shoot, relative to the root, to the stress (Table 1 and 2). Indeed, shoots display high susceptibility to water shortage, even under mild-moderate

conditions of drought stress, leading to potential halting of growth while roots continue to grow and elongate (Sharp *et al*., 2004; van der Weele *et al*., 2000). The reduction in both cell division and cell expansion during drought stress contributes equivalently to the decline in shoot growth (Arve *et al*., 2011). During water stress conditions, plants often shift resource allocation towards increasing root biomass while reducing allocation to the shoot, given the essential role of roots in water and nutrient absorption (Eziz *et al*., 2017; Poorter *et al*., 2012). Nevertheless, this growth pattern is strongly species-specific. This phenomenon has been demonstrated in crop plants including *B. napus* (Channaoui *et al*., 2017; Li *et al*., 2018; Sharif *et al*., 2018; Yang *et al*., 2007), *Triticum aestivum* L. (Khan *et al*., 2010), and *Glycine max* L. (Creelman *et al*., 1990; Specht *et al*., 2001), highlighting the distinct adaptive strategies displayed by different plant species in response to water shortage.

The successful integration of QTL into breeding strategies has led to increased production in numerous crop species, including *B. napus* (Fletcher *et al*., 2015; Ishimaru *et al*., 2001; Ren *et al*., 2021; Verma *et al*., 2020). In this study, 18 QTL (4 QTL from the 1901 population and 14 QTL from the 1904 population) linked to drought stress tolerance were identified on 6 different chromosomes (Table 3). The results of this study show that the certain QTL responsible for drought stress tolerance and contributing to various traits co-localize on the same chromosome and within the same genomic region or neighboring regions (qDSI-FS-A4-10 and qDSI-FWP-A4-10 on A4, qRD-FS-A2-7 and qRD-FWP-A2-7 on A2, and qRD-FS-A3-2, qRD-FWP-A3-2, qRD-DS-A3-2, and qRD-DWP-A3-2 on A3) (Figure 4). This suggests that specific genomic regions might harbor clusters of genes that regulate multiple traits simultaneously, confirming the interplay of various traits in water stress tolerance (Lu *et al*., 2008). A

recent study by Raman *et al*. (2022) has echoed these observations; specific traits like number of days required for flowering, height of plants, and seed yield in water-stressed *B. napus* plants are controlled by shared genomic regions. A similar result was reported by Gad *et al*. (2021), where the length of both roots and shoots of *B. napus* were associated with co-localized QTLs. Overall, these independent studies highlight the interconnected nature of diverse attributes contributing to drought stress tolerance and overall performance in plants.

In our research, the identified QTL linked to drought stress tolerance were located on chromosomes A2, A3, A4, A9, C3, and C7 (Table 4). These chromosomes might play an important role in allowing plants to cope with water shortage. In water-stressed *B. napus* plants, days to flower, root pulling force, and yield were linked to QTL located on chromosome A3 (Fletcher *et al*. 2015), carbon allocation connected to drought avoidance was linked to chromosomes A2, A4, A9 and C7 (Mekonnen *et al*. 2020), while shoot length and root: shoot ratio were associated to QTL on chromosomes A2 and C3 (Gad *et al*. 2021). More recently, Raman *et al*. (2022) reported QTL associated with water use efficiency in *B. napus* located on the A9 chromosome. Although the genomic regions of the majority of these QTL do not directly overlap with the QTL reported in our study, there are a few instances where the genomic regions of the identified QTL in our study precisely align with those found in previous research related to water stress tolerance. The QTL regions of qRD-FS-A2-7 and qRD-FWP-A2-7 showed complete alignment with the QTL region of qcR/s-2-1 (root: shoot ratio), as reported by Gad *et al*. (2021). Additionally, the QTL region of qDSI-FR-A4-10 overlapped with the QTL associated with plant height in the A4 chromosome, which was previously reported by Mekonnen *et al*. (2020). Based on this, it is plausible that

the identified QTL regions might play a significant role in enhancing drought stress tolerance in *B. napus*.

Of the 2,154 putative candidate genes identified for all the 18 QTL, we identified 213 as being associated with drought tolerance (Supplementary Table S1) based on previous reports (Alexieva *et al*., 2001; Chen & Jiang, 2010; Hasanuzzaman *et al*., 2020; Ma *et al*., 2004; Vishwakarma *et al*., 2017; Zhu, 2016).

The antioxidant machinery plays a key role in water stress tolerance, with catalases and peroxidases being the most important enzymes (Carvalho, 2008). A03p63280.1_BnaDAR located in QTL qRD-FR-A3-2-2 is orthologous to catalase. Enhanced activity of catalase, which is a major scavenger of H_2O_2, was observed in water-stressed *Oryza sativa* L. (Sharma & Dubey, 2005), *Cicer arietinum* L. (Mafakheri *et al*., 2011), and *B. napus* (Abedi & Pakniyat, 2010). A03p63220.1_BnaDAR located in QTL qRD-FR-A3-2-2 is orthologous to ascorbate peroxidase scavenging of H_2O_2 using ascorbate as a substrate (Miao *et al*., 2006), while BnaA03T0167800WE is orthologous to glutathione peroxidase in QTLqRD-FS-A3-2, qRD-DS-A3-2, qRD-FWPA3-2, and qRD- DWP-A3-2. The relevance of the antioxidant system for water stress tolerance is also supported by cytochrome P450. Besides playing a role in hormone and secondary metabolite biosynthesis, it is heavily involved in the synthesis of antioxidants, which collectively contribute to plants' ability to withstand drought stress (Pandian *et al*., 2020). A03p65090.1_BnaDAR, A03p65100.1_BnaDAR, A03p65110.1_BnaDAR, and A03p64120.1_BnaDAR in QTL qRD-FR-A3-2-2, A04p33900.1_BnaDAR and A04p33910.1_BnaDAR in QTL qDSI-FS-A4-10 and qDSI-FWP-A4-10, and C03p56000.1_BnaDAR and C03p56010.1_BnaDAR in QTL

qRD-FR-C3-11 and qRD-DR-C3-11 are all orthologous to cytochrome P450. Enhanced activity of cytochrome P450 under water-stressed conditions was reported in several crop species, including *Medicago truncatula* L. (Xia *et al*., 2021), *O. sativa* (Cai *et al*., 2015), *T. aestivum*, and *Zea maize* L. (Li & Wei, 2020).

Plant growth regulators are also essential for regulating drought responses in plants. Among them, ABA is widely recognized as the primary hormone responsible for mediating stress responses (Mehrotra *et al*., 2014; Tuteja, 2007). Three ABA receptor-like genes were reported in QTL qDSI-FS-A4-10, qDSI-FWP-A4-10, and qDSI-FR-C7-22-2, qDSI-DR-C7-22 (A04p29340.1_BnaDAR, A04p30720.1_BnaDAR, and C07p29150.1_BnaDAR respectively). Abscisic acid receptor-like genes are also involved in drought stress tolerance mechanisms in *T. aestivum* (Mao *et al*., 2022), *Z. maize* (He *et al*., 2018), *Brassica juncea* L. (Cheng *et al*., 2019). Stomatal conductance is controlled by ion fluxes in guard cells through Ca^{2+}-dependent and Ca^{2+}-independent pathways, both mediated by ABA (Murata *et al*., 2001) and it has been extensively studied throughout the past few decades (Assmann, 2003; Cutler *et al*., 2010). One ABA-induced stomatal closure gene (A04p34310.1_BnaDAR) was also identified in qDSI-FS-A4-10 and qDSI-FWP-A4-10.

Besides ABA, several studies have highlighted the role of auxin in modulating drought stress tolerance mechanisms in plants, particularly through the restructuring of physiological and metabolic turnover under drought conditions (Korver *et al*., 2018; Rosquete *et al*., 2013). Genes responsible for controlling auxin-related drought tolerance mechanisms initiate these pathways by engaging auxin-responsive factors (Roosjen *et al*., 2017). These auxin-responsive factors mainly regulate root

development, floral development, and senescence (Hagen & Guilfoyle, 2002; Roosjen *et al*., 2017). Genes encoding auxin-responsive factors were identified in qRD-FR-A3-2-2 (A03p63140.1_BnaDAR and A03p63160.1_BnaDAR), qDSI-FR-A4-10 (A04p34440.1_BnaDAR), qDSI-FS-A4-10, and qDSI-FWP-A4-10 (A04p34570.1_BnaDAR). In the auxin stress signaling pathway, AUX/IAA also plays a crucial role (Gretchen & Guilfoyle, 2002; Szemenyei *et al*., 2008). A gene encoding AUX/IAA family protein was identified in qRD-FR-A3-2-2. Apart from the mentioned phytohormones, several studies have reported the involvement of ethylene in drought tolerance mechanisms in *G. max* (Arraes *et al*., 2015), *Brassica rapa* L. (Seo *et al*., 2010), and *Gossypium hirsutum* L. (Liu & Zhang, 2017). In our study, genes encoding several ethylene-responsive transcription factors were identified in several QTL (A03p61590.1_BnaDAR and A03p64990.1_BnaDAR in qRD-FR-A3-2-2, A04p33510.1_BnaDAR in qDSI-FR-A4-10, C07p30930.1_BnaDAR and C07p30970.1_BnaDAR in qDSI-FR-C7-22-1). These findings collectively emphasize the intricate crosstalk and interplay among various phytohormones in shaping plants' adaptive strategies to survive drought stress.

Late embryogenesis abundant (LEA) proteins have been recognized for their role in facilitating desiccation tolerance of embryonic tissues in seeds (Dalal *et al*., 2009). Several studies have also indicated their involvement in enhancing water stress tolerance in vegetative tissues (Cheng *et al*., 2002; Dalal *et al*., 2009; Tunnacliffe & Wise, 2007). The substantial abundance of water-interacting residues in LEA proteins enhances the capture of water molecules. This process holds particular significance during developmental phases where death of tissues occurs (Olvera-Carrillo *et al*., 2010). In this study, several genes encoding LEA proteins were identified

(A03p63420.1_BnaDAR in qRD-FR-A3-2-2, A04p27480.1_BnaDAR, A04p28050.1_BnaDAR, A04p31460.1_BnaDAR, A04p32280.1_BnaDAR, and A04p34290.1_BnaDAR in qDSI-FS-A4-10 and qDSI-FWP-A4-10, and C03p54920.1_BnaDAR in qRD-FR-C3-11 and qRD-DR-C3-11). These findings support the contribution of LEA proteins in not only desiccation tolerance during seed embryogenesis but also enhancing water stress resilience in vegetative tissues.

In the linkage map of both populations, a possible homoeologous translocation between two chromosomes was observed. In the 1901 population, linkage group 4 is shared between A1 and C1 chromosomes. In the linkage map of the 1904 population, linkage group 6 is shared between A7 and C6 chromosomes. *Brassica napus* is an allotetraploid plant species that comprises two closely related diploid genomes. These diploid genomes are prone to intergenomic homologous recombination, a process that can lead to complex changes in the genomic structure (Larkan *et al*., 2016). A possible translocation between these chromosomes has been reported several times in the past. In a study based on the pangenome of *B. napus* constructed from 53 accessions and the Darmor-*bzh* reference genome, Hurgobin *et al*. (2018) reported that homoeologous exchange events occur more frequently in chromosomes A1, A2, A3, and A9 and C1, C2, C3, C8, and C9. Research findings indicate that in early generations, there is evidence of homoeologous non-reciprocal transposition occurring between A1 and C1 chromosomes. Moreover, this transposition appears to persist and can be transmitted across subsequent generations (Gaeta *et al*., 2007). Besides the transposition involving A1 and C1, Udall *et al*. (2005) documented a potential homoeologous reciprocal transposition occurring between A7 and C6 in the male parents that they examined in a study conducted with four mapping populations of *B. napus* to identify chromosomal

rearrangements. In a study related to the *Brassica – Leptosphaeria* pathosystem, Larkan *et al*. (2016) also reported a possible reciprocal translocation between A7 and C6 chromosomes. These homoeologous translocations have garnered significant attention due to their potential impacts on enhancing seed yield (Osborn *et al*., 2003; Sharpe *et al*., 1995), increasing disease resistance (Larkan *et al*., 2016; Zhao *et al*., 2006), and influencing flowering time (Pires *et al*., 2004) in *B. napus.*

5.0 CONCLUSION AND FUTURE PERSPECTIVES

The aim of this study is to understand the genetic control of drought stress tolerance in *Brassica napus* L. by utilizing 10 % PEG as an osmotic agent. Eighteen QTL located on 6 distinct chromosomes were identified. Collectively, 2154 putative candidate genes were identified across all the identified QTL. Among them, 213 genes were chosen as being directly associated with drought stress tolerance based on nine functional annotations (antioxidants, phytohormones, osmotic adjustments, LEA proteins, photosynthesis, respiration, signal transduction, hydraulic conductance, and growth and development). This study also documented a possible homoeologous translocation between the A1 and C1 chromosomes, as well as between the A7 and C6 chromosomes. However, additional investigations are needed to validate whether this observed phenomenon is indeed the result of homoeologous translocation between these chromosomes.

Genotypes exhibiting higher DSI values could serve as valuable candidates for differential gene expression studies. Furthermore, candidate genes proposed in this study could be validated by using candidate gene knockout or overexpression study. Drought stress tolerance in plants involves numerous biochemical pathways, making gene expression studies crucial for unraveling the intricate interactions within these regulatory networks. Another recommendation is to replicate this experiment in field settings, employing a rain shed system. Conducting this experiment in various fields throughout western Canada will enhance the precision and accuracy of the study. These approaches could represent significant strides in the integration of QTL associated with drought stress tolerance into commercially available *B. napus* varieties.

Plant response to water stress is governed by an intricate genetic network, and studies on drought tolerance in *B. napus* are scarce. Therefore, the identification of QTL and candidate genes involved in water stress tolerance is an important advancement in creating more resilient germplasm that can endure drought. Besides providing indications on the level of drought stress tolerance of selected *B. napus* germplasm, this study also identified QTL associated with drought tolerance. These results can be incorporated into future breeding initiatives to select plant material with the ability to effectively cope with water stress.

6.0 REFERENCES

Abedi, T., & Pakniyat, H. (2010). Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L.). *Czech Journal of Genetics and Plant Breeding*, *46*(1), 27–34. doi: 10.17221/67/2009-cjgpb

Acar, O., Türkan, I., & özdemir, F. (2001). Superoxide dismutase and peroxidase activities in drought sensitive and resistant barley (*Hordeum vulgare* L.) varieties. *Acta Physiologiae Plantarum*, *23*(3), 351–356. doi: 10.1007/s11738-001-0043-8

Ahmar, S., Gill, R. A., Jung, K. H., Faheem, A., Qasim, M. U., Mubeen, M., & Zhou, W. (2020). Conventional and molecular techniques from simple breeding to speed breeding in crop plants: Recent advances and future outlook. *International Journal of Molecular Sciences*, *21*(7). doi: 10.3390/ijms21072590

Ainsworth, E. A., & Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising (CO_2): Mechanisms and environmental interactions. *Plant, Cell and Environment*, *30*(3), 258–270. doi: 10.1111/j.1365-3040.2007.01641.x

Akhunov, E., Nicolet, C., & Dvorak, J. (2009). Single nucleotide polymorphism genotyping in polyploid wheat with the Illumina GoldenGate assay. *Theoretical and Applied Genetics*, *119*(3), 507–517. doi: 10.1007/s00122-009-1059-5

Akond, Z., Alam, Md. J., Hasan, M. N., Uddin, Md. S., Alam, M., & Mollah, N. H. (2019). A Comparison on Some Interval Mapping Approaches for QTL Detection. *Bioinformation*, *15*(2), 90–94. doi: 10.6026/97320630015090

Alexieva, V., Sergiev, I., Mapelli, S., & Karanov, E. (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant, Cell and Environment*, *24*(12), 1337–1344. doi: 10.1046/j.1365-3040.2001.00778.x

Ali, S., Hayat, K., Iqbal, A., & Xie, L. (2020). Implications of abscisic acid in the drought stress tolerance of plants. *Agronomy*, *10*(9), 1323–1351. doi:10.3390/agronomy10091323

Almeida, G. D., Makumbi, D., Magorokosho, C., Nair, S., Borém, A., Ribaut, J. M., ... & Babu, R. (2013). QTL mapping in three tropical maize populations reveals a set of constitutive and adaptive genomic regions for drought tolerance. *Theoretical and Applied Genetics*, *126*, 583-600. doi: 10.1007/s00122-012-2003-7

Alonso-Cantabrana, H., Cousins, A. B., Danila, F., Ryan, T., Sharwood, R. E., von Caemmerer, S., & Furbank, R. T. (2018). Diffusion of CO_2 across the mesophyll-bundle sheath cell interface in a C4 plant with genetically reduced PEP carboxylase activity. *Plant Physiology*, *178*(1), 72–81. doi: 10.1104/pp.18.00618

Alvarado, G., Rodriguez, F. M., Pacheco, A., Burgueño, J., Crossa, J., Vargas, M., ... & Lopez-Cruz, M. A. (2020). META-R: A software to analyze data from multi-

environment plant breeding trials. *The Crop Journal*, *8*(5), 745-756. doi: doi.org/10.1016/j.cj.2020.03.010

Amthor, J. S. (2000). The McCree-de Wit-Penning de Vries-Thornley respiration paradigms: 30 Years later. *Annals of Botany*, *86*(1), 1–20. doi:10.1006/anbo.2000.1175

Aranzana, M. J., Kim, S., Zhao, K., Bakker, E., Horton, M., Jakob, K., ... & Nordborg, M. (2005). Genome-wide association mapping in *Arabidopsis* identifies previously known flowering time and pathogen resistance genes. *PLoS genetics*, *1*(5). doi: 10.1371/journal.pgen.0010060

Arnon, D. I., & Hoagland, D. R. (1939). A comparison of water culture and soil as media for crop production. *Science*, *89*(2318), 512-514. doi: 10.1126/science.89.2318.512

Arraes, F. B. M., Beneventi, M. A., Lisei de Sa, M. E., Paixao, J. F. R., Albuquerque, E. V. S., Marin, S. R. R., ... & Grossi-de-Sa, M. F. (2015). Implications of ethylene biosynthesis and signaling in soybean drought stress tolerance. *BMC plant biology*, *15*, 1-20. doi: 10.1186/s12870-015-0597-z

Arve, L. E., Torre, S., Olsen, J. E., & Tanino, K. K. (2011). Stomatal responses to drought stress and air humidity. In *abiotic stress in plants-Mechanisms and adaptations*. IntechOpen.

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. In *Plant Physiology*, *141*(2), 391–396. doi:10.1104/pp.106.082040

Assefa, Y., Prasad, P. V., Foster, C., Wright, Y., Young, S., Bradley, P., ... & Ciampitti, I. A. (2018). Major management factors determining spring and winter canola yield in North America. *Crop Science*, *58*(1), 1-16. doi: 10.2135/cropsci2017.02.0079

Ashraf, M., & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, *59*(2), 206–216. doi:10.1016/j.envexpbot.2005.12.006

Assmann, S. M. (2003). OPEN STOMATA1 opens the door to ABA signaling in *Arabidopsis* guard cells. *Trends in plant science*, *8*(4), 151-153. doi: 10.1016/S1360-1385(03)00052-9

Atkin, O. K., & Macherel, D. (2009). The crucial role of plant mitochondria in orchestrating drought tolerance. *Annals of Botany*, *103*(4), 581–597. doi:10.1093/aob/mcn094

Balasubramanian, S., Schwartz, C., Singh, A., Warthmann, N., Kim, M. C., Maloof, J. N., Loudet, O., Trainer, G. T., Dabi, T., Borevitz, J. O., Chory, J., & Weigel, D. (2009). QTL mapping in new *Arabidopsis thaliana* advanced intercross-recombinant inbred lines. *PLoS ONE*, *4*(2). doi: 10.1371/journal.pone.0004318

Bandurska, H., Niedziela, J., Pietrowska-Borek, M., Nuc, K., Chadzinikolau, T., & Radzikowska, D. (2017). Regulation of proline biosynthesis and resistance to drought

stress in two barley (*Hordeum vulgare* L.) genotypes of different origin. *Plant Physiology and Biochemistry*, *118*, 427–437. doi: 10.1016/j.plaphy.2017.07.006

Bartels, D., & Sunkar, R. (2005). Drought and salt tolerance in plants. *Critical Reviews in Plant Sciences*, *24*(1), 23–58. doi: 10.1080/07352680590910410

Batieno, B. J., Eric, D., Jean-Baptiste, T., Bao-Lam, H., Issa, D., Timothy, J. C., Kwadwo, O., Philip, R., & Tinga, J. O. (2016). Application of marker-assisted backcrossing to improve cowpea (*Vigna unguiculata* L. Walp) for drought tolerance. *Journal of Plant Breeding and Crop Science*, *8*(12), 273–286. doi: 10.5897/jpbcs2016.0607

Batley, J., Edwards, D. (2007). SNP Applications in Plants. In Oraguzie, N.C., Rikkerink, E.H.A., Gardiner, S.E., De Silva, H.N. (eds) Association Mapping in Plants. New York: Springer. doi: 10.1007/978-0-387-36011-9_6

Batley, J., Jewell, E., & Edwards, D. (2007). Automated Discovery of Single Nucleotide Polymorphism and Simple Sequence Repeat Molecular Genetic Markers. *Methods in Molecular Biology*, *406*, 473–494. doi: 10.1007/978-1-59745-535-0_23

Beudez, N., Doussan, C., Lefeuve-Mesgouez, G., & Mesgouez, A. (2013). Influence of Three Root Spatial Arrangement on Soil Water Flow and Uptake. Results from an Explicit and an Equivalent, Upscaled, Model. *Procedia Environmental Sciences*, *19*, 37–46. doi: 10.1016/j.proenv.2013.06.005

Bayer, M. M., Rapazote-Flores, P., Ganal, M., Hedley, P. E., Macaulay, M., Plieske, J., ... & Waugh, R. (2017). Development and evaluation of a barley 50k iSelect SNP array. *Frontiers in plant science*, *8*, 1792. doi: 10.3389/fpls.2017.01792

Biehler, K., & Fock, H. (1996). Evidence for the contribution of the Mehler-peroxidase reaction in dissipating excess electrons in drought-stressed wheat. *Plant physiology*, *112*(1), 265-272. doi: 10.1104/pp.112.1.265

Bleby, T. M., Mcelrone, A. J., & Jackson, R. B. (2010). Water uptake and hydraulic redistribution across large woody root systems to 20 m depth. *Plant, cell & environment*, *33*(12), 2132-2148. doi: 10.1111/j.1365-3040.2010.02212.x

Bloom, A. J., Chapin, F. S., & Mooney, H. A. (1985). Resource Limitation in Plants--An Economic Analogy. *Annual Review of Ecology and Systematics*, *16*, 363–392. doi: 10.1146/annurev.es.16.110185.002051

Blum, A. (2017). Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. *Plant Cell and Environment*, *40*(1), 4–10. doi: 10.1111/pce.12800

Bonsal, B., & Regier, M. (2007). Historical comparison of the 2001/2002 drought in the Canadian Prairies. *Climate Research*, *33*(3), 229–242. doi: 10.3354/cr033229

Booth, E. J., & Gunstone, F. D. (2004). Rapeseeds and rapeseed oil: agronomy, production, and trade. In FD. Gunstone (Ed.), *Rapeseed and Canola Oil Production, Processing, Properties and Uses* (pp. 1 - 16). Oxford: Blackwell.

Bot, A., Nachtergaele, F., & Young, A. (2000). *Land resource potential and constraints at regional and country levels* (No. 90). Food & Agriculture Org..

Bouman, B. A. M., & Tuong, T. P. (2001). Field water management to save water and increase its productivity in irrigated lowland rice. *Agricultural Water Management*, *49*, 11–30. doi: 10.1016/S0378-3774(00)00128-1

Bowler, C., Montagu, M. van, & Inze, D. (1992). Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology*, *43*, 83–116. doi: 10.1146/annurev.pp.43.060192.000503

Brauner, P. C., Schipprack, W., Utz, H. F., Bauer, E., Mayer, M., Schön, C. C., & Melchinger, A. E. (2019). Testcross performance of doubled haploid lines from European flint maize landraces is promising for broadening the genetic base of elite germplasm. *Theoretical and Applied Genetics*, *132*(6), 1897–1908. doi: 10.1007/s00122-019-03325-0

Brisson, N., & Casals, M. L. (2005). Leaf dynamics and crop water status throughout the growing cycle of durum wheat crops grown in two contrasted water budget conditions. *Agronomie*, *25*(1), 151–158. doi: 10.1051/agro:2004066

Burbidge, A., Grieve, T. M., Jackson, A., Thompson, A., McCarty, D. R., & Taylor, I. B. (1999). Characterization of the ABA-deficient tomato mutant notabilis and its relationship with maize Vp14. *Plant Journal*, *17*(4), 427–431. doi: 10.1046/j.1365-313X.1999.00386.x

Cai, S., Jiang, G., Ye, N., Chu, Z., Xu, X., Zhang, J., & Zhu, G. (2015). A key ABA catabolic gene, *OsABA8ox3*, is involved in drought stress resistance in rice. *PLoS One*, *10*(2), e0116646. doi: 10.1371/journal.pone.0116646

Caldwell, M. M., Dawson, T. E., & Richards, J. H. (1998). Hydraulic lift: consequences of water efflux from the roots of plants. *Oecologia*, *113*, 151-161. doi: doi.org/10.1007/s004420050363

Campos, H., Cooper, M., Habben, J. E., Edmeades, G. O., & Schussler, J. R. (2004). Improving drought tolerance in maize: A view from industry. *Field Crops Research*, *90*(1), 19–34. doi: 10.1016/j.fcr.2004.07.003

Cano, F. J., Sánchez-Gómez, D., Rodríguez-Calcerrada, J., Warren, C. R., Gil, L., & Aranda, I. (2013). Effects of drought on mesophyll conductance and photosynthetic limitations at different tree canopy layers. *Plant, Cell and Environment*, *36*(11), 1961–1980. doi: 10.1111/pce.12103

Cao, Q., Li, G., & Liu, F. (2022). Elevated CO_2 enhanced water use efficiency of wheat to progressive drought stress but not on maize. *Frontiers in Plant Science*, *13*. doi: 10.3389/fpls.2022.953712

Carminati, A., Vetterlein, D., Weller, U., Vogel, H.-J., & Oswald, S. E. (2009). When Roots Lose Contact. *Vadose Zone Journal*, *8*(3), 805–809. doi: 10.2136/vzj2008.0147

Caverzan, A., Passaia, G., Rosa, S. B., Ribeiro, C. W., Lazzarotto, F., & Margis-Pinheiro, M. (2012). Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. *Genetics and molecular biology*, *35*(4), 1011–1019. doi: 10.1590/s1415-47572012000600016

Chalhoub, B., Denoeud, F., Liu, S., Parkin, I. A., Tang, H., Wang, X., ... & Wincker, P. (2014). Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. *science*, *345*(6199), 950-953. doi: 10.1126/science.125343

Channaoui, S., El Kahkahi, R., Charafi, J., Mazouz, H., El Fechtali, M., & Nabloussi, A. (2017). Germination and seedling growth of a set of rapeseed (*Brassica napus*) varieties under drought stress conditions. *International Journal of Environment, Agriculture and Biotechnology*, *2*(1). doi: 10.22161/ijeab/2.1.61

Chaves, M. M., Flexas, J., & Pinheiro, C. (2009). Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Annals of Botany*, *103*(4), 551–560. doi: 10.1093/aob/mcn125

Chaves, M. M., Maroco, J. P., & Pereira, J. S. (2003). Understanding plant responses to drought - From genes to the whole plant. *Functional Plant Biology*, *30*(3), 239–264. doi: 10.1071/FP02076

Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P. P., Osório, M. L., ... & Pinheiro, C. (2002). How plants cope with water stress in the field? Photosynthesis and growth. *Annals of botany*, *89*(7), 907-916. doi: 10.1093/aob/mcf105

Cheema, J., & Dicks, J. (2009). Computational approaches and software tools for genetic linkage map estimation in plants. *Briefings in bioinformatics*, *10*(6), 595-608. doi: 10.1093/bib/bbp045

Chen, H., & Jiang, J. G. (2010). Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. *Environmental Reviews*, *18*(1), 309–319. doi: 10.1139/A10-014

Cheng, A., Ismail, I., Osman, M., Hashim, H., Samahah, N., & Zainual, M. (2017). Rapid and targeted introgression of fgr gene through marker-assisted 1 backcrossing in rice (*Oryza sativa* L.). *Genome*, *60*(12), 1045–1050. doi: 10.1139/gen-2017-0100

Cheng, C., Zhong, Y., Cai, Z., Su, R., & Li, C. (2019). Genome-wide identification and gene expression analysis of ABA receptor family genes in *Brassica juncea* var. tumida. *Genes*, *10*(6), 470. doi: 10.3390/genes10060470

Cheng, Z., Targolli, J., Huang, X., & Wu, R. (2002). Wheat LEA genes, PMA80 and PMA1959, enhance dehydration tolerance of transgenic rice (*Oryza sativa* L.). *Molecular Breeding*, *10*, 71-82. doi: 10.1023/A:1020329401191

Chesnokov, Y. V., Kosolapov, V. M., & Savchenko, I. V. (2020). Morphological genetic markers in plants. *Russian Journal of Genetics*, *56*, 1406-1415. doi: 10.1134/S1022795420120042

Clarke, W. E., Higgins, E. E., Plieske, J., Wieseke, R., Sidebottom, C., Khedikar, Y., ... & Parkin, I. A. (2016). A high-density SNP genotyping array for *Brassica napus* and its ancestral diploid species based on optimised selection of single-locus markers in the allotetraploid genome. *Theoretical and Applied Genetics*, *129*, 1887-1899. doi: 10.1007/s00122-016-2746-7

Cole, C. T. (2003). Genetic Variation in Rare and Common Plants. *Annual Review of Ecology, Evolution, and Systematics*, *34*, 213–237. doi: 10.1146/annurev.ecolsys.34.030102.151717

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, *142*, 169–196. doi: 10.1007/s10681-005-1681-5

Coryell, V. H., Jessen, H., Schupp, J. M., Webb, D., Keim, P., & Webb, D. (1999). Allele-specific hybridization markers for soybean. *Theoretical and Applied Genetics*, *98*, 690–696.

Costa, R. C. L. D., Lobato, A. K. D. S., & Silveira, J. A. G. D. (2011). ABA-mediated proline synthesis in cowpea leaves exposed to water deficiency and rehydration. *Turkish Journal of Agriculture and Forestry*, *35*(3), 309-317. doi: 10.3906/tar-0911-409

Creelman, R. A., Mason, H. S., Bensen, R. J., Boyer, J. S., & Mullet, J. E. (1990). Water deficit and abscisic acid cause differential inhibition of shoot versus root growth in soybean seedlings: analysis of growth, sugar accumulation, and gene expression. *Plant Physiology*, *92*(1), 205-214. doi: 10.1104/pp.92.1.205

Crepieux, S., Lebreton, C., Servin, B., & Charmet, G. (2004). Quantitative trait loci (QTL) detection in multicross inbred designs: recovering QTL identical-by-descent status information from marker data. *Genetics*, *168*(3), 1737-1749. doi: 10.1534/genetics.104.028993

Crocetti, L., Forkel, M., Fischer, M., Jurečka, F., Grlj, A., Salentinig, A., ... & Dorigo, W. (2020). Earth Observation for agricultural drought monitoring in the Pannonian Basin (southeastern Europe): current state and future directions. *Regional Environmental Change*, *20*, 1-17. doi: 10.1007/s10113-020-01710-w

Cruz de Carvalho, M. H. (2008). Drought stress and reactive oxygen species: production, scavenging and signaling. *Plant signaling & behavior*, *3*(3), 156-165. doi: 10.4161/psb.3.3.5536

Cutler, S. R., Rodriguez, P. L., Finkelstein, R. R., & Abrams, S. R. (2010). Abscisic acid: emergence of a core signaling network. *Annual review of plant biology*, *61*, 651-679. doi: 10.1146/annurev-arplant-042809-112122

Dalal, M., Tayal, D., Chinnusamy, V., & Bansal, K. C. (2009). Abiotic stress and ABA-inducible Group 4 *LEA* from *Brassica napus* plays a key role in salt and drought

tolerance. *Journal of biotechnology*, *139*(2), 137-145. doi: 10.1016/j.jbiotec.2008.09.014

Dalton-Morgan, J., Hayward, A., Alamery, S., Tollenaere, R., Mason, A. S., Campbell, E., ... & Batley, J. (2014). A high-throughput SNP array in the amphidiploid species *Brassica napus* shows diversity in resistance genes. *Functional & Integrative Genomics*, *14*, 643-655. doi: 10.1007/s10142-014-0391-2

de Dorlodot, S., Forster, B., Pagès, L., Price, A., Tuberosa, R., & Draye, X. (2007). Root system architecture: opportunities and constraints for genetic improvement of crops. In *Trends in Plant Science, 12*(10), 474–481. doi: 10.1016/j.tplants.2007.08.012

de Jong van Lier, Q., van Dam, J. C., Metselaar, K., de Jong, R., & Duijnisveld, W. H. M. (2008). Macroscopic root water uptake distribution using a matric flux potential approach. *Vadose Zone Journal*, *7*(3), 1065–1078. doi: 10.2136/vzj2007.0083

De Swaef, T., Pieters, O., Appeltans, S., Borra-Serrano, I., Coudron, W., Couvreur, V., ... & Wyffels, F. (2022). On the pivotal role of water potential to model plant physiological processes. *in silico Plants*, *4*(1), diab038.doi: 10.1093/insilicoplants/diab038

Deak, K. I., & Malamy, J. (2005). Osmotic regulation of root system architecture. *Plant Journal*, *43*(1), 17–28. doi: 10.1111/j.1365-313X.2005.02425.x

Deschamps, S., Llaca, V., & May, G. D. (2012). Genotyping-by-sequencing in plants. *Biology*, *1*(3), 460–483. doi: 10.3390/biology1030460

Dibike, Y., Prowse, T., Bonsal, B., & O'Neil, H. (2017). Implications of future climate on water availability in the western Canadian river basins. *International Journal of Climatology*, *37*(7), 3247–3263. doi: 10.1002/joc.4912

dos Reis, S. P., Lima, A. M., & de Souza, C. R. B. (2012). Recent molecular advances on downstream plant responses to abiotic stress. *International Journal of Molecular Sciences, 13*(7), 8628–8647). doi: 10.3390/ijms13078628

Durstewitz, G., Polley, A., Plieske, J., Luerssen, H., Graner, E. M., Wieseke, R., & Ganal, M. W. (2010). SNP discovery by amplicon sequencing and multiplex SNP genotyping in the allopolyploid species *Brassica napus*. *Genome*, *53*(11), 948-956. doi: 10.1139/G10-079

Dusenge, M. E., Duarte, A. G., & Way, D. A. (2019). Plant carbon metabolism and climate change: elevated CO_2 and temperature impacts on photosynthesis, photorespiration and respiration. *New Phytologist, 221*(1), 32–49. doi: 10.1111/nph.15283

Duvick, D. N. (2005). The contribution of breeding to yield advances in maize (*Zea mays* L.). *Advances in agronomy*, *86*, 83-145. doi: 10.1016/S0065-2113(05)86002-X

Edwards, D., Forster, J.W., Chagné, D., Batley, J. (2007). What Are SNPs?. In: Oraguzie, N.C., Rikkerink, E.H.A., Gardiner, S.E., De Silva, H.N. (eds) Association Mapping in Plants. New York: Springer. doi: 10.1007/978-0-387-36011-9_3

El-Badri, A. M., Batool, M., Mohamed, I. A., Khatab, A., Sherif, A., Wang, Z., ... & Zhou, G. (2021). Modulation of salinity impact on early seedling stage via nano-priming application of zinc oxide on rapeseed (*Brassica napus* L.). *Plant Physiology and Biochemistry*, *166*, 376-392. doi: 10.1016/j.plaphy.2021.05.040

Elferjani, R., & Soolanayakanahally, R. (2018). Canola responses to drought, heat, and combined stress: shared and specific effects on carbon assimilation, seed yield, and oil composition. *Frontiers in Plant Science*, *9*. doi: 10.3389/fpls.2018.01224

Erb, T. J., & Zarzycki, J. (2018). A short history of RubisCO: the rise and fall (?) of Nature's predominant CO_2 fixing enzyme. *Current opinion in biotechnology*, *49*, 100-107. doi: 10.1016/j.copbio.2017.07.017

Eskin, N. A. M., & McDonald, B. E. (1991). Canola oil. *Nutrition bulletin*, *16*(3), 138-146. doi: 10.1111/j.1467-3010.1991.tb01048.x

Eziz, A., Yan, Z., Tian, D., Han, W., Tang, Z., & Fang, J. (2017). Drought effect on plant biomass allocation: A meta-analysis. *Ecology and Evolution*, *7*(24), 11002–11010. doi: 10.1002/ece3.3630

Fang, Y., & Xiong, L. (2015). General mechanisms of drought response and their application in drought resistance improvement in plants. *Cellular and Molecular Life Sciences*, *72*(4), 673–689. doi: 10.1007/s00018-014-1767-0

Farooq, M., Hussain, M., Wahid, A., & Siddique, K. H. M. (2012). Drought stress in plants: an overview. *Plant responses to drought stress: From morphological to molecular features*, *12*, 1-33. doi: 10.1007/978-3-642-32653-0_1

Feddes, R. A., Kowalik, P., Kolinska-Malinka, K., & Zaradny, H. (1976). Simulation of field water uptake by plants using a soil water dependent root extraction function. *Journal of Hydrology*, *31*(13), 13–26. doi: 10.1016/0022-1694(76)90017-2

Felcher, K. J., Coombs, J. J., Massa, A. N., Hansey, C. N., Hamilton, J. P., Veilleux, R. E., Buell, C. R., & Douches, D. S. (2012). Integration of two diploid potato linkage maps with the potato genome sequence. *PLoS ONE*, *7*(4). doi: 10.1371/journal.pone.0036347

Feng, W., Lindner, H., Robbins, N. E., & Dinneny, J. R. (2016). Growing out of stress: The role of cell- and organ-scale growth control in plant water-stress responses. *Plant Cell*, *28*(8), 1769–1782. doi: 10.1105/tpc.16.00182

Fenta, B. A., Beebe, S. E., Kunert, K. J., Burridge, J. D., Barlow, K. M., Lynch, J. P., & Foyer, C. H. (2014). Field phenotyping of soybean roots for drought stress tolerance. *Agronomy*, *4*(3), 418–435. doi: 10.3390/agronomy4030418

Finnegan, P.M., Soole, K.L., Umbach, A.L. (2004). Alternative mitochondrial electron transport proteins in higher plants. In: Day, D.A., Millar, A.H., Whelan, J. (eds) Plant mitochondria: from genome to function. Advances in photosynthesis and respiration. Dordrecht: Springer. doi: 10.1007/978-1-4020-2400-9_9

Flamholz, A. I., Prywes, N., Moran, U., Davidi, D., Bar-On, Y. M., Oltrogge, L. M., Alves, R., Savage, D., & Milo, R. (2019). Revisiting Trade-offs between Rubisco Kinetic Parameters. *Biochemistry*, *58*(31), 3365–3376. doi: 10.1021/acs.biochem.9b00237

Fletcher, R., Conventry, J., & Kott, L. S. (1998). Double haploid technology for spring and winter *Brassica napus*. Department of Plant Agriculture. Technical Bulletin, OAC publication, University of Guelph, Canada.

Fletcher, R. S., Mullen, J. L., Heiliger, A., & McKay, J. K. (2015). QTL analysis of root morphology, flowering time, and yield reveals trade-offs in response to drought in *Brassica napus*. *Journal of Experimental Botany*, *66*(1), 245-256. doi: 10.1093/jxb/eru423

Flexas, J., Baron, M., Bota, J., Ducruet, J. M., Galle, A., Galmes, J., ... & Medrano, H. (2009). Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted Vitis hybrid Richter-110 (*V. berlandieri*× *V. rupestris*). *Journal of experimental Botany*, *60*(8), 2361-2377. doi: 10.1093/jxb/erp069

Flexas, J., Galmes, J., Ribas-Carbo, M., Medrano, H. (2005). The effects of water stress on plant respiration. In: Lambers, H., Ribas-Carbo, M. (eds) Plant respiration. Advances in photosynthesis and respiration. Dordrecht: Springer. doi: 10.1007/1-4020-3589-6_6

Flexas, J., & Medrano, H. (2002). Energy dissipation in C3 plants under drought. *Functional Plant Biology*, *29*(10), 1209–1215. doi: 10.1071/FP02015

Food and agriculture organization of the United State. (2021). The impact of disasters and crises on agriculture and food security: 2021. In *The impact of disasters and crises on agriculture and food security: 2021*. FAO. Retrieved from https://doi.org/10.4060/cb3673en

Foisset, N., Delourme, R., Barret, P., Hubert, N., Landry, B. S., & Renard, M. (1996). Molecular-mapping analysis in *Brassica napus* using isozyme, RAPD and RFLP markers on a doubled-haploid progeny. *Theoretical and Applied Genetics*, *93*, 1017-1025. doi: 10.1007/BF00230119

Franks, S. J. (2011). Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. *New Phytologist*, *190*(1), 249–257. doi: 10.1111/j.1469-8137.2010.03603.x

Franks, S. J., Sim, S., & Weis, A. E. (2007). Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences*, *104*(4), 1278-1282. doi: 10.1073/pnas.0608379104

Gad, M., Chao, H., Li, H., Zhao, W., Lu, G., & Li, M. (2021). QTL mapping for seed germination response to drought stress in *Brassica napus*. *Frontiers in Plant Science*, *11*, 2276. doi: 10.3389/fpls.2020.629970

Gaeta, R. T., Pires, J. C., Iniguez-Luy, F., Leon, E., & Osborn, T. C. (2007). Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype. *The Plant Cell*, *19*(11), 3403-3417. doi: 10.1105/tpc.107.054346

Galmes, J., Ribas-Carbó, M., Medrano, H., & Flexas, J. (2007). Response of leaf respiration to water stress in Mediterranean species with different growth forms. *Journal of Arid Environments*, *68*(2), 206–222. doi: 10.1016/j.jaridenv.2006.05.005

Ganal, M. W., Polley, A., Graner, E. M., Plieske, J., Wieseke, R., Luerssen, H., & Durstewitz, G. (2012). Large SNP arrays for genotyping in crop plants. *Journal of Biosciences*, *37*(5), 821–828. doi: 10.1007/s12038-012-9225-3

Girma, F. S., & Krieg, D. R. (1992). Osmotic Adjustment in Sorghum 1. Mechanisms of Diurnal Osmotic Potential Changes. *Plant Physiology*, *99*, 577–582. doi: 10.1104/pp.99.2.577

Ghashghaie, J., Duranceau, M., Badeck, F. W., Cornic, G., Adeline, M. T., & Deleens, E. (2001). $\delta^{13}C$ of CO_2 respired in the dark in relation to $\delta^{13}C$ of leaf metabolites: comparison between *Nicotiana sylvestris* and *Helianthus annuus* under drought. *Plant, Cell & Environment*, *24*(5), 505-515. doi: 10.1046/j.1365-3040.2001.00699.x

Gliessman, S. (2020). A vision for future food and agriculture systems. *Agroecology and Sustainable Food Systems*, *44*(2), 137-138. doi: 10.1080/21683565.2020.1676967

Gomez-Casanovas, N., Blanc-Betes, E., Gonzalez-Meler, M. A., & Azcon-Bieto, J. (2007). Changes in respiratory mitochondrial machinery and cytochrome and alternative pathway activities in response to energy demand underlie the acclimation of respiration to elevated CO_2 in the invasive *Opuntia ficus-indica*. *Plant Physiology*, *145*(1), 49-61. doi: 10.1104/pp.107.103911

Gratani, L., Varone, L., & Bonito, A. (2007). Environmental induced variations in leaf dark respiration and net photosynthesis of *Quercus ilex* L. *Photosynthetica*, *45*(4), 633–636. doi: 10.1007/s11099-007-0109-y

Grzesiak, M. T., Janowiak, F., Szczyrek, P., Kaczanowska, K., Ostrowska, A., Rut, G., Hura, T., Rzepka, A., & Grzesiak, S. (2016). Impact of soil compaction stress combined with drought or waterlogging on physiological and biochemical markers in two maize hybrids. *Acta Physiologiae Plantarum*, *38*(5). doi: 10.1007/s11738-016-2128-4

Guo, S., Zou, J., Li, R., Long, Y., Chen, S., & Meng, J. (2012). A genetic linkage map of *Brassica carinata* constructed with a doubled haploid population. *Theoretical and applied genetics*, *125*, 1113-1124. doi: 10.1007/s00122-012-1898-3

Guo, Y. M., Samans, B., Chen, S., Kibret, K. B., Hatzig, S., Turner, N. C., ... & Snowdon, R. J. (2017). Drought-tolerant *Brassica rapa* shows rapid expression of gene networks for general stress responses and programmed cell death under simulated drought stress. *Plant molecular biology reporter*, *35*(4), 416-430. doi: 10.1007/s11105-017-1032-4

Hagen, G., & Guilfoyle, T. (2002). Auxin-responsive gene expression: genes, promoters and regulatory factors. *Plant molecular biology*, *49*, 373-385. doi: 10.1023/A:1015207114117

Haley, C. S., & Knott, S. A. (1992). A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*, *69*, 315–324. doi: 10.1038/hdy.1992.131

Hare, P. D., Cress, W. A., & van Staden1, J. (1999). Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. *Journal of Experimental Botany*, *50*(333), 413–434. doi: doi.org/10.1093/jxb/50.333.413

Harpaz-Saad, S., Azoulay, T., Arazi, T., Ben-Yaakov, E., Mett, A., Shiboleth, Y. M., ... & Eyal, Y. (2007). Chlorophyllase is a rate-limiting enzyme in chlorophyll catabolism and is posttranslationally regulated. *The Plant Cell*, *19*(3), 1007-1022. doi: 10.1105/tpc.107.050633

Hasan, N., Choudhary, S., Naaz, N., Sharma, N., & Laskar, R. A. (2021). Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. *Journal of Genetic Engineering and Biotechnology*, *19*(1), 1–26. doi: 10.1186/s43141-021-00231-1

Hasanuzzaman, M., Bhuyan, M. B., Zulfiqar, F., Raza, A., Mohsin, S. M., Mahmud, J. A., ... & Fotopoulos, V. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants*, *9*(8), 681. doi: 10.3390/antiox9080681

Hasanuzzaman, M., Hossain, M.A., da Silva, J.A.T., Fujita, M. (2012). Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: Venkateswarlu, B., Shanker, A., Shanker, C., Maheswari, M. (eds) Crop stress and its management: perspectives and strategies. Dordrecht: Springer. doi: 10.1007/978-94-007-2220-0_8

Haupt-Herting, S., & Fock, H. P. (2002). Oxygen exchange in relation to carbon assimilation in water-stressed leaves during photosynthesis. *Annals of Botany*, *89*(SPEC. ISS.), 851–859. doi: 10.1093/aob/mcf023

Hayward, A., Mason, A. S., Dalton-Morgan, J., Zander, M., Edwards, D., & Batley, J. (2012). SNP discovery and applications in Brassica napus. *Journal of Plant Biotechnology*, *39*(1), 49–61. doi: 10.5010/JPB.2012.39.1.049

He, M., He, C. Q., & Ding, N. Z. (2018). Abiotic stresses: General defenses of land plants and chances for engineering multistress tolerance. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.01771

Henmi, T., Miyao, M., & Yamamoto, Y. (2004). Release and reactive-oxygen-mediated damage of the oxygen-evolving complex subunits of PSII during photoinhibition. *Plant and cell physiology*, *45*(2), 243-250. doi: 10.1093/pcp/pch027

Hergert, G. W., Margheim, J. F., Pavlista, A. D., Martin, D. L., Supalla, R. J., & Isbell, T. A. (2016). Yield, irrigation response, and water productivity of deficit to fully irrigated spring canola. *Agricultural Water Management*, *168*, 96-103. doi: 10.1016/j.agwat.2016.02.003

Hernández, I., Cela, J., Alegre, L., Munné-Bosch, S. (2012). Antioxidant defenses against drought stress. In: Aroca, R. (eds) Plant responses to drought stress. Heidelberg: Springer. doi: 10.1007/978-3-642-32653-0_9

Hu, H., & Xiong, L. (2014). Genetic engineering and breeding of drought-resistant crops. *Annual Review of Plant Biology*, *65*, 715–741. doi: 10.1146/annurev-arplant-050213-040000

Hu, X., Khan, I., Jiao, Q., Zada, A., & Jia, T. (2021). Chlorophyllase, a common plant hydrolase enzyme with a long history, is still a puzzle. *Genes*, *12*(12). doi: 10.3390/genes12121871

Huehn, M. (2011). On the bias of recombination fractions, Kosambis and Haldanes distances based on frequencies of gametes. *Genome*, *54*(3), 196–201. doi: 10.1139/G10-109

Hurgobin, B., Golicz, A. A., Bayer, P. E., Chan, C. K. K., Tirnaz, S., Dolatabadian, A., ... & Edwards, D. (2018). Homoeologous exchange is a major cause of gene presence/absence variation in the amphidiploid *Brassica napus*. *Plant biotechnology journal*, *16*(7), 1265-1274. doi: 10.1111/pbi.12867

Igwe, D. O., Ihearahu, O. C., Osano, A. A., Acquaah, G., & Ude, G. N. (2021). Genetic diversity and population assessment of *Musa* L. (Musaceae) employing CDDP markers. *Plant Molecular Biology Reporter*, *39*(4), 801–820. doi: 10.1007/s11105-021-01290-x

Ishimaru, K., Shirota, K., Higa, M., & Kawamitsu, Y. (2001). Identification of quantitative trait loci for adaxial and abaxial stomatal frequencies in *Oryza sativa*. *Plant Physiology and Biochemistry*, *39*(2), 173–177. doi: 10.1016/S0981-9428(00)01232-8

Jaleel, C. A., Manivannan, P., Lakshmanan, G. M. A., Gomathinayagam, M., & Panneerselvam, R. (2008). Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficits. *Colloids and Surfaces B: Biointerfaces*, *61*(2), 298–303. doi: 10.1016/j.colsurfb.2007.09.008

Jansen, R. C. (1993). Interval mapping of multiple quantitative trait loci. *Genetics*, *135*(1), 205–211. doi: 10.1093/genetics/135.1.205

Jensen, R. G. (2000). Activation of Rubisco regulates photosynthesis at high temperature and CO_2. *Proceedings of the National Academy of Sciences*, *97*(24), 12937-12938. doi: 10.1073/pnas.97.24.12937

Jiang, G. L. (2013). Molecular markers and marker-assisted breeding in plants. In: Anderson, S. B. (eds) Plant Breeding from Laboratories to Fields. IntechOpen. doi: 10.5772/52583

Joehanes, R., & Nelson, J. C. (2008). QGene 4.0, an extensible Java QTL-analysis platform. *Bioinformatics*, *24*(23), 2788-2789. doi: 10.1093/bioinformatics/btn523

Johnston, A. M., Tanaka, D. L., Miller, P. R., Brandt, S. A., Nielsen, D. C., Lafond, G. P., & Riveland, N. R. (2002). Oilseed crops for semiarid cropping systems in the

northern great plains. *Agronomy Journal*, *94(2)*, 231–240. doi: 10.2134/agronj2002.2310

Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K., & Shinozaki, K. (1999). Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nature biotechnology*, *17*(3), 287-291. doi: 10.1038/7036

Katche, E., Gaebelein, R., Idris, Z., Vasquez-Teuber, P., Lo, Y. tzu, Nugent, D., Batley, J., & Mason, A. S. (2021). Stable, fertile lines produced by hybridization between allotetraploids *Brassica juncea* (AABB) and *Brassica carinata* (BBCC) have merged the A and C genomes. *New Phytologist*, *230*(3), 1242–1257. doi: 10.1111/nph.17225

Kavar, T., Maras, M., Kidrič, M., Šuštar-Vozlič, J., & Meglič, V. (2008). Identification of genes involved in the response of leaves of *Phaseolus vulgaris* to drought stress. *Molecular Breeding*, *21*(2), 159–172. doi: 10.1007/s11032-007-9116-8

Kebriyaee, D., Kordrostami, M., Rezadoostami, M. H., & Lahiji, H. S. (2012). QTL analysis of agronomic traits in rice using SSR and AFLP markers. *Notulae Scientia Biologicae*, *4*(2), 116–123. doi: 10.15835/nsb427501

Khan, A. S., Allah, S. U., & Sadique, S. (2010). Genetic variability and correlation among seedling traits of wheat (*Triticum aestivum*) under water stress. *International Journal of Agriculture and Biology*, *12*(2), 247-250. doi:

Khan, A., Pan, X., Najeeb, U., Tan, D. K. Y., Fahad, S., Zahoor, R., & Luo, H. (2018). Coping with drought: stress and adaptive mechanisms, and management through cultural and molecular alternatives in cotton as vital constituents for plant stress resilience and fitness. *Biological research*, *51*(1). doi: 10.1186/s40659-018-0198-z

Killi, D., Bussotti, F., Raschi, A., & Haworth, M. (2017). Adaptation to high temperature mitigates the impact of water deficit during combined heat and drought stress in C3 sunflower and C4 maize varieties with contrasting drought tolerance. *Physiologia Plantarum*, *159*(2), 130–147. doi: 10.1111/ppl.12490

Kim, H., Lei, P., Wang, A., Liu, S., Zhao, Y., Huang, F., Yu, Z., Zhu, G., He, Z., Tan, D., Wang, H., & Meng, F. (2021). Genetic diversity of castor bean (*Ricinus communis* L.) revealed by ISSR and RAPD markers. *Agronomy*, *11*(3). doi: 10.3390/agronomy11030457

Kim, T. H., Bohmer, M., Hu, H., Nishimura, N., & Schroeder, J. I. (2010). Guard cell signal transduction network: Advances in understanding abscisic acid, CO_2, and Ca^{2+} signaling. *Annual Review of Plant Biology*, *61*, 561–591. doi: 10.1146/annurev-arplant-042809-112226

Koevoets, I. T., Venema, J. H., Elzenga, J. T. M., & Testerink, C. (2016). Roots withstanding their environment: exploiting root system architecture responses to abiotic stress to improve crop tolerance. *Frontiers in plant science*, *7*, 1335. doi: 10.3389/fpls.2016.01335

Korver, R. A., Koevoets, I. T., & Testerink, C. (2018). Out of shape during stress: a key role for auxin. *Trends in plant science*, *23*(9), 783-793. doi: 10.1016/j.tplants.2018.05.011

Koscielny, C. B., Gardner, S. W., & Duncan, R. W. (2018). Impact of high temperature on heterosis and general combining ability in spring canola (*Brassica napus* L.). *Field Crops Research*, *221*, 61–70. doi: 10.1016/j.fcr.2018.02.014

Kottmann, L., Wilde, P., & Schittenhelm, S. (2016). How do timing, duration, and intensity of drought stress affect the agronomic performance of winter rye? *European Journal of Agronomy*, *75*, 25–32. doi: 10.1016/j.eja.2015.12.010

Kpoghomou, B. K., Sapra, V. T., & Beyl, C. A. (1990). Screening for drought tolerance: soybean germination and its relationship to seedling responses. *Journal of Agronomy and Crop Science*, *164*(3), 153-159. doi: 10.1111/j.1439-037X.1990.tb00801.x

Ku, M. S., SCHMITT, M. R., & EDWARDS, G. E. (1979). Quantitative determination of RuBP carboxylase–oxygenase protein in leaves of several C3 and C4 plants. *Journal of Experimental Botany*, *30*(1), 89-98. doi: 10.1093/jxb/30.1.89

Kutcher, H. R., Warland, J. S., & Brandt, S. A. (2010). Temperature and precipitation effects on canola yields in Saskatchewan, Canada. *Agricultural and Forest Meteorology*, *150*(2), 161–165. doi: 10.1016/j.agrformet.2009.09.011

Laboda, T. (2000). Gas exchange of spring barley and wheat grown under mild water shortage. *Photosynthetica*, *38*(3), 429–432. doi: 10.1023/A:1010942108576

Lambers, H., Chapin, F. S., & Pons, T. L. (2008). Plant physiological ecology. New York: Springer. doi: 10.1007/978-0-387-78341-3

Lander, E. S., & Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, *121*(1), 185–199. doi: 10.1093/genetics/121.1.185

Larkan, N. J., Yu, F., Lydiate, D. J., Rimmer, S. R., & Borhan, M. H. (2016). Single R gene introgression lines for accurate dissection of the *Brassica-Leptosphaeria* pathosystem. *Frontiers in Plant Science*, *7*, 1771. doi: 10.3389/fpls.2016.01771

Lawlor, D. W. (1976). Water stress induced changes in photosynthesis, photorespiration, respiration and CO_2 compensation concentration of wheat. *Photosynthetica*, *10*(03), 378–387.

Lawlor, D. W., & Cornic, G. (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, Cell and Environment*, *25*(2), 275–294. doi: 10.1046/j.0016-8025.2001.00814.x

Lee, J. W., Kim, I. H., & Woyengo, T. A. (2020). Toxicity of canola-derived glucosinolate degradation products in pigs-A review. *Animals*, *10*(12), 2337. doi:

Levy, D., Fogelman, E., Itzhak, Y., Ma, Q., Turner, D. W., Cowling, W. A., Ma, Y., Turner, Q., & Cowling, D. W. (2006). Osmotic adjustment in leaves of *Brassica*

oilseeds in response to water deficit. *Canadian Journal of Plant Science*, *86*(2), 389–397. doi: 10.4141/P05-10

Li, H., Ye, G., & Wang, J. (2007). A modified algorithm for the improvement of composite interval mapping. *Genetics*, *175*(1), 361–374. doi: 10.1534/genetics.106.066811

Li, J., Lv, J., Liu, P., Chen, P., Wang, J., & Li, J. (2019). Genome survey and high-resolution backcross genetic linkage map construction of the ridgetail white prawn *Exopalaemon carinicauda* applications to QTL mapping of growth traits. *BMC Genomics*, *20*(1). doi: 10.1186/s12864-019-5981-x

Li, J., Zeng, L., Cheng, Y., Lu, G., Fu, G., Ma, H., ... & Li, C. (2018). Exogenous melatonin alleviates damage from drought stress in *Brassica napus* L.(rapeseed) seedlings. *Acta Physiologiae Plantarum*, *40*, 1-11. doi: 10.1007/s11738-017-2601-8

Li, P., Chen, J., & Wu, P. (2011). Agronomic characteristics and grain yield of 30 spring wheat genotypes under drought stress and nonstress conditions. *Agronomy Journal*, *103*(6), 1619–1628. doi: 10.2134/agronj2011.0013

Li, X., Han, Y., Wei, Y., Acharya, A., Farmer, A. D., Ho, J., Monteros, M. J., & Brummer, E. C. (2014). Development of an alfalfa SNP array and its use to evaluate patterns of population structure and linkage disequilibrium. *PLoS ONE*, *9*(1). doi: 10.1371/journal.pone.0084329

Li, Y., & Wei, K. (2020). Comparative functional genomics analysis of cytochrome P450 gene superfamily in wheat and maize. *BMC plant biology*, *20*, 1-22. doi: 10.1186/s12870-020-2288-7

Li, Y., Ye, W., Wang, M., & Yan, X. (2009). Climate change and drought: a risk assessment of crop-yield impacts. *Research*, *39*(1), 31–46. doi: 10.2307/24870421

Li, Z., Mei, S., Mei, Z., Liu, X., Fu, T., Zhou, G., & Tu, J. (2014). Mapping of QTL associated with waterlogging tolerance and drought resistance during the seedling stage in oilseed rape (*Brassica napus*). *Euphytica*, *197*(3), 341-353. doi: 10.1007/s10681-014-1070-z

Lillemo, M., Mather, D., Appels, R., … Akhunov, E. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant Biotechnology Journal*, *12*(6), 787–796. doi: 10.1111/pbi.12183

Lipiec, J., Doussan, C., Nosalewicz, A., & Kondracka, K. (2013). Effect of drought and heat stresses on plant growth and yield: A review. *International Agrophysics*, *27*(4), 463–477. doi: 10.2478/intag-2013-0017

Liu, C., & Zhang, T. (2017). Expansion and stress responses of the AP2/EREBP superfamily in cotton. *BMC genomics*, *18*(1), 1-16. doi: 10.1186/s12864-017-3517-9

Liu, H. S., Li, F. M., & Xu, H. (2004). Deficiency of water can enhance root respiration rate of drought-sensitive but not drought-tolerant spring wheat. *Agricultural Water Management*, *64*(1), 41-48. doi: 10.1016/S0378-3774(03)00143-4

Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K., & Shinozaki, K. (1998). Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought-and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *The Plant Cell*, *10*, 1391–1406. doi: 10.1105/tpc.10.8.1391

Lohani, N., Jain, D., Singh, M. B., & Bhalla, P. L. (2020). Engineering multiple abiotic stress tolerance in canola, *Brassica napus*. *Frontiers in plant science*, *11*. doi: 10.3389/fpls.2020.00003

Lu, G., Cao, J., Yu, X., Xiang, X., & Chen, H. (2008). Mapping QTLs for root morphological traits in *Brassica rapa* L. based on AFLP and RAPD markers. *Journal of applied genetics*, *49*, 23-31. doi: 10.1007/BF03195245

Lynch, J. P. (2013). Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. *Annals of botany*, *112*(2), 347-357. doi: 10.1093/aob/mcs293

Ma, Q., Turner, D. W., Levy, D., & Cowling, W. A. (2004). Solute accumulation and osmotic adjustment in leaves of *Brassica* oilseeds in response to soil water deficit. *Australian Journal of Agricultural Research*, *55*(9), 939–945. doi:10.1071/AR03183

Mafakheri, A., Siosemardeh, A.F., Bahramnejad, B., Struik, P.C. and Sohrabi, Y. (2010) Effect of Drought Stress on Yield, Proline and Chlorophyll Contents in Three Chickpea Cultivars. *Journal of Crop Science*, *4*, 777-780.

Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P. C., & Sohrabi, Y. (2011). Effect of drought stress and subsequent recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea (*'Cicer arietinum'*) cultivars. *Australian Journal of Crop Science*, *5*(10), 1255-1260. doi: 10.3316/informit.746357591676684

Makino, A., Mae, T., & Ohira, K. (1983). Photosynthesis and ribulose 1, 5-bisphosphate carboxylase in rice leaves: changes in photosynthesis and enzymes involved in carbon assimilation from leaf development through senescence. *Plant Physiology*, *73*(4), 1002-1007. doi: 10.1104/pp.73.4.1002

Mao, H., Jian, C., Cheng, X., Chen, B., Mei, F., Li, F., ... & Kang, Z. (2022). The wheat ABA receptor gene TaPYL1-1B contributes to drought tolerance and grain yield by increasing water-use efficiency. *Plant Biotechnology Journal*, *20*(5), 846-861. doi: 10.1111/pbi.13764

Maracchi, G. (2000). Agricultural Drought — A practical approach to definition, assessment and mitigation strategies. In: Vogt, J.V., Somma, F. (eds) Drought and drought mitigation in europe. advances in natural and technological hazards research. Dordrecht: Springer. doi: 10.1007/978-94-015-9472-1_5

Maroco, J. P., Rodrigues, M. L., Lopes, C., & Chaves, M. M. (2002). Limitations to leaf photosynthesis in field-grown grapevine under drought - metabolic and modeling approaches. *Functional Plant Biology*, *29*(4), 451–459. doi: 10.1071/PP01040

Martignago, D., Rico-Medina, A., Blasco-Escámez, D., Fontanet-Manzaneque, J. B., & Caño-Delgado, A. I. (2020). Drought resistance by engineering plant tissue-specific responses. *Frontiers in plant science*, *10*, 1676. doi: 10.3389/fpls.2019.01676

Mason, A. S., Higgins, E. E., Snowdon, R. J., Batley, J., Stein, A., Werner, C., & Parkin, I. A. P. (2017). A user guide to the *Brassica* 60K Illumina Infinium™ SNP genotyping array. *Theoretical and Applied Genetics*, *130*(4), 621–633. doi: 10.1007/s00122-016-2849-1

Mason, A. S., Takahira, J., Atri, C., Samans, B., Hayward, A., Cowling, W. A., ... & Nelson, M. N. (2015). Microspore culture reveals complex meiotic behaviour in a trigenomic *Brassica* hybrid. *BMC Plant Biology*, *15*(1), 1-15. doi: 10.1186/s12870-015-0555-9

Massacci, A., Nabiev, S. M., Pietrosanti, L., Nematov, S. K., Chernikova, T. N., Thor, K., & Leipner, J. (2008). Response of the photosynthetic apparatus of cotton (*Gossypium hirsutum*) to the onset of drought stress under field conditions studied by gas-exchange analysis and chlorophyll fluorescence imaging. *Plant Physiology and Biochemistry*, *46*(2), 189–195. doi: 10.1016/j.plaphy.2007.10.006

Masud, M. B., Khaliq, M. N., & Wheater, H. S. (2017). Future changes to drought characteristics over the Canadian prairie provinces based on NARCCAP multi-RCM ensemble. *Climate Dynamics*, *48*(8), 2685–2705. doi: 10.1007/s00382-016-3232-2

Mathew, B., Léon, J., Dadshani, S., Pillen, K., Sillanpää, M. J., & Naz, A. A. (2021). Importance of correcting genomic relationships in single-locus QTL mapping model with an advanced backcross population. *G3: Genes, Genomes, Genetics*, *11*(6). doi: 10.1093/g3journal/jkab105

McCouch, S. R., Wright, M. H., Tung, C. W., Maron, L. G., McNally, K. L., Fitzgerald, M., ... & Mezey, J. (2016). Open access resources for genome-wide association mapping in rice. *Nature communications*, *7*(1). doi: 10.1038/ncomms10532

McVetty, P.B.E., Duncan, R.W. (2015). Canola, rapeseed, and mustard: for biofuels and bioproducts. In: Cruz, V.M.V., Dierig, D.A. (eds) Industrial crops. handbook of plant breeding. New York: Springer. doi: 10.1007/978-1-4939-1447-0_7

McVetty, P. B. E., Fernando, W. G. D., Scarth, R., & Li, G. (2006). Red river 1826 Roundup Ready™ high erucic acid, low glucosinolate summer rape. *Canadian Journal of Plant Science*, ***86***(4), 1179–1180. doi: 10.4141/P06-072

Mehrotra, R., Bhalothia, P., Bansal, P., Basantani, M. K., Bharti, V., & Mehrotra, S. (2014). Abscisic acid and abiotic stress tolerance - Different tiers of regulation. *Journal of Plant Physiology*, *171*(7), 486–496. doi: 10.1016/j.jplph.2013.12.007

Mekonnen, M. D., Mullen, J. L., Arathi, H. S., Assefa, Y., McKay, J. K., & Byrne, P. F. (2020). Quantitative trait locus mapping for carbon isotope ratio and root pulling force in canola. *Agrosystems, Geosciences & Environment*, *3*(1). doi: 10.1002/agg2.20095

Meyer, S., & Genty, B. (1999). Heterogeneous inhibition of photosynthesis over the leaf surface of Rosa rubiginosa L. during water stress and abscisic acid treatment: induction of a metabolic component by limitation of CO_2 diffusion. *Planta*, *210*, 126-131. doi: 10.1007/s004250050661

Miao, Y., Lv, D., Wang, P., Wang, X. C., Chen, J., Miao, C., & Song, C. P. (2006). An *Arabidopsis* glutathione peroxidase functions as both a redox transducer and a scavenger in abscisic acid and drought stress responses. *The Plant Cell*, *18*(10), 2749-2766. doi: 10.1105/tpc.106.044230

Michel, B. E. (1983). Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant physiology*, *72*(1), 66-70. doi: 10.1104/pp.72.1.66

Michel, B. E., & Kaufmann, M. R. (1973). The osmotic potential of polyethylene glycol 6000. *Plant physiology*, *51*(5), 914-916. doi: 10.1104/pp.51.5.914

Miles, C. M., & Wayne, M. (2008). Quantitative Trait Locus (QTL) analysis. *Nature Education*, *1*(208).

Millar, A. H., Whelan, J., Soole, K. L., & Day, D. A. (2011). Organization and regulation of mitochondrial respiration in plants. *Annual Review of Plant Biology*, *62*, 79–104. doi: 10.1146/annurev-arplant-042110-103857

Mishra, A. K., & Singh, V. P. (2010). A review of drought concepts. *Journal of hydrology*, *391*(1-2), 202-216. doi: 10.1016/j.jhydrol.2010.07.012

Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends in Plant Science*, *11*(1), 15–19. doi: 10.1016/j.tplants.2005.11.002

Mittler, R. (2017). ROS Are Good. *Trends in Plant Science*, *22*(1), 11–19. doi: 10.1016/j.tplants.2016.08.002

Miziorko, H. M., & Lorimer, G. H. (1983). Ribulose-1,5-bisphosphate carboxylase-oxygenase. *Annual review of biochemistry*, *52*, 507–535. doi: 10.1146/annurev.bi.52.070183.002451

Mohan, M., Nair, S., Bhagwat, A., Krishna, T. G., Yano, M., Bhatia, C. R., & Sasaki, T. (1997). Genome mapping, molecular markers and marker-assisted selection in crop plants. *Molecular Breeding*, *3*, 87–103. doi: 10.1023/A:1009651919792

Moragues, M., Comadran, J., Waugh, R., Milne, I., Flavell, A. J., & Russell, J. R. (2010). Effects of ascertainment bias and marker number on estimations of barley diversity from high-throughput SNP genotype data. *Theoretical and Applied Genetics*, *120*(8), 1525–1534. doi: 10.1007/s00122-010-1273-1

Morgan, J. M. (1984). Osmoregulation and water stress in higher plants. *Annual review of plant physiology*, *35*(1), 299-319. doi: 10.1146/annurev.pp.35.060184.001503

Morgante, M., & Salamini, F. (2003). From plant genomics to breeding practice. *Current Opinion in Biotechnology*, *14*(2), 214–219. doi: 10.1016/S0958-1669(03)00028-4

Morrell, P. L., Buckler, E. S., & Ross-Ibarra, J. (2012). Crop genomics: advances and applications. *Nature Reviews Genetics*, *13*(2), 85–96. doi: 10.1038/nrg3097

Morton, N. E. (1996). Logarithm of odds (lods) for linkage in complex inheritance (polygenic linkage). *Proceedings of National Academy of Sciences*, *93*(8), 3471–3476. doi: 10.1073/pnas.93.8.3471

Munné-Bosch, S., & Alegre, L. (2004). Die and let live: leaf senescence contributes to plant survival under drought stress. *Functional Plant Biology*, *31*(3), 203-216. doi: 10.1071/FP03236

Murata, Y., Pei, Z.-M., Mori, I. C., & Schroeder, J. (2001). Abscisic acid activation of plasma membrane ca 2+ channels in guard cells requires cytosolic NADPH and is differentially disrupted upstream and downstream of reactive oxygen species production in abi1-1 and abi2-1 protein phosphatase 2C mutants. *The Plant Cell*, *13*(11), 2513–2523. doi: 10.1105/tpc.010210

Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M., ... & Baloch, F. S. (2018). DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment*, *32*(2), 261-285. doi: 10.1080/13102818.2017.1400401

Nagaharu, U. (1935) Genome Analysis in Brassica with Special Reference to the Experimental Formation of *B. Napus* and Peculiar Mode of Fertilization. *Japanese Journal of Botany*, 7, 389-452.

Nagaharu, U. J. J. J. B., & Nagaharu, N. J. J. J. B. (1935). Genome analysis in Brassica with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *Japanese Journal of Botany*, *7*(7), 389-452.

Navari-Izzo, F., Quartacci, M. F., Pinzino, C., Rascio, N., Vazzana, C., & Sgherri, C. L. (2000). Protein dynamics in thylakoids of the desiccation-tolerant plant *Boea hygroscopica* during dehydration and rehydration. *Plant Physiology*, *124*(3), 1427-1436. doi: 10.1104/pp.124.3.1427

Nie, Y., Ji, W., & Ma, S. (2019). Assessment of heterosis based on genetic distance estimated using SNP in common wheat. *Agronomy*, *9*(2). doi: 10.3390/agronomy9020066

Noctor, G., & Foyer, C. H. (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annual review of plant biology*, *49*(1), 249-279. doi: 10.1146/annurev.arplant.49.1.249

Norouzi, M., Toorchi, M., Salekdeh, G. H., Mohammadi, S. A., Neyshabouri, M. R., & Aharizad, S. (2008). Effect of water deficit on growth, grain yield and osmotic adjustment in rapeseed. *Journal of Food Agriculture and Environment*, *6*(2), 132-138.

Novick, K. A., Ficklin, D. L., Baldocchi, D., Davis, K. J., Ghezzehei, T. A., Konings, A. G., ... & Wood, J. D. (2022). Confronting the water potential information gap. *Nature Geoscience*, *15*(3), 158-164. doi: 10.1038/s41561-022-00909-2

Nuccio, M. L., Paul, M., Bate, N. J., Cohn, J., & Cutler, S. R. (2018). Where are the drought tolerant crops? An assessment of more than two decades of plant biotechnology effort in crop improvement. *Plant science*, *273*, 110-119. doi: 10.1016/j.plantsci.2018.01.020

Olvera-Carrillo, Y., Campos, F., Reyes, J. L., Garciarrubio, A., & Covarrubias, A. A. (2010). Functional analysis of the group 4 late embryogenesis abundant proteins reveals their relevance in the adaptive response during water deficit in Arabidopsis. *Plant physiology*, *154*(1), 373-390. doi: 10.1104/pp.110.158964

Orsavova, J., Misurcova, L., Vavra Ambrozova, J., Vicha, R., & Mlcek, J. (2015). Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *International journal of molecular sciences*, *16*(6), 12871-12890. doi: 10.3390/ijms160612871

Osakabe, Y., Osakabe, K., Shinozaki, K., & Tran, L. S. P. (2014). Response of plants to water stress. *Frontiers in plant science*, *5*, 86. doi: 10.3389/fpls.2014.00086

Osborn, T. C., Butrulle, D. V., Sharpe, A. G., Pickering, K. J., Parkin, I. A., Parker, J. S., & Lydiate, D. J. (2003). Detection and effects of a homeologous reciprocal transposition in *Brassica napus*. *Genetics*, *165*(3), 1569-1577. doi: 10.1093/genetics/165.3.1569

Ouyang, W., Struik, P. C., Yin, X., & Yang, J. (2017). Stomatal conductance, mesophyll conductance, and trans piration efficiency in relation to leaf anatomy in rice and wheat genotypes under drought. *Journal of Experimental Botany*, *68*(18), 5191–5205. doi: 10.1093/jxb/erx314

Pallardy, S. G. (2008). Absorption of Water and Ascent of Sap. In *Physiology of woody plants* (pp. 287–323).

Pandey, M. K., Agarwal, G., Kale, S. M., Clevenger, J., Nayak, S. N., Sriswathi, M., ... & Varshney, R. K. (2017). Development and evaluation of a high density genotyping 'Axiom_Arachis' array with 58 K SNPs for accelerating genetics and breeding in groundnut. *Scientific Reports*, *7*(1), 1-10. doi: 10.1038/srep40577

Pandian, B. A., Sathishraj, R., Djanaguiraman, M., Prasad, P. V., & Jugulam, M. (2020). Role of cytochrome P450 enzymes in plant stress response. *Antioxidants*, *9*(5), 454. doi: 10.3390/antiox9050454

Papendick, R.I. & Campbell, G.S. (1981) Theory and measurementsof water potential. In: Parr, J.F., Gardner, W.R. & Elliott, L.F.(Eds.)Water potential relations in soil microbiology. Madison:Soil Science Society of America. doi: 10.2136/sssaspecpub9.c1

Parkin, I. A., Gulden, S. M., Sharpe, A. G., Lukens, L., Trick, M., Osborn, T. C., & Lydiate, D. J. (2005). Segmental structure of the Brassica napus genome based on comparative analysis with *Arabidopsis thaliana*. *Genetics*, *171*(2), 765-781. doi: 10.1534/genetics.105.042093

Parry, M. A. J., Andralojc, P. J., Khan, S., Lea, P. J., & Keys, A. J. (2002). Rubisco activity: effects of drought stress. *Annals of Botany*, *89*(7), 833–839. doi: 10.1093/aob/mcf103

Patakas, A., Nikolaou, N., Zioziou, E., Radoglou, K., & Noitsakis, B. (2002). The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines. *Plant Science*, *163*(2), 361–367. www.elsevier.com/locate/plantsci

Peleman, J. D., Wye, C., Zethof, J., Sorensen, A. P., Verbakel, H., van Oeveren, J., Gerats, T., & van der Voort, J. R. (2005). Quantitative trait locus (QTL) isogenic recombinant analysis: A method for high-resolution mapping of QTL within a single population. *Genetics*, *171*(3), 1341–1352. doi: .1534/genetics.105.045963

Pelloux, J., Jolivet, Y., Fontaine, V., Banvoy, J., & Dizengremel, P. (2001). Changes in Rubisco and Rubisco activase gene expression and polypeptide content in *Pinus halepensis* M. subjected to ozone and drought. *Plant, Cell and Environment*, *24*(1), 123–131. doi: 10.1046/j.1365-3040.2001.00665.x

Peltzer, D., Dreyer, E., & Polle, A. (2002). Differential temperature dependencies of antioxidative enzymes in two contrasting species: *Fagus sylvatica* and *Coleus blumei*. *Plant Physiology and Biochemistry*, *40*, 141–150. doi: 10.1016/S0981-9428(01)01352-3

Per, T. S., Khan, N. A., Reddy, P. S., Masood, A., Hasanuzzaman, M., Khan, M. I. R., & Anjum, N. A. (2017). Approaches in modulating proline metabolism in plants for salt and drought stress tolerance: Phytohormones, mineral nutrients and transgenics. *Plant Physiology and Biochemistry*, *115*, 126–140. doi: 10.1016/j.plaphy.2017.03.018

Pereira, L. S. (2005). Water and agriculture: facing water scarcity and environmental challenges. *Agricultural Engineering International: CIGR Journal*, *7*.

Philip, J. R. (1966). Plant water relations: some physical aspects. *Annual Review of Plant Physiology*, *17*(1), 245-268. doi: 10.1146/annurev.pp.17.060166.001333

Pires, J. C., Zhao, J., Schranz, M. E., Leon, E. J., Quijada, P. A., Lukens, L. N., & Osborn, T. C. (2004). Flowering time divergence and genomic rearrangements in resynthesized *Brassica* polyploids (Brassicaceae). *Biological Journal of the Linnean Society*, *82*(4), 675-688. doi: 10.1111/j.1095-8312.2004.00350.x

Polania, J. A., Poschenrieder, C., Beebe, S., & Rao, I. M. (2016). Effective use of water and increased dry matter partitioned to grain contribute to yield of common bean improved for drought resistance. *Frontiers in Plant Science*, *7*. doi: 10.3389/fpls.2016.00660

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation

and environmental control. *New Phytologist*, 193(1), 30-50). doi: 10.1111/j.1469-8137.2011.03952.x

Portis Jr, A. R. (1995). The regulation of Rubisco by Rubisco activase. *Journal of Experimental Botany*, *46*, 1285-1291. doi: 10.1093/jxb/46.special_issue.1285

Qaderi, M. M., Kurepin, L. v., & Reid, D. M. (2006). Growth and physiological responses of canola (*Brassica napus*) to three components of global climate change: Temperature, carbon dioxide and drought. *Physiologia Plantarum*, *128*(4), 710–721. doi: 10.1111/j.1399-3054.2006.00804.x

Raboanatahiry, N., Li, H., Yu, L., & Li, M. (2021). Rapeseed (*Brassica napus*): Processing, utilization, and genetic improvement. *Agronomy*, *11*(9), 1776. doi: 10.3390/agronomy11091776

Rafalski, A. (2002). Applications of single nucleotide polymorphisms in crop genetics. *Current Opinion in Plant Biology*, *5*(2), 94–100. doi: 10.1016/S1369-5266(02)00240-6

Raghavendra, A. S., & Padmasree, K. (2003). Beneficial interactions of mitochondrial metabolism with photosynthetic carbon assimilation. *Trends in Plant Science*, *8*(11), 546–553. doi: 10.1016/j.tplants.2003.09.015

Raman, H., Raman, R., Kilian, A., Detering, F., Long, Y., Edwards, D., ... & Lydiate, D. (2013). A consensus map of rapeseed (*Brassica napus* L.) based on diversity array technology markers: applications in genetic dissection of qualitative and quantitative traits. *BMC genomics*, *14*, 1-13. doi: 10.1186/1471-2164-14-277

Raman, H., Raman, R., Pirathiban, R., McVittie, B., Sharma, N., Liu, S., ... & Zhang, Y. (2022). Multienvironment QTL analysis delineates a major locus associated with homoeologous exchanges for water-use efficiency and seed yield in canola. *Plant, Cell & Environment*, *45*(7), 2019-2036. doi: 10.1111/pce.14337

Reddy, A. R., Chaitanya, K. V., & Vivekanandan, M. (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology*, *161*(11), 1189–1202. doi: 10.1016/j.jplph.2004.01.013

Rempel, C. B., Hutton, S. N., & Jurke, C. J. (2014). Clubroot and the importance of canola in Canada. *Canadian Journal of Plant Pathology*, *36*, 19-26. doi: 10.1080/07060661.2013.864336

Ren, X., Wang, J., Liu, L., Sun, G., Li, C., Luo, H., & Sun, D. (2016). SNP-based high density genetic map and mapping of *btwd1* dwarfing gene in barley. *Scientific reports*, *6*(1), 31741. doi: 10.1038/srep31741

Ren, Y., Liu, J., Zhang, J., Dreisigacker, S., Xia, X., & Geng, H. (2021). QTL mapping of drought tolerance at germination stage in wheat using the 50 K SNP array. *Plant Genetic Resources: Characterisation and Utilisation*, *19*(5), 453–460. doi: 10.1017/S1479262121000551

Ribas-Carbo, M., Taylor, N. L., Giles, L., Busquets, S., Finnegan, P. M., Day, D. A., ... & Flexas, J. (2005). Effects of water stress on respiration in soybean leaves. *Plant Physiology*, *139*(1), 466-473. doi: 10.1104/pp.105.065565

Rice, J. P., Saccone, N. L., & Corbett, J. (2008). Model-based methods for linkage analysis. *Advances in Genetics*, *60*, 155–173). doi: 10.1016/S0065-2660(07)00407-5

Rimmer, S.R., Scarth, R., and McVetty, P. (1998). Sentry summer rape. *Canadian Journal of Plant Science*, **78**, 615-616. doi: 10.4141/P97-12

Rodriguez-Dominguez, C. M., Buckley, T. N., Egea, G., de Cires, A., Hernandez-Santana, V., Martorell, S., & Diaz-Espejo, A. (2016). Most stomatal closure in woody species under moderate drought can be explained by stomatal responses to leaf turgor. *Plant, Cell & Environment*, *39*(9), 2014-2026. doi: 10.1111/pce.12774

Roosjen, M., Paque, S., & Weijers, D. (2018). Auxin response factors: output control in auxin biology. *Journal of experimental botany*, *69*(2), 179-188. doi: 10.1093/jxb/erx237

Rosquete, M. R., Von Wangenheim, D., Marhavý, P., Barbez, E., Stelzer, E. H., Benková, E., ... & Kleine-Vehn, J. (2013). An auxin transport mechanism restricts positive orthogravitropism in lateral roots. *Current biology*, *23*(9), 817-822. doi: 10.1016/j.cub.2013.03.064

Rousseau-Gueutin, M., Belser, C., Da Silva, C., Richard, G., Istace, B., Cruaud, C., ... & Aury, J. M. (2020). Long-read assembly of the *Brassica napus* reference genome Darmor-bzh. *GigaScience*, *9*(12). doi: 10.1093/gigascience/giaa137

Salinas Ruíz, J., Montesinos López, O.A., Hernández Ramírez, G., Crossa Hiriart, J. (2023). Generalized Linear Mixed Models for Non-normal Responses. In: Generalized Linear Mixed Models with Applications in Agriculture and Biology. Cham: Springer. doi: 10.1007/978-3-031-32800-8_4

Russo, M., Yan, F., Stier, A., Klasen, L., & Honermeier, B. (2021). Erucic acid concentration of rapeseed (*Brassica napus* L.) oils on the German food retail market. *Food science & nutrition*, *9*(7), 3664-3672. doi: 10.1002/fsn3.2327

Rymen, B., Kawamura, A., Schäfer, S., Breuer, C., Iwase, A., Shibata, M., ... & Sugimoto, K. (2017). ABA suppresses root hair growth via the OBP4 transcriptional regulator. *Plant Physiology*, *173*(3), 1750-1762. doi: 10.1104/pp.16.01945

Saeedipour, S. (2013). Relationship of grain yield, ABA and proline accumulation in tolerant and sensitive wheat cultivars as affected by water stress. *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*, *83*(3), 311–315. doi: 10.1007/s40011-012-0147-5

Scheben, A., Verpaalen, B., Lawley, C. T., Chan, C. K. K., Bayer, P. E., Batley, J., & Edwards, D. (2019). CropSNPdb: a database of SNP array data for *Brassica* crops and hexaploid bread wheat. *The Plant Journal*, *98*, 142–152. doi: 10.1111/tpj.14194

Schranz, M. E., Lysak, M. A., & Mitchell-Olds, T. (2006). The ABC's of comparative genomics in the Brassicaceae: building blocks of crucifer genomes. *Trends in Plant Science*, *11*(11), 535–542. doi: 10.1016/j.tplants.2006.09.002

Schroeder, Julian. I., & Hagiawara, S. (1989). Cytosolic calcium regulates ion channels in the plasma membrane of Vicia faba guard cells. *Nature* , *338*, 427–430. doi: 10.1038/338427a0

Scoffoni, C., Albuquerque, C., Brodersen, C. R., Townes, S. V., John, G. P., Bartlett, M. K., ... & Sack, L. (2017). Outside-xylem vulnerability, not xylem embolism, controls leaf hydraulic decline during dehydration. *Plant physiology*, *173*(2), 1197-1210. doi: 10.1104/pp.16.01643

Seki, M., Kamei, A., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2003). Molecular responses to drought, salinity and frost: Common and different paths for plant protection. *Current Opinion in Biotechnology*, *14*(2), 194–199. doi: 10.1016/S0958-1669(03)00030-2

Seki, M., Umezawa, T., Urano, K., & Shinozaki, K. (2007). Regulatory metabolic networks in drought stress responses. *Current Opinion in Plant Biology*, *10*(3), 296–302. doi: 10.1016/j.pbi.2007.04.014

Semagn, K., Bjørnstad, Å., & Ndjiondjop, M. N. (2006). An overview of molecular marker methods for plants. *African Journal of Biotechnology*, *5*(25), 2540–2568.

Seo, Y. J., Park, J. B., Cho, Y. J., Jung, C., Seo, H. S., Park, S. K., ... & Song, J. T. (2010). Overexpression of the ethylene-responsive factor gene *BrERF4* from *Brassica rapa* increases tolerance to salt and drought in *Arabidopsis* plants. *Molecules and Cells*, *30*, 271-277. doi: 10.1007/s10059-010-0114-z

Seymour, D. K., Filiault, D. L., Henry, I. M., Monson-Miller, J., Ravi, M., Pang, A., ... & Maloof, J. N. (2012). Rapid creation of *Arabidopsis* doubled haploid lines for quantitative trait locus mapping. *Proceedings of the National Academy of Sciences*, *109*(11), 4227-4232. doi: 10.1073/pnas.1117277109

Sgherri, C., Pinzino, C., & Nvari Izzo, F. (1996). Sunflower seedlings subjected to increasing stress by water deficit: changes in superoxide production related to the composition of thylakoid membranes. *Physiologia Plantarum*, *96*(3), 446-452. doi: 10.1111/j.1399-3054.1996.tb00457.x

Shabala, S. N., & Lew, R. R. (2002). Turgor regulation in osmotically stressed *Arabidopsis* epidermal root cells. Direct support for the role of inorganic ion uptake as revealed by concurrent flux and cell turgor measurements. *Plant physiology*, *129*(1), 290-299. doi: 10.1104/pp.020005

Sharif, P., Seyedsalehi, M., Paladino, O., Van Damme, P., Sillanpää, M., & Sharifi, A. A. (2018). Effect of drought and salinity stresses on morphological and physiological characteristics of canola. *International Journal of Environmental Science and Technology*, *15*, 1859-1866. doi: 10.1007/s13762-017-1508-7

Sharma, P., & Dubey, R. S. (2005). Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. *Plant Growth Regulation*, *46*(3), 209–221. doi: 10.1007/s10725-005-0002-2

Sharp, R. E., Poroyko, V., Hejlek, L. G., Spollen, W. G., Springer, G. K., Bohnert, H. J., & Nguyen, H. T. (2004). Root growth maintenance during water deficits: physiology to functional genomics. *Journal of experimental botany*, *55*(407), 2343-2351. doi: 10.1093/jxb/erh276

Sharpe, A. G., Parkin, I. A. P., Keith, D. J., & Lydiate, D. J. (1995). Frequent nonreciprocal translocations in the amphidiploid genome of oilseed rape (*Brassica napus*). *Genome*, *38*(6), 1112-1121. doi: 10.1139/g95-148

Shavrukov, Y., Kurishbayev, A., Jatayev, S., Shvidchenko, V., Zotova, L., Koekemoer, F., ... & Langridge, P. (2017). Early flowering as a drought escape mechanism in plants: how can it aid wheat production?. *Frontiers in plant science*, *8*. doi: 10.3389/fpls.2017.01950

Shinozaki, K., & Yamaguchi-Shinozaki, K. (2007). Gene networks involved in drought stress response and tolerance. *Journal of Experimental Botany*, *58*(2), 221–227. doi: 10.1093/jxb/erl164

Smyth, S. J., Gusta, M., Belcher, K., Phillips, P. W., & Castle, D. (2011). Environmental impacts from herbicide tolerant canola production in Western Canada. *Agricultural Systems*, *104*(5), 403-410. doi: 10.1016/j.agsy.2011.01.004

Song, J. M., Guan, Z., Hu, J., Guo, C., Yang, Z., Wang, S., ... & Guo, L. (2020). Eight high-quality genomes reveal pan-genome architecture and ecotype differentiation of *Brassica napus*. *Nature Plants*, *6*(1), 34-45. doi: 10.1038/s41477-019-0577-7

Specht, J. E., Chase, K., Macrander, M., Graef, G. L., Chung, J., Markwell, J. P., ... & Lark, K. G. (2001). Soybean response to water: a QTL analysis of drought tolerance. *Crop science*, *41*(2), 493-509. doi: 10.2135/cropsci2001.412493x

Statistics Canada. (2021). Production of principal field crops, November 2021. Retrieved from https://www150.statcan.gc.ca/n1/daily-quotidien/211203/dq211203b-eng.pdf

Stefansson, B. R., & Kondra, Z. P. (1975). Tower summer rape. *Canadian Journal of Plant Science*, *55*(1), 343-344.

Sun, C., Dong, Z., Zhao, L., Ren, Y., Zhang, N., & Chen, F. (2020). The Wheat 660K SNP array demonstrates great potential for marker-assisted selection in polyploid wheat. *Plant Biotechnology Journal*, *18*(6), 1354-1360. doi: 10.1111/pbi.13361

Sumberg, J., & Giller, K. E. (2022). What is 'conventional'agriculture?. *Global Food Security*, *32*, doi: 10.1016/j.gfs.2022.100617

Syvanen, A. C. (2001). Accessing Genetic Variation: Genotyping Single nucleotide polymorphisms. *Nature reviews genetics*, *2*, 930–942. doi: 10.1038/35103535

Szemenyei, H., Hannon, M., & Long, J. A. (2008). TOPLESS mediates auxin-dependent transcriptional repression during *Arabidopsis* embryogenesis. *Science*, *319*(5868), 1384-1386. doi: 10.1126/science.11514

Szeto, K., Zhang, X., White, R. E., & Brimelow, J. (2016). The 2015 extreme drought in Western Canada. *Bulletin of the American Meteorological Society*, *97*(12), S42–S46. doi: 10.1175/BAMS-D-16-0147.1

Tahkokorpi, M., Taulavuori, K., Laine, K., & Taulavuori, E. (2007). After-effects of drought-related winter stress in previous and current year stems of *Vaccinium myrtillus* L. *Environmental and Experimental Botany*, *61*(1), 85–93. doi: 10.1016/j.envexpbot.2007.03.003

Takahashi, F., Kuromori, T., Urano, K., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2020). Drought stress responses and resistance in plants: from cellular responses to long-distance intercellular communication. *Frontiers in Plant Science*, *11*, 1–14. doi: 10.3389/fpls.2020.556972

Takahashi, F., Suzuki, T., Osakabe, Y., Betsuyaku, S., Kondo, Y., Dohmae, N., ... & Shinozaki, K. (2018). A small peptide modulates stomatal control via abscisic acid in long-distance signalling. *Nature*, *556*(7700), 235-238. doi: 10.1038/s41586-018-0009-2

Tan, Cai. B., Schwartz, S. H., Zeevaart, J. A. D., & Mccarty, D. R. (1997). Genetic control of abscisic acid biosynthesis in maize. *Proceedings of the National Academy of Science*, *94*, 12235–12240. doi: 10.1073/pnas.94.22.12235

Tan, Y. de, & Fornage, M. (2008). Mapping functions. *Genetica*, *133*(3), 235–246. doi: 10.1007/s10709-007-9207-9

Tanksley, S. D., & Nelson, J. C. (1996). Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theoritical and Applied Genetics*, *92*, 191–203. doi: 10.1007/BF00223376

Tardieu, F., Bruckler, L., & Lafolie, F. (1992). Root clumping may affect the root water potential and the resistance to soil-root water transport. *Plant and Soil*, *140*, 291–301. doi: 10.1007/BF00010606

Tenaillon, M. I., Sawkins, M. C., Long, A. D., Gaut, R. L., Doebley, J. F., & Gaut, B. S. (2001). Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays* ssp. *mays* L.). *Proceedings of the National Academy of Sciences*, *98*(16), 9161-9166. doi: 10.1073/pnas.151244298

Tesfaye, M., Feyissa, T., Hailesilassie, T., Kanagarajan, S., & Zhu, L. H. (2023). Genetic diversity and population structure in ethiopian mustard (*Brassica carinata* A. Braun) as revealed by single nucleotide polymorphism markers. *Genes*, *14*(9), 1757. doi: 10.3390/genes14091757

Thambugala, D., Pozniak, C. J., Kumar, S., Burt, A. J., Wise, I. L., Smith, M. A., ... & McCartney, C. A. (2021). Genetic analysis of oviposition deterrence to orange wheat

blossom midge in spring wheat. *Theoretical and Applied Genetics*, *134*, 647-660. doi: 10.1007/s00122-020-03720-y

The economic impact of canola on the Canadian economy (2016). *Report for the Canola Council of Canada*. Manitoba. Retrieved from https://www.canolacouncil.org/download/131/economic-impact/17818/economic-impact-report-canada_december-2020

Tomkowiak, A., Bocianowski, J., Kwiatek, M., & Kowalczewski, P. Ł. (2020). Dependence of the heterosis effect on genetic distance, determined using various molecular markers. *Open Life Sciences*, *15*, 1–11. doi: 10.1515/biol-2020-0001

Trenberth, K. E., Dai, A., van der Schrier, G., Jones, P. D., Barichivich, J., Briffa, K. R., & Sheffield, J. (2014). Global warming and changes in drought. *Nature Climate Change*, *4*(1), 17–22. doi: 10.1038/nclimate2067

Trenberth, K. E., Smith, L., Qian, T., Dai, A., & Fasullo, J. (2007). Estimates of the global water budget and its annual cycle using observational and model data. *Journal of Hydrometeorology*, *8*(4), 758-769. doi: 10.1175/JHM600.1

Trick, M., Long, Y., Meng, J., & Bancroft, I. (2009). Single nucleotide polymorphism (SNP) discovery in the polyploid *Brassica napus* using Solexa transcriptome sequencing. *Plant Biotechnology Journal*, *7*(4), 334–346. doi: 10.1111/j.1467-7652.2008.00396.x

Troggio, M., Malacarne, G., Coppola, G., Segala, C., Cartwright, D. A., Pindo, M., ... & Velasco, R. (2007). A dense single-nucleotide polymorphism-based genetic linkage map of grapevine (*Vitis vinifera* L.) anchoring Pinot Noir bacterial artificial chromosome contigs. *Genetics*, *176*(4), 2637-2650. doi: 10.1534/genetics.106.067462

Troy, T. J., Kipgen, C., & Pal, I. (2015). The impact of climate extremes and irrigation on US crop yields. *Environmental Research Letters*, *10*(5). doi: 10.1088/1748-9326/10/5/054013

Tunnacliffe, A., & Wise, M. J. (2007). The continuing conundrum of the LEA proteins. *Naturwissenschaften*, *94*(10), 791-812. doi: 10.1007/s00114-007-0254-y

Tuteja, N. (2007). Abscisic acid and abiotic stress signaling. *Plant Signaling and Behavior*, *2*(3), 135–138. doi: 10.4161/psb.2.3.4156

Udall, J. A., Quijada, P. A., & Osborn, T. C. (2005). Detection of chromosomal rearrangements derived from homeologous recombination in four mapping populations of *Brassica napus* L. *Genetics*, *169*(2), 967-979. doi: 10.1534/genetics.104.033209

Unterseer, S., Bauer, E., Haberer, G., Seidel, M., Knaak, C., Ouzunova, M., ... & Schön, C. C. (2014). A powerful tool for genome analysis in maize: development and evaluation of the high density 600 k SNP genotyping array. *BMC genomics*, *15*(1), 1-15. doi: 10.1186/1471-2164-15-823

van Bueren, E. T. L., Backes, G., de Vriend, H., & Østergård, H. (2010). The role of molecular markers and marker assisted selection in breeding for organic agriculture. *Euphytica*, *175*(1), 51–64. doi: 10.1007/s10681-010-0169-0

Van Der Weele, C. M., Spollen, W. G., Sharp, R. E., & Baskin, T. I. (2000). Growth of *Arabidopsis thaliana* seedlings under water deficit studied by control of water potential in nutrient-agar media. *Journal of experimental botany*, *51*(350), 1555-1562. doi: 10.1093/jexbot/51.350.1555

Verma, A., Niranjana, M., Jha, S. K., Mallick, N., Agarwal, P., & Vinod. (2020). QTL detection and putative candidate gene prediction for leaf rolling under moisture stress condition in wheat. *Scientific Reports*, *10*(1). doi: 10.1038/s41598-020-75703-4

Vishwakarma, K., Upadhyay, N., Kumar, N., Yadav, G., Singh, J., Mishra, R. K., ... & Sharma, S. (2017). Abscisic acid signaling and abiotic stress tolerance in plants: a review on current knowledge and future prospects. *Frontiers in plant science*, *8*, 161. doi: 10.3389/fpls.2017.00161

Vos, P. G., Uitdewilligen, J. G. A. M. L., Voorrips, R. E., Visser, R. G. F., & van Eck, H. J. (2015). Development and analysis of a 20K SNP array for potato (*Solanum tuberosum*): an insight into the breeding history. *Theoretical and Applied Genetics*, *128*(12), 2387–2401. doi: 10.1007/s00122-015-2593-y

Wan, J., Griffiths, R., Ying, J., McCourt, P., & Huang, Y. (2009). Development of drought-tolerant canola (*Brassica napus* L.) through genetic modulation of ABA-mediated stomatal responses. *Crop Science*, *49*(5), 1539–1554). doi: 10.2135/cropsci2008.09.0568

Wang, J., Lydiate, D. J., Parkin, I. A. P., Falentin, C., Delourme, R., Carion, P. W. C., & King, G. J. (2011). Integration of linkage maps for the Amphidiploid *Brassica napus* and comparative mapping with *Arabidopsis* and *Brassica rapa*. *BMC Genomics*, *12*. doi: 10.1186/1471-2164-12-101

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., ... & Akhunov, E. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant biotechnology journal*, *12*(6), 787-796. doi: 10.1111/pbi.12183

Wang, X., Chen, L., Wang, A., Wang, H., Tian, J., Zhao, X., ... & Li, M. (2016). Quantitative trait loci analysis and genome-wide comparison for silique related traits in *Brassica napus*. *BMC plant biology*, *16*(1), 1-15. doi: 10.1186/s12870-016-0759-7

Wang, X., Du, T., Huang, J., Peng, S., & Xiong, D. (2018). Leaf hydraulic vulnerability triggers the decline in stomatal and mesophyll conductance during drought in rice. *Journal of Experimental Botany*, *69*(16), 4033–4045. doi: 10.1093/jxb/ery188

Wang, X., Li, Q., Xie, J., Huang, M., Cai, J., Zhou, Q., Dai, T., & Jiang, D. (2021). Abscisic acid and jasmonic acid are involved in drought priming-induced tolerance to drought in wheat. *The Crop Journal*, *9*(1), 120–132. doi: 10.1016/j.cj.2020.06.002

Wang, X., Liu, H., Yu, F., Hu, B., Jia, Y., Sha, H., & Zhao, H. (2019). Differential activity of the antioxidant defence system and alterations in the accumulation of osmolyte and reactive oxygen species under drought stress and recovery in rice (*Oryza sativa* L.) tillering. *Scientific Reports*, *9*(1). doi: 10.1038/s41598-019-44958-x

Wendlinger, C., Hammann, S., & Vetter, W. (2014). Various concentrations of erucic acid in mustard oil and mustard. *Food chemistry*, *153*, 393-397. doi: 10.1016/j.foodchem.2013.12.073

Werner, C., Correia, O., & Beyschlag, W. (1999). Two different strategies of Mediterranean macchia plants to avoid photoinhibitory damage by excessive radiation levels during summer drought. *Acta Oecologica*, *20*(1), 15–23. doi: 10.1016/S1146-609X(99)80011-3

Whalen, A., Gorjanc, G., & Hickey, J. M. (2019). Parentage assignment with genotyping-by-sequencing data. *Journal of Animal Breeding and Genetics*, *136*(2), 102-112. doi: 10.1111/jbg.12370

Wheater, H., & Gober, P. (2013). Water security in the Canadian Prairies: Science and management challenges. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *371*. doi: 10.1098/rsta.2012.0409

Wheaton, E., Kulshreshtha, S., Wittrock, V., & Koshida, G. (2008). Dry times: hard lessons from the Canadian drought of 2001 and 2002. *The Canadian Geographer/Le Géographe canadien*, *52*(2), 241-262. doi: 10.1111/j.1541-0064.2008.00211.x

Wijewardene, I., Shen, G., & Zhang, H. (2021). Enhancing crop yield by using Rubisco activase to improve photosynthesis under elevated temperatures. *Stress Biology*, *1*(1). doi: 10.1007/s44154-021-00002-5

Willekens, H., Chamnongpol, S., Davey, M., Schraudner, M., van Montagu, M., & Inzé, D. (1997). Catalase is a sink for H_2O_2 and is indispensable for stress defence in C3 plants. *The EMBO Journal*, *16*(16), 4806–4816. doi: 10.1093/emboj/16.16.4806

Winter, P., & Kahl, G. (1995). Molecular marker technologies for plant improvement. *World journal of microbiology & biotechnology*, *11*(4), 438–448. doi: 10.1007/BF00364619

Wright, I. J., Reich, P. B., Atkin, O. K., Lusk, C. H., Tjoelker, M. G., & Westoby, M. (2006). Irradiance, temperature and rainfall influence leaf dark respiration in woody plants: Evidence from comparisons across 20 sites. *New Phytologist*, *169*(2), 309–319. doi: 10.1111/j.1469-8137.2005.01590.x

Wright, T. I., Burnett, A. C., Griffiths, H., Kadner, M., Powell, J. S., Oliveira, H. R., & Leigh, F. J. (2020). Identification of quantitative trait loci relating to flowering time, flag leaf and awn characteristics in a novel *Triticum dicoccum* mapping population. *Plants*, *9*(7), 829. doi: 10.3390/plants9070829

Wu, W., Ma, B. L., & Whalen, J. K. (2018). Enhancing rapeseed tolerance to heat and drought stresses in a changing climate: perspectives for stress adaptation from root

system architecture. *Advances in Agronomy*, *151*, 87–157. doi: 10.1016/bs.agron.2018.05.002

Wu, Y., Bhat, P. R., Close, T. J., & Lonardi, S. (2008). Efficient and accurate construction of genetic linkage maps from the minimum spanning tree of a graph. *PLoS genetics*, *4*(10), e1000212. doi: doi.org/10.1371/journal.pgen.1000212

Xiong, L., Wang, R. G., Mao, G., & Koczan, J. M. (2006). Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid. *Plant Physiology*, *142*(3), 1065–1074. doi: 10.1104/pp.106.084632

Xia, Y., Yang, J., Ma, L., Yan, S., & Pang, Y. (2021). Genome-wide identification and analyses of drought/salt-responsive cytochrome P450 genes in *Medicago truncatula*. *International Journal of Molecular Sciences*, *22*(18), 9957. doi: 10.3390/ijms22189957

Xue, J. Y., Wang, Y., Chen, M., Dong, S., Shao, Z. Q., & Liu, Y. (2020). Maternal inheritance of U's triangle and evolutionary process of *Brassica* mitochondrial genomes. *Frontiers in Plant Science*, *11*(805). doi: 10.3389/fpls.2020.00805

Yamaguchi-Shinozakiaib, K., & Shinozaki, K. (1994). A novel cis-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *The Plant Cell*, *6*, 251–264. doi: 10.1105/tpc.6.2.251

Yamane, K., Hayakawa, K., Kawasaki, M., Taniguchi, M., & Miyake, H. (2003). Bundle sheath chloroplasts of rice are more sensitive to drought stress than mesophyll chloroplasts. *Journal of plant physiology*, *160*(11), 1319-1327. doi: 10.1078/0176-1617-01180

Yang, J., Liu, K., Wang, Z., Du, Y., & Zhang, J. (2007). Water-saving and high-yielding irrigation for lowland rice by controlling limiting values of soil water potential. *Journal of Integrative Plant Biology*, *49*(10), 1445–1454. doi: 10.1111/j.1672-9072.2007.00555.x

You, J., & Chan, Z. (2015). ROS regulation during abiotic stress responses in crop plants. *Frontiers in plant science*, *6*, 1092. doi: 10.3389/fpls.2015.01092

Young, N.D. (1994). Constructing a plant genetic linkage map with DNA markers. In: Phillips, R.L., Vasil, I.K. (eds) DNA-based markers in plants. Advances in Cellular and Molecular Biology of Plants. Dordrecht: Springer. doi: 10.1007/978-94-011-1104-1_3

Yusa, A., Berry, P., Cheng, J. J., Ogden, N., Bonsal, B., Stewart, R., & Waldick, R. (2015). Climate change, drought and human health in Canada. *International journal of environmental research and public health*, *12*(7), 8359-8412. doi: 10.3390/ijerph120708359

Zagdanska, B. (1995). Respiratory energy demand for protein turnover and ion transport in wheat leaves upon water deficit. *Physiologia Plantarum*, *95*(3), 428–436. doi: 10.1111/j.1399-3054.1995.tb00859.x

Zargar, M., Zavarykina, T., Voronov, S., Pronina, I., & Bayat, M. (2022). The recent development in technologies for attaining doubled haploid plants *in vivo*. *Agriculture* , *12*(10). doi: 10.3390/agriculture12101595

Zargar, S. M., Gupta, N., Nazir, M., Mahajan, R., Malik, F. A., Sofi, N. R., ... & Salgotra, R. K. (2017). Impact of drought on photosynthesis: Molecular perspective. *Plant gene*, *11*, 154-159. doi: 10.1016/j.plgene.2017.04.003

Zhang, B., Lewis, J. A., Kovacs, F., Sattler, S. E., Sarath, G., & Kang, C. (2023). Activity of Cytosolic Ascorbate Peroxidase (APX) from *Panicum virgatum* against ascorbate and phenylpropanoids. *International Journal of Molecular Sciences*, *24*(2), 1778. doi: 10.3390/ijms24021778

Zhang, C., Gou, L., Hu, S., & Lu, N. (2022). A thermodynamic formulation of water potential in soil. *Water Resources Research*, *58*(9). doi: 10.1029/2022WR032369

Zhang, H., Zhao, Y., & Zhu, J. K. (2020). Thriving under stress: how plants balance growth and the stress response. *Developmental Cell*, *55*(5), 529-543. doi: 10.1016/j.devcel.2020.10.012

Zhang, J., & Davies, W. J. (1987). Increased synthesis of ABA in partially dehydrated root tips and ABA transport from roots to leaves. *Journal of Experimental Botany*, *38*(12), 2015-2023. doi: 10.1093/jxb/38.12.2015

Zhang, J., Mason, A. S., Wu, J., Liu, S., Zhang, X., Luo, T., ... & Yan, G. (2015). Identification of putative candidate genes for water stress tolerance in canola (*Brassica napus*). *Frontiers in Plant Science*, *6*, 1058. doi:10.3389/fpls.2015.01058

Zhang, J., Zheng, H. G., Aarti, A., Pantuwan, G., Nguyen, T. T., Tripathy, J. N., ... & Nguyen, H. T. (2001). Locating genomic regions associated with components of drought resistance in rice: comparative mapping within and across species. *Theoretical and applied Genetics*, *103*, 19-29. doi: 10.1007/s001220000534

Zhao, J., Udall, J. A., Quijada, P. A., Grau, C. R., Meng, J., & Osborn, T. C. (2006). Quantitative trait loci for resistance to *Sclerotinia sclerotiorum* and its association with a homeologous non-reciprocal transposition in *Brassica napus* L. *Theoretical and Applied Genetics*, *112*, 509-516. doi: 10.1007/s00122-005-0154-5

Zhou, Y., Lam, H. M., & Zhang, J. (2007). Inhibition of photosynthesis and energy dissipation induced by water and high light stresses in rice. *Journal of Experimental Botany*, *58*(5), 1207–1217. doi: 10.1093/jxb/erl291

Zhu, J. K. (2016). Abiotic stress signaling and responses in plants. *Cell*, *167*(2), 313–324. doi: 10.1016/j.cell.2016.08.029

Zhu, M., Simons, B., Zhu, N., Oppenheimer, D. G., & Chen, S. (2010). Analysis of abscisic acid responsive proteins in *Brassica napus* guard cells by multiplexed isobaric tagging. *Journal of Proteomics*, *73*(4), 790–805. doi: 10.1016/j.jprot.2009.11.002

Zlatev, Z. S., Lidon, F. C., Ramalho, J. C., & Yordanov, I. T. (2006). Comparison of resistance to drought of three bean cultivars. *BIOLOGIA PLANTARUM*, *50*(3), 389–394.

SUPPLEMENTARY TABLES

Supplemental table S1: Selected candidate genes related to drought stress tolerance

Marker name	Biological functional category	Gene name	QTL
Antioxidant			
BnaA03T0171000WE	Antioxidant	Cell differentiation family, Rcd1-like	qRD-FR-A3-2-1
A03p61580.1_BnaDAR	Antioxidant	Tocopherol cyclase	qRD-FR-A3-2-2
A03p61900.1_BnaDAR	Antioxidant	Meiosis arrest female protein 1	qRD-FR-A3-2-2
A03p61970.1_BnaDAR	Antioxidant	Peroxidase	qRD-FR-A3-2-2
A03p62160.1_BnaDAR	Antioxidant	Aldo-keto reductase, putative	qRD-FR-A3-2-2
A03p62410.1_BnaDAR	Antioxidant	Aldehyde dehydrogenase	qRD-FR-A3-2-2
A03p62420.1_BnaDAR	Antioxidant	Aldehyde dehydrogenase	qRD-FR-A3-2-2
A03p62540.1_BnaDAR	Antioxidant	DJ-1 family protein	qRD-FR-A3-2-2
A03p62610.1_BnaDAR	Antioxidant	CBS domain-containing protein CBSX1, chloroplastic	qRD-FR-A3-2-2
A03p62660.1_BnaDAR	Antioxidant	V-type proton ATPase subunit H	qRD-FR-A3-2-2
A03p63220.1_BnaDAR	Antioxidant	Ascorbate peroxidase	qRD-FR-A3-2-2
A03p63280.1_BnaDAR	Antioxidant	Catalase	qRD-FR-A3-2-2
A03p64210.1_BnaDAR	Antioxidant	Cytochrome P450	qRD-FR-A3-2-2
A03p64240.1_BnaDAR	Antioxidant	Peroxidase	qRD-FR-A3-2-2
A03p64270.1_BnaDAR	Antioxidant	Salicylate O-methyltransferase	qRD-FR-A3-2-2
A03p64980.1_BnaDAR	Antioxidant	CBS domain-containing protein CBSX1, chloroplastic	qRD-FR-A3-2-2
A03p65090.1_BnaDAR	Antioxidant	Cytochrome P450 like protein	qRD-FR-A3-2-2
A03p65100.1_BnaDAR	Antioxidant	Cytochrome P450 like protein	qRD-FR-A3-2-2
A03p65110.1_BnaDAR	Antioxidant	Cytochrome P450-like protein	qRD-FR-A3-2-2
A03p65220.1_BnaDAR	Antioxidant	Peroxidase	qRD-FR-A3-2-2
A03p65780.1_BnaDAR	Antioxidant	Cytochrome P450 79B1	qRD-FR-A3-2-2
A03p65860.1_BnaDAR	Antioxidant	Alpha-ketoglutarate-dependent dioxygenase alkB-like protein 2	qRD-FR-A3-2-2
A03p66410.1_BnaDAR	Antioxidant	Peroxidase 14	qRD-FR-A3-2-2
A03p64120.1_BnaDAR	Antioxidant	Cytochrome P450	qRD-FR-A3-2-2
A03p64130.1_BnaDAR	Antioxidant	Phytochrome A	qRD-FR-A3-2-2
A03p64140.1_BnaDAR	Antioxidant	Aldehyde dehydrogenase	qRD-FR-A3-2-2
A03p64690.1_BnaDAR	Antioxidant	Zinc finger CCCH domain protein	qRD-FR-A3-2-2

A03p64760.1_BnaDAR	Antioxidant	L-ascorbate oxidase-like protein	qRD-FR-A3-2-2
A03p64830.1_BnaDAR	Antioxidant	Heat stress transcription factor	qRD-FR-A3-2-2
A03p66330.1_BnaDAR	Antioxidant	Heat shock 70 kDa protein	qRD-FR-A3-2-2
BnaA03T0149300WE	Antioxidant	Cytochrome P450	qRD-FS-A3-2, qRD-DS-A3-2, qRD-FWPA3-2, and qRD- DWP-A3-2
BnaA03T0149500WE	Antioxidant	Cytochrome P450	qRD-FS-A3-2, qRD-DS-A3-2, qRD-FWPA3-2, and qRD- DWP-A3-2
BnaA03T0155300WE	Antioxidant	Iron/manganese superoxide dismutases, C-terminal domain	qRD-FS-A3-2, qRD-DS-A3-2, qRD-FWPA3-2, and qRD- DWP-A3-2
BnaA03T0162800WE	Antioxidant	Cytochrome P450	qRD-FS-A3-2, qRD-DS-A3-2, qRD-FWPA3-2, and qRD- DWP-A3-2
BnaA03T0162900WE	Antioxidant	Cytochrome P450	qRD-FS-A3-2, qRD-DS-A3-2, qRD-FWPA3-2, and qRD- DWP-A3-2
BnaA03T0163900WE	Antioxidant	Cytochrome P450	qRD-FS-A3-2, qRD-DS-A3-2, qRD-FWPA3-2, and qRD- DWP-A3-2
BnaA03T0164400WE	Antioxidant	Eukaryotic cytochrome b561	qRD-FS-A3-2, qRD-DS-A3-2, qRD-FWPA3-2, and qRD- DWP-A3-2
BnaA03T0163400WE	Antioxidant	WRKY DNA -binding domain	qRD-FS-A3-2, qRD-DS-A3-2, qRD-FWPA3-2, and qRD- DWP-A3-2
BnaA03T0167800WE	Antioxidant	Glutathione peroxidase	qRD-FS-A3-2, qRD-DS-A3-2, qRD-FWPA3-2, and qRD- DWP-A3-2
A04p25650.1_BnaDAR	Antioxidant	Stellacyanin	qDSI-FR-A4-10
A04p25720.1_BnaDAR	Antioxidant	Retinol dehydrogenase 13	qDSI-FR-A4-10
A04p32180.1_BnaDAR	Antioxidant	transcription factor VOZ1-like	qDSI-FR-A4-10
A04p28390.1_BnaDAR	Antioxidant	Peroxidase	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p28720.1_BnaDAR	Antioxidant	HSP20-like chaperones superfamily protein	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p29270.1_BnaDAR	Antioxidant	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p29370.1_BnaDAR	Antioxidant	Peroxidase	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p29380.1_BnaDAR	Antioxidant	Peroxidase	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p29740.1_BnaDAR	Antioxidant	Zinc finger protein-like protein	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p30870.1_BnaDAR	Antioxidant	Heat shock transcription factor	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p30890.1_BnaDAR	Antioxidant	Heat shock transcription factor	qDSI-FS-A4-10 and qDSI-FWP-A4-10

A04p30900.1_BnaDAR	Antioxidant	Heat shock transcription factor	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p31090.1_BnaDAR	Antioxidant	Glycerol-3-phosphate dehydrogenase [NAD(P)+]	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p31650.1_BnaDAR	Antioxidant	Peroxidase	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p31860.1_BnaDAR	Antioxidant	Cytochrome P450 83a1	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p31870.1_BnaDAR	Antioxidant	Cytochrome P450, putative	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p32070.1_BnaDAR	Antioxidant	Cytochrome P450, putative	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p32730.1_BnaDAR	Antioxidant	Peroxidase	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p33490.1_BnaDAR	Antioxidant	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p33590.1_BnaDAR	Antioxidant	BnaA04g26000D protein	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p33900.1_BnaDAR	Antioxidant	Cytochrome P450, putative	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p33910.1_BnaDAR	Antioxidant	Cytochrome P450, putative	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p34510.1_BnaDAR	Antioxidant	ATP-dependent zinc metalloprotease FtsH	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p34540.1_BnaDAR	Antioxidant	Cytochrome b5	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p34550.1_BnaDAR	Antioxidant	Cytochrome P450	qDSI-FS-A4-10 and qDSI-FWP-A4-10
C03p55000.1_BnaDAR	Antioxidant	Serine/threonine-protein kinase ATR	qRD-FR-C3-11 and qRD-DR-C3-11
C03p55290.1_BnaDAR	Antioxidant	Cytochrome b561 and DOMON domain-containing protein	qRD-FR-C3-11 and qRD-DR-C3-11
C03p55600.1_BnaDAR	Antioxidant	DBH-like monooxygenase	qRD-FR-C3-11 and qRD-DR-C3-11
C03p56000.1_BnaDAR	Antioxidant	Cytochrome P450 like protein	qRD-FR-C3-11 and qRD-DR-C3-11
C03p56010.1_BnaDAR	Antioxidant	Cytochrome P450 family protein	qRD-FR-C3-11 and qRD-DR-C3-11
C07p30530.1_BnaDAR	Antioxidant	14.7 kDa heat shock protein	qDSI-FR-C7-22-1
C07p30540.1_BnaDAR	Antioxidant	Small heat shock protein, chloroplastic	qDSI-FR-C7-22-1
C07p30570.1_BnaDAR	Antioxidant	Heat shock 22 kDa protein, mitochondrial	qDSI-FR-C7-22-1
C07p30600.1_BnaDAR	Antioxidant	NADPH--cytochrome P450 reductase 1	qDSI-FR-C7-22-1
C07p30630.1_BnaDAR	Antioxidant	Peroxidase	qDSI-FR-C7-22-1
C07p30850.1_BnaDAR	Antioxidant	NADPH:quinone oxidoreductase	qDSI-FR-C7-22-1
C07p31230.1_BnaDAR	Antioxidant	Small heat shock protein, chloroplastic	qDSI-FR-C7-22-1
C07p31250.1_BnaDAR	Antioxidant	Small heat shock protein, chloroplastic	qDSI-FR-C7-22-1

C07p31290.1_BnaDAR	Antioxidant	Small heat shock protein, chloroplastic	qDSI-FR-C7-22-1
C07p29370.1_BnaDAR	Antioxidant	F-box family protein	qDSI-FR-C7-22-2 and qDSI-DR-C7-22
C07p29580.1_BnaDAR	Antioxidant	meiosis arrest female protein 1	qDSI-FR-C7-22-2 and qDSI-DR-C7-22
C07p29730.1_BnaDAR	Antioxidant	ATPase P	qDSI-FR-C7-22-2 and qDSI-DR-C7-22
C07p29930.1_BnaDAR	Antioxidant	Aldo-keto reductase	qDSI-FR-C7-22-2 and qDSI-DR-C7-22
Hormones			
BnaA02T0077700WE	Hormone	PP2C	qDSI-FR-C7-22-2 and qDSI-DR-C7-22
BnaA03T0174100WE	Hormone	Zinc finger C-x8-C-x5-C-x3-H type (and similar)	qRD-FR-A3-2-1
BnaA03T0175700WE	Hormone	AUX/IAA family	qRD-FR-A3-2-1
A03p61590.1_BnaDAR	Hormone	Ethylene-responsive transcription factor ERF043	qRD-FR-A3-2-2
A03p61940.1_BnaDAR	Hormone	NAD-dependent epimerase/dehydratase	qRD-FR-A3-2-2
A03p62070.1_BnaDAR	Hormone	Indole-3-acetic acid-amido synthetase GH3.3	qRD-FR-A3-2-2
A03p62080.1_BnaDAR	Hormone	Indole-3-acetic acid-amido synthetase GH3.3	qRD-FR-A3-2-2
A03p62090.1_BnaDAR	Hormone	Indole-3-acetic acid-amido synthetase GH3.3	qRD-FR-A3-2-2
A03p63130.1_BnaDAR	Hormone	Auxin-induced protein 15A	qRD-FR-A3-2-2
A03p63140.1_BnaDAR	Hormone	Auxin-responsive family protein	qRD-FR-A3-2-2
A03p63160.1_BnaDAR	Hormone	Auxin-responsive family protein	qRD-FR-A3-2-2
A03p64070.1_BnaDAR	Hormone	Auxin-induced protein	qRD-FR-A3-2-2
A03p64780.1_BnaDAR	Hormone	Salicylic acid-binding protein 2	qRD-FR-A3-2-2
A03p64990.1_BnaDAR	Hormone	Ethylene-responsive transcription factor	qRD-FR-A3-2-2
A03p65710.1_BnaDAR	Hormone	Glycine-rich domain-containing protein 2	qRD-FR-A3-2-2
A03p66050.1_BnaDAR	Hormone	Pyruvate dehydrogenase E1 component subunit alpha	qRD-FR-A3-2-2
A03p63470.1_BnaDAR	Hormone	Guanine nucleotide-binding protein subunit beta	qRD-FR-A3-2-2
A03p62900.1_BnaDAR	Hormone	Ethylene-responsive transcription factor	qRD-FR-A3-2-2
A03p63450.1_BnaDAR	Hormone	Serine/threonine-protein kinase pakH	qRD-FR-A3-2-2
A03p64970.1_BnaDAR	Hormone	Floral homeotic protein APETALA 2	qRD-FR-A3-2-2
A03p65590.1_BnaDAR	Hormone	AP2-like ethylene-responsive transcription factor ANT	qRD-FR-A3-2-2

A03p65870.1_BnaDAR	Hormone	Ethylene-responsive transcription factor ERF060	qRD-FR-A3-2-2
A03p66520.1_BnaDAR	Hormone	ATP phosphoribosyltransferase	qRD-FR-A3-2-2
BnaA03T0155600WE	Hormone	Ferric reductase NAD binding domain	qRD-FS-A3-2, qRD-DS-A3-2, qRD-FWPA3-2, and qRD- DWP-A3-2
BnaA03T0158900WE	Hormone	Auxin responsive protein	qRD-FS-A3-2, qRD-DS-A3-2, qRD-FWPA3-2, and qRD- DWP-A3-2
BnaA03T0175700WE	Hormone	AUX/IAA family	qRD-FS-A3-2, qRD-DS-A3-2, qRD-FWPA3-2, and qRD- DWP-A3-2
A04p34350.1_BnaDAR	Hormone	Jasmonic acid-amido synthetase JAR1	qDSI-FR-A4-10
A04p34440.1_BnaDAR	Hormone	Auxin response factor, putative (DUF688)	qDSI-FR-A4-10
A04p33510.1_BnaDAR	Hormone	Ethylene-responsive transcription factor 13	qDSI-FR-A4-10
A04p32080.1_BnaDAR	Hormone	Calmodulin binding protein	qDSI-FR-A4-10
A04p29220.1_BnaDAR	Hormone	Auxin transporter-like protein	qDSI-FR-A4-10
A04p27440.1_BnaDAR	Hormone	Methyl esterase 17	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p28300.1_BnaDAR	Hormone	Auxin-responsive family protein	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p29340.1_BnaDAR	Hormone	Abscisic acid receptor like	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p30720.1_BnaDAR	Hormone	Abscisic acid receptor like	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p31810.1_BnaDAR	Hormone	Zinc finger AN1 domain stress-associated protein	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p33190.1_BnaDAR	Hormone	Protein AUXIN-REGULATED GENE INVOLVED IN ORGAN SIZE	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p33810.1_BnaDAR	Hormone	LOB domain-containing protein, putative	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p34570.1_BnaDAR	Hormone	Auxin-responsive family protein	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p34310.1_BnaDAR	Hormone	Abscisic acid-induced stomatal closure	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p33460.1_BnaDAR	Hormone	F-box protein DOR	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p29970.1_BnaDAR	Hormone	Protein phosphatase 2C	qDSI-FS-A4-10 and qDSI-FWP-A4-10
C03p54930.1_BnaDAR	Hormone	Ethylene-responsive transcription factor	qRD-FR-C3-11 and qRD-DR-C3-11
C03p55410.1_BnaDAR	Hormone	Transport inhibitor response 1 protein	qRD-FR-C3-11 and qRD-DR-C3-11
C03p55640.1_BnaDAR	Hormone	12-oxophytodienoate reductase	qRD-FR-C3-11 and qRD-DR-C3-11
C07p30930.1_BnaDAR	Hormone	Ethylene-responsive transcription factor	qDSI-FR-C7-22-1

C07p30970.1_BnaDAR	Hormone	Ethylene-responsive transcription factor 5	qDSI-FR-C7-22-1
C03p55320.1_BnaDAR	Hormone	WRKY transcription factor 1	qRD-FR-C3-11 and qRD-DR-C3-11
C07p29150.1_BnaDAR	Hormone	Abscisic acid receptor like	qDSI-FR-C7-22-2 and qDSI-DR-C7-22
C07p29920.1_BnaDAR	Hormone	Ethylene-responsive transcription factor RAP2-2	qDSI-FR-C7-22-2 and qDSI-DR-C7-22
LEA proteins			
A03p63420.1_BnaDAR	LEA proteins	Late embryogenesis abundant protein	qRD-FR-A3-2-2
A04p27480.1_BnaDAR	LEA proteins	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p28050.1_BnaDAR	LEA proteins	Late embryogenesis abundant protein ECP63	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p31460.1_BnaDAR	LEA proteins	Late embryogenesis abundant protein M10	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p32280.1_BnaDAR	LEA proteins	Late embryogenesis abundant domain-containing protein / LEA domain-containing protein	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p34290.1_BnaDAR	LEA proteins	Late embryogenesis abundant protein	qDSI-FS-A4-10 and qDSI-FWP-A4-10
C03p54920.1_BnaDAR	LEA proteins	Late embryogenesis abundant protein, LEA-18	qRD-FR-C3-11 and qRD-DR-C3-11
Photosynthesis			
A03p62390.1_BnaDAR	Photosynthesis	Photosystem II CP43 reaction center protein	qRD-FR-A3-2-2
A03p64500.1_BnaDAR	Photosynthesis	F-box/kelch-repeat protein At5g39560	qRD-FR-A3-2-2
A03p64680.1_BnaDAR	Photosynthesis	Photosystem II oxygen-evolving enhancer protein	qRD-FR-A3-2-2
A04p30300.1_BnaDAR	Photosynthesis	Ribulose bisphosphate carboxylase/oxygenase activase	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p31220.1_BnaDAR	Photosynthesis	Stomatal closure actin-binding-like protein	qDSI-FS-A4-10 and qDSI-FWP-A4-10
C03p55360.1_BnaDAR	Photosynthesis	Chlorophyll a-b binding protein, chloroplastic	qRD-FR-C3-11 and qRD-DR-C3-11
C07p28750.1_BnaDAR	Photosynthesis	ATP synthase gamma chain	qDSI-FR-C7-22-2 and qDSI-DR-C7-22
C07p28760.1_BnaDAR	Photosynthesis	ATP synthase subunit b 2	qDSI-FR-C7-22-2 and qDSI-DR-C7-22
Osmotic adjustment			
A03p63440.1_BnaDAR	Osmotic adjustment	Proline--tRNA ligase	qRD-FR-A3-2-2
A03p61470.1_BnaDAR	Osmotic adjustment	V-type proton ATPase proteolipid subunit	qRD-FR-A3-2-2
A03p61510.1_BnaDAR	Osmotic adjustment	Potassium channel protein	qRD-FR-A3-2-2

A03p61830.1_BnaDAR	Osmotic adjustment	Alpha-aminoadipic semialdehyde synthase	qRD-FR-A3-2-2
A03p61840.1_BnaDAR	Osmotic adjustment	Alpha-aminoadipic semialdehyde synthase	qRD-FR-A3-2-2
A04p31580.1_BnaDAR	Osmotic adjustment	Dihydroxy-acid dehydratase	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p34530.1_BnaDAR	Osmotic adjustment	proline-rich receptor-like protein kinase PERK2	qDSI-FS-A4-10 and qDSI-FWP-A4-10
C03p56040.1_BnaDAR	Osmotic adjustment	Proline iminopeptidase	qRD-FR-C3-11 and qRD-DR-C3-11
Respiration			
BnaA03T0172800WE	Respiration	ATP synthase	qRD-FR-A3-2-1
BnaA03T0173200WE	Respiration	ATP synthase	qRD-FR-A3-2-1
A03p63090.1_BnaDAR	Respiration	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 9	qRD-FR-A3-2-2
A03p64570.1_BnaDAR	Respiration	NAD(P)H dehydrogenase (Quinone)	qRD-FR-A3-2-2
A04p30310.1_BnaDAR	Respiration	succinate dehydrogenase assembly factor 1, mitochondrial-like	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A04p30320.1_BnaDAR	Respiration	Succinate dehydrogenase assembly factor 1, mitochondrial	qDSI-FS-A4-10 and qDSI-FWP-A4-10
Signal transduction			
BnaA03T0170700WE	Signal transduction	Myb-like DNA-binding domain	qRD-FR-A3-2-1
A03p64640.1_BnaDAR	Signal transduction	Calcium uniporter, mitochondrial	qRD-FR-A3-2-2
A03p64650.1_BnaDAR	Signal transduction	Calcium uniporter, mitochondrial	qRD-FR-A3-2-2
A04p31330.1_BnaDAR	Signal transduction	Calmodulin-binding protein 25	qDSI-FS-A4-10 and qDSI-FWP-A4-10
C07p29430.1_BnaDAR	Signal transduction	Cullin	qDSI-FR-C7-22-2 and qDSI-DR-C7-22
Growth and development			
BnaA03T0171600WE	Growth and development	Cyclin-dependent kinase inhibitor	qRD-FR-A3-2-1
A03p61390.1_BnaDAR	Growth and development	Elongation factor 4	qRD-FR-A3-2-2
A03p61490.1_BnaDAR	Growth and development	Transcriptional corepressor LEUNIG	qRD-FR-A3-2-2
A04p33530.1_BnaDAR	Growth and development	Protein ARABIDILLO 1	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A09p02830.1_BnaDAR	Growth and development	Callose synthase	qDSI-DS-A9-5
C07p28700.1_BnaDAR	Growth and development	Transcription initiation factor TFIID subunit 14b	qDSI-FR-C7-22-2 and qDSI-DR-C7-22
Hydraulic conductance			
A03p63290.1_BnaDAR	Hydraulic conductance	Aquaporin	qRD-FR-A3-2-2
A04p34170.1_BnaDAR	Hydraulic conductance	Aquaporin	qDSI-FR-A4-10
A04p28130.1_BnaDAR	Hydraulic conductance	Aquaporin	qDSI-FS-A4-10 and qDSI-FWP-A4-10

C07p31070.1_BnaDAR	Hydraulic conductance	Aquaporin-like protein	qDSI-FR-C7-22-1
Other			
A04p34180.1_BnaDAR	autophagy	ATG8-interacting protein 1	qDSI-FS-A4-10 and qDSI-FWP-A4-10
aC03p55720.1_BnaDAR	Senescence	Triphosphate tunel metalloenzyme 3	qRD-FR-C3-11 and qRD-DR-C3-11
A03p61630.1_BnaDAR	molecular	ATP-dependent DNA helicase	qRD-FR-A3-2-2
C03p55650.1_BnaDAR	alterations of the composition of fatty acids and, subsequently, the Na+/K+ ratio	Enoyl-[acyl carrier-protein] reductase	qRD-FR-C3-11 and qRD-DR-C3-11
C03p54760.1_BnaDAR	transcriptional and post-transcriptional regulation of organellar gene expression in chloroplast and mitochondria	Mitochondrial transcription termination factor family protein	qRD-FR-C3-11 and qRD-DR-C3-11
A04p30730.1_BnaDAR	Cannot tell specifically	Dehydration-responsive element-binding protein like	qDSI-FS-A4-10 and qDSI-FWP-A4-10
A09p02860.1_BnaDAR	decreased epidermal permeability	Acetyl-coenzyme A synthetase	qDSI-DS-A9-5
C03p54700.1_BnaDAR	decreased epidermal permeability	Long chain acyl-CoA synthetase	qRD-FR-C3-11 and qRD-DR-C3-11
C03p54960.1_BnaDAR	play critical roles in cellular processes, such as folding of newly synthesized polypeptides, refolding of misfolded or aggregated proteins, translocation of proteins across organelles membranes, assembling or disassembling complex structure of a protein, controlling the activity of regulatory proteins, and assisting in the	Heat shock protein 70	qRD-FR-C3-11 and qRD-DR-C3-11

	degradation of proteins		
C03p55650.1_BnaDAR	alterations of the composition of fatty acids and, subsequently, the Na+/K+ ratio	Enoyl-[acyl carrier-protein] reductase	qRD-FR-C3-11 and qRD-DR-C3-11