CHAPTER THREE

Enhancing Rapeseed Tolerance to Heat and Drought Stresses in a Changing Climate: Perspectives for Stress Adaptation from Root System Architecture

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Abstract

Globally, the increase in climatic variability is responsible for more frequent extreme heat and drought stress events. Heat and water stress have a devastating impact on plant growth, development, and formation of the yield components of rapeseed/canola, an important oilseed crop for human consumption and a renewable feedstock for biodiesel production. The growing demand for rapeseed/canola will not be met unless strategies are rapidly developed to sustain and improve crop yields and product quality in the context of global climate change. Therefore, the objective of this review is to discuss the development of rapeseed/canola varieties with enhanced tolerance to heat and drought stresses, as the most promising solution to improve crop productivity in the future. The genetic basis of stress tolerance has provided plant breeders with new options for efficient breeding programs that support high yield potentials under both favorable and stressful environments. This review provides an update on recent advances in characterizing the response of rapeseed/canola to heat and drought stresses, from the molecular level (i.e., signal transduction) to phenotypes at the whole-plant and agroecosystem scales. Of particular interest is the ability of the root system to alleviate abiotic stressors. Roots, as the "hidden half" of the plant, play a central role in acquiring water and nutrients (i.e., improving water and nutrient use efficiencies) as well as anchoring the crop so it can resist lodging and tolerate heat and drought stresses. Considering the urgent need to achieve sustainable production of rapeseed/canola under changing climatic conditions, it is essential to determine how the root system can mitigate abiotic stress. Modern methodologies to quantify root system architecture are presented. Finally, we describe root-specific tolerance mechanisms to abiotic stress and explain how this information can be used to direct breeding programs and decide on agronomic practices that support sustainable rapeseed/canola production now and under future climate change scenarios.

1. INTRODUCTION

Rapeseed (*Brassica* species) has been grown to produce edible and nonedible oils for thousands of years (Canola Council of Canada, 2013). *Brassica juncea*, *Brassica napus*, and *Brassica rapa* are the three main species used for edible oil production (McVetty and Duncan, 2015). Global

production of rapeseed/canola has expanded rapidly in the past 60 years, and rapeseed is now the second largest oilseed crop after soybean, with over 22.7 million tonnes of canola/rapeseed oil produced annually (FAO, 2016, Fig. 1). Different *Brassica* oilseed species are grown in or adapted to climate zones of the world. *B. napus* is mainly grown in cooler temperate regions, such as Australia, Europe, Canada, and northern China, whereas *B. juncea* and *B. rapa* are produced in warmer regions, including India and northwest China (McVetty and Duncan, 2015).

Canada is the leading producer of canola in the world, followed by China and India (Fig. 2). The name of canola came from "Can" (for Canada) and "ola" (for oil low acid); it is distinguished from the conventional rapeseed by low erucic acid content in the oil (<2%) and low glucosinolate concentration (<30 μmol/g) in the meal (Stefansson and Downey, 1995). Canola was developed by Canadian plant breeders in the mid-1970s as a new crop for healthy human oil and nutritious animal feed, using traditional plant breeding techniques. By the mid-2010s, canola accounted for 13%–16% of the world's vegetable oil production and contributed to the rural economy in many regions of North America. For example, canola has become the largest field crop in Canada, with more than 8 million ha of harvested area (Canola Council of Canada, 2017) valued at $26 billion in Canadian dollars. The edible and industrial oils derived from canola are in demand due to their high oil content, and the high-protein meal remaining after the oil extraction process is sold as an animal feed supplement. Canola germinates at soil temperature as low as at ≥5°C and early growth occurs at cooler temperatures, resulting in widespread adoption of this oilseed in cropping systems of temperate climatic zones (Booth and Gunstone, 2004; McVetty and Duncan, 2015).

Although *Brassica* oilseed production and acreage in the world have been increasing steadily, questions remain about the ability of rapeseed/canola production to keep pace with the edible oil demands of the growing global population of 9.1 billion by 2050 (Tilman et al., 2002; Wu and Ma, 2015; FAO, 2007, 2016, Fig. 1). In addition, *Brassica* crops are susceptible to abiotic stresses and ongoing climate change threatens rapeseed/canola production in many parts of the world (Lobell and Gourdji, 2012). Of particular concern for rapeseed/canola crops is more frequent heat stress, drought stress, and a combination of heat and drought stresses that are predicted in the foreseeable future (IPCC, 2007), and which are the dominant environment-induced abiotic stressors linked to catastrophic loss of crop

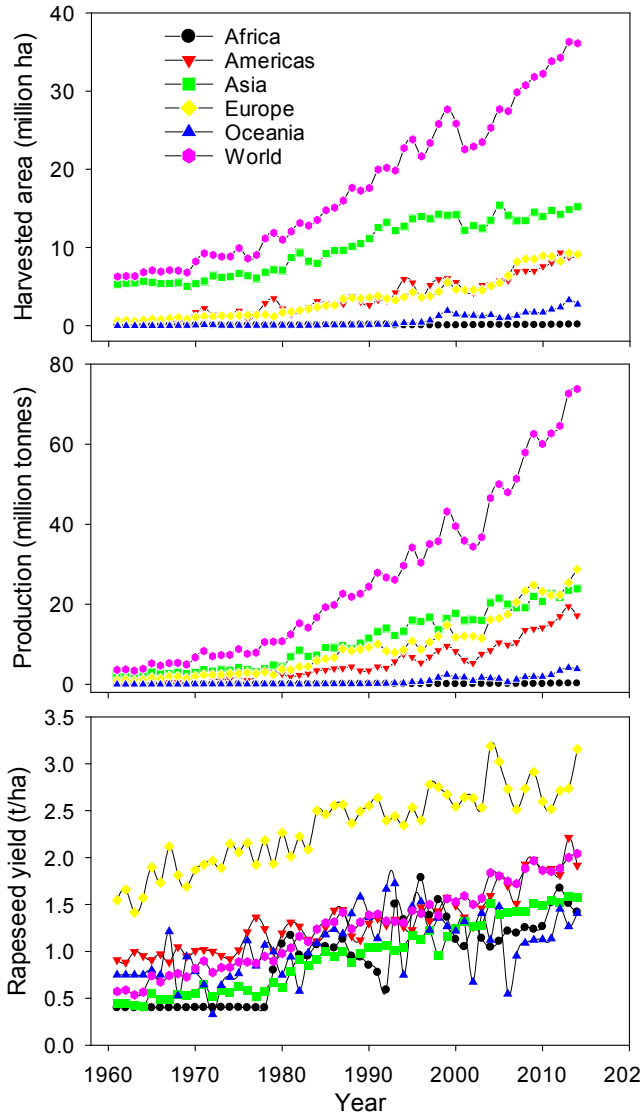

Figure 1 Temporal changes in harvested area, total production, and average yield of oilseed from rapeseed/canola crop from 1961 to 2014. *Data available: http://faostat.fao.org/site/626/default.aspx*.

productivity (Tilman et al., 2002; Ma and Zheng, 2016; Wu and Ma, 2016; Wu et al., 2017). For instance, a temperature increase of 3−4°C could lead to a crop yield loss of 15%−35% in Africa and Asia, and 25%−35% in the Middle East (Ortiz et al., 2008). Similarly, the estimated economically

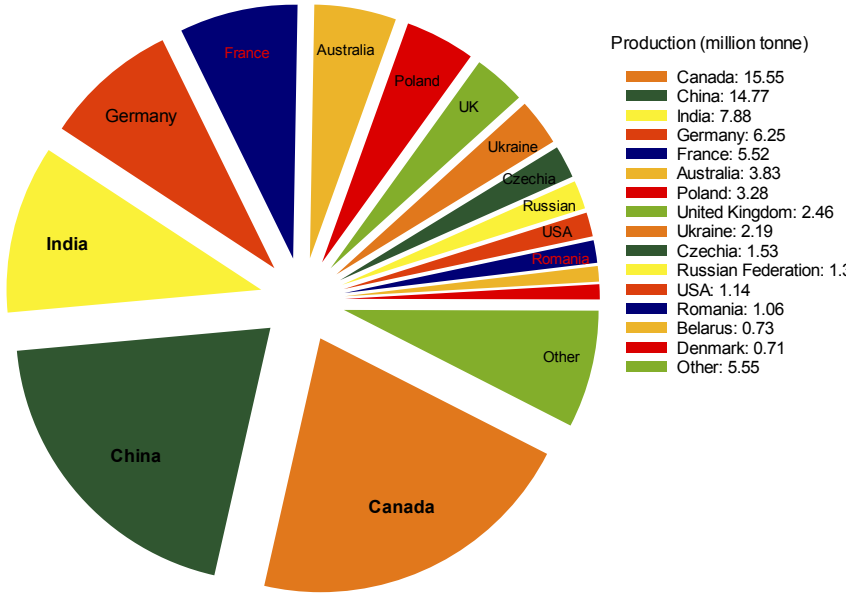

Figure 2 Global production of rapeseed/canola crops from top-producing countries in 2014. *Data available:* http://faostat.fao.org/site/626/default.aspx.

agricultural losses by drought stress could be $50 billion, and drought in combination with heat stress could result in economic losses of $200 billion in overall agricultural production (Suzuki et al., 2014). Genetic improvement of *Brassica* oilseeds to withstand abiotic stress, leading to the development of climate-adapted cultivars, has become a major focus of rapeseed/canola breeding programs worldwide (Fischer and Edmeades, 2010).

Plants exhibit tolerance, avoidance, or escape abiotic stress through acclimation mechanisms that evolved with natural selection or by purposeful selection in agricultural breeding programs (Levitt, 1980; Chaves et al., 2003; Reddy et al., 2004; Li et al., 2017). At the molecular level, plants express or repress genes when they experience heat and drought stresses, initiating a cascade of biochemical, physiological, and morphological responses that increase their probability of surviving the stress (Ding et al., 2009; Seo et al., 2012; Augustine et al., 2015; Liu et al., 2016). Hundreds of genes, proteins, quantitative trait loci (QTLs), and microRNA are known to modulate the heat and drought responses and tolerance in *Brassica* species or other important crops, and these were discovered by genetic, genomic, and molecular methods (Lu et al., 2008; Fletcher et al., 2015; Zhang et al., 2016; ArifUzZaman et al., 2017).

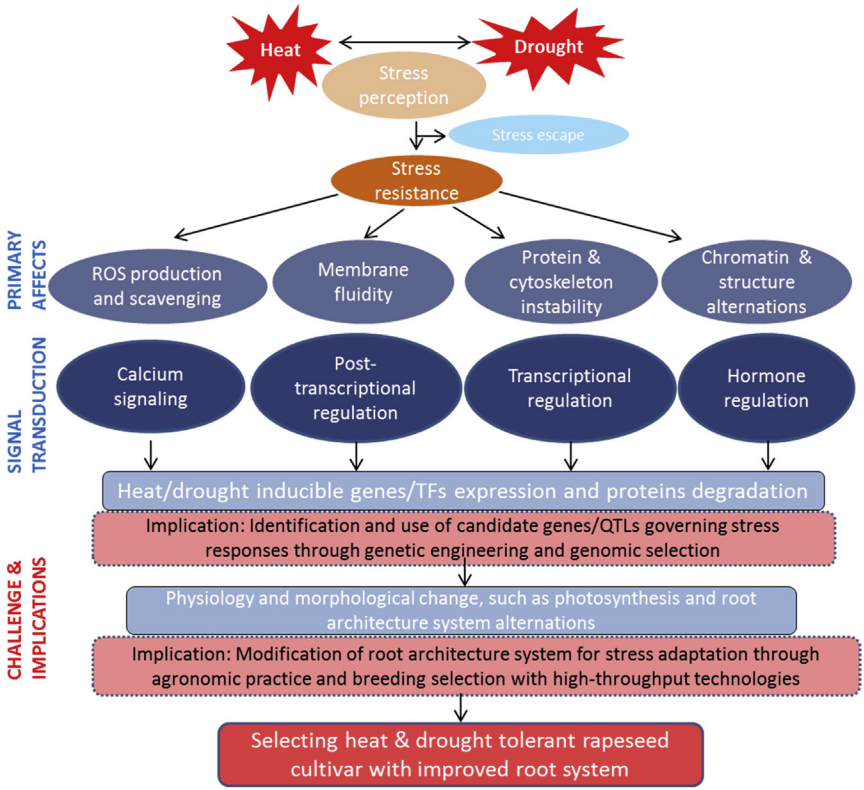

Figure 3 Illustration of heat and drought responses with primary affects and signal transduction, and overview of global approach to improve the stress tolerance with manipulation of root system architecture in rapeseed/canola. Transcription factors (TFs) refer to transcription factors. *ROS*, reactive oxygen species; *QTL*, quantitative trait loci.

Most expression products of stress-responsive genes are proteins involved in signal transduction and transcriptional regulation that ensure cellular integrity and proper functioning, such as protein kinase and transcription factors (TFs) (Sato et al., 2014; Ohama et al., 2017, Fig. 3). Other important expression products include diverse stress-responsive functional proteins that maintain the cellular membrane by osmotic adjustment and scavenging of reactive oxygen species (ROS), as well as late embryogenesis abundant proteins (Singh and Jwa, 2013; Kaur and Asthir, 2017). TFs have received the most attention for their ability to alleviate heat and drought stresses. For example, the heat-shock TF a1s (HsfA1) genes have been regarded as "master regulators" in transcriptional networks and have a critical role in

regulation of heat- and drought-responsive TFs, especially for dehydration-responsive element-binding protein 2A (DREB2A), a well-known TF (Sakuma et al., 2006a, 2006b; Yoshida et al., 2011, Table 1). Many QTLs related to heat and drought stresses have been mapped (Hu and Xiong, 2014), but only a few have been cloned and verified. The QTL deeper rooting 1 (DRO1) on chromosome 9 in rice controls root growth angle, and is the first root QTL cloned in crops that has potential for drought and heat avoidance (Uga et al., 2011, 2013). MicroRNA emerges as another important regulator of plant development and plant stress responses (Zhang and Wang, 2015). The direct link between microRNA and plant tolerance to heat and drought stresses was established with the identification of microRNA398, which targets the Cu/Zn superoxide dismutases (SODs) through hormone regulations and signal transduction processes (Sunkar et al., 2012; Guan et al., 2013), whereas the microR169 and microR394 perform similar functions in *Brassica* species and *Arabidopsis* (Song et al., 2013; Bhardwaj et al., 2014). Overall, the key drought- and heat-responsive genes/molecules mentioned above are instrumental in guiding plant responses to abiotic stresses, such as through altering plant phenology (e.g., flowering time adjustment), canopy structure (temperature adjustment within canopy), water transport activity (aquaporin function modification), and root system architecture (RSA) (e.g., deep rooting, more branching, or different angle of lateral roots) in response to environmental conditions (Franco–Zorrilla et al., 2014; Mallory et al., 2015; Wang et al., 2016a; Shriram et al., 2016).

Roots anchor the plant and are responsible for water and nutrient acquisition from soil, which is essential for plant survival and productivity. We now understand that abiotic stress affects roots more than aboveground components, as roots are the primary organs to exhibit specific cell defense responses to drought and other stressors (Hashimoto et al., 2004; Jeong et al., 2010). For instance, drought stress is first detected in the root tissue and results in the exchange of hydraulic and nonhydraulic response signals between roots and shoots (Comstock, 2002; Jiang and Hartung, 2008). The subsequent abscisic acid (ABA) regulation through the xylem sap is responsible for triggering stomatal closure, which allows plants to tolerate heat and drought stresses (Comstock, 2002). RSA, defined as the spatial and temporal configuration and structure of a plant root system in the soil (De Dorlodot et al., 2007), regulates soil water uptake (Osmont et al., 2007; Aroca et al., 2012; Lynch, 2013). For instance, field and lab studies illustrate that a deep root system provides more heat and drought tolerance

Table 1 Major Transcription Factor Gene Families Expressed in Plants Exposed to Heat and Drought Stresses

Gene Family	Gene	Donor	Acceptor	Enhanced Tolerance	References
AREB/ABF	AREB1/AREB2/ABF3	*Arabidopsis*	*Arabidopsis*	Drought	Yoshida et al. (2015)
AP2/ERFBP	VrDREB2A	*Vigna radiata*	*Arabidopsis*	Drought	Chen et al. (2016b)
	LcDREB3a	*Leymus chinensis*	*Arabidopsis*	Drought	Peng et al. (2013)
	JERF3	*Solanum lycopersicum*	*Oryza sativa*	Drought	Zhang et al. (2010)
	OsERF4a	*O. sativa*	*O. sativa*	Drought	Joo et al. (2013)
	AtDREB1A	*Arabidopsis*	*O. sativa*	Drought	Ravikumar et al. (2014)
	EaDREB2	*Erianthus arundinaceus*	*Sugarcane*	Drought	Augustine et al. (2015)
MYB	BnaMYB78	*Arabidopsis*	*Brassica napus*	Heat and drought; modulates reactive oxygen species (ROS)−dependent cell death	Chen et al. (2016a)
	AtMYB15	*Arabidopsis*	*Arabidopsis*	Drought	Ding et al. (2009)
	GmMYBJ1	*Glycine max*	*Arabidopsis*	Drought	Su et al. (2014)
	TaMYB3R1	*Triticum aestivum*	*Arabidopsis*	Drought	Cai et al. (2015)
	LeAN2	*Lycopersicum esculentum*	*Solanum lycopersicum*	Heat	Meng et al. (2015)
	AtMYB44	*Arabidopsis*	*G. max*	Drought	Seo et al. (2012)
WRKY	BnaWRKY147/166/210	*B. napus*	*B. napus*	Drought and low temperature	He et al. (2016b)
	TaWRKY79	*T. aestivum*	*Arabidopsis*	Drought	Qin et al. (2013)
	GsWRKY20	*Glycine soja*	*Arabidopsis*	Drought	Luo et al. (2013)
	MtWRKY76	*Medicago truncatula*	*M. truncatula*	Drought	Liu et al. (2016)
	TaWRKY1/TaWRKY33	*T. aestivum*	*Arabidopsis*	Drought; heat	He et al. (2016a)

NAC	NAC 14	*Sinapis alba*	*Brassica juncea*	Drought	Phukan et al. (2016)
	NAC 19	*B. juncea*	*B. juncea*	Drought	Phukan et al. (2016)
	NAC 55	*B. napus*	*B. napus*	Heat and drought; modulating ROS accumulation and cell death	Niu et al. (2016)
	NAC09	*Arabidopsis*	*Arabidopsis*	Heat	Guan et al. (2014)
	ZmSNAC1	*Zea mays*	*Arabidopsis*	Drought	Lu et al. (2012)
	TaNAC67	*T. aestivum*	*Arabidopsis*	Drought	Mao et al. (2014)
	TaNAC29	*T. aestivum*	*Arabidopsis*	Drought	Huang et al. (2015)
	SNAC1	*O. sativa*	*T. aestivum*	Drought	Saad et al. (2013)
	OsNAP	*O. sativa*	*O. sativa*	Drought	Chen et al. (2014)
bZIP	Bol008071/ Bol033132/ Bol042729	*Brassica oleracea*	*B. oleracea*	Temperature	Hwang et al. (2016)
	ZnbZIP17	*Z. mays*	*Z. mays*	Heat	Jia et al. (2009)
	OsAREB1	*O. sativa*	*Arabidopsis*	Heat	Jin et al. (2010)
	OsbZIP16	*O. sativa*	*O. sativa*	Drought	Chen et al. (2012)
	ZmbZIP72	*Z. mays*	*Arabidopsis*	Drought	Ying et al. (2012)
	TabZIP60	*T. aestivum*	*Arabidopsis*	Drought	Zhang et al. (2015b)
	OsbZIP71	*O. sativa*	*O. sativa*	Drought	Liu et al. (2014)
	OsTZF1	*O. sativa*	*O. sativa*	Drought; stress −responsive genes	Jan et al. (2013)
Zinc finger proteins	TaMYB33	*T. aestivum*	*Arabidopsis*	Drought	Qin et al. (2012)
	TaPIMP1	*T. aestivum*	*Nicotiana tabacum*	Drought	Liu et al. (2011)
	Several HSF/HSP transcripts and heat-related marker genes	*B. napus*	*B. napus*	Heat-responsive gene	Yu et al. (2014)

(*Continued*)

Table 1 Major Transcription Factor Gene Families Expressed in Plants Exposed to Heat and Drought Stresses—cont'd

Gene Family	Gene	Donor	Acceptor	Enhanced Tolerance	References
HSF/HSP or other protein	Several heat-shock proteins and some CWM genes	*Brassica rapa*	*B. rapa*	Heat-responsive gene	Yang et al. (2006)
	HSP21	*Arabidopsis*	*Arabidopsis*	Heat-shock protein; interacts with pTAC5	Zhong et al. (2013)
	HsfA1	*Arabidopsis*	*Arabidopsis*	Heat-shock protein; enhances heat stress −induced gene expression	Yoshida et al. (2011)
	DREB2A	*Arabidopsis*	*Arabidopsis*	Enhance heat stress −induced gene expression; interacts with NF−Y subunits	Sato et al. (2014)
	HsfA3	*Arabidopsis*	*Arabidopsis*	Heat-shock protein	Schramm et al. (2008)
	HsfA2	*Arabidopsis*	*Arabidopsis*	Heat-shock protein	Liu et al.(2013)
	HsfB1/HsfB2b	*Arabidopsis*	*Arabidopsis*	Heat-shock protein	Ikeda et al. (2011)
	MBF1	*Arabidopsis*	*Arabidopsis*	Heat-shock protein	Suzuki et al. (2011)
	LIHSFA1	*Lilium longiflorum*	*Arabidopsis*	Heat; interacts with LIHSFA2	Gong et al. (2014)
	TaHSF3	*T. aestivum*	*Arabidopsis*	Heat	Zhang et al. (2013)
	CarHSFB2	*Cicer arietinum*	*Arabidopsis*	Heat	Ma et al. (2016b)

to rice than a shallow root system (Uga et al., 2011, 2013; Arai–Sanoh et al., 2014). Therefore, RSA must be described to understand rapeseed/canola responses to abiotic stresses (Lynch, 2013; Wu and Ma, 2018).

Despite the mounting evidence that roots are an early-detection system of heat and drought stresses, RSA was ignored by rapeseed/canola breeders and agronomists who focused on selection for high-yielding varieties and disease resistance outcomes (Wu and Ma, 2016). This is largely because roots are intrinsically complex tissues that are inherently difficult to characterize in terms of morphology and functionality within the edaphic environment, and the contribution of RSA to yield is poorly documented compared to the established relationships between aboveground plant parts and yield (de Dorlodot et al., 2007). However, RSA modification has the potential to simultaneously improve crop productivity and crop tolerance to abiotic stresses (Garnett et al., 2009; Bellini et al., 2014; Wang et al., 2015) and technical advances now make it possible to quantify root structure in a nondestructive manner and examine root functions in situ (Frasier et al., 2016; Postic and Claude, 2016; van Dusschoten et al., 2016; Rogers et al., 2016; Wu and Ma, 2018). Still, there remain major research gaps regarding the phenotypic characteristics that reflect a good RSA for climate-adapted cultivars of rapeseed/canola, which need to be defined before root-based knowledge can be integrated into future breeding programs.

This review will (1) summarize the genetic basis of drought and heat tolerance mechanisms of rapeseed/canola from the molecular level to the whole plant and agroecosystem scales (Section 2), (2) present modern methodologies for describing RSA and evaluating root functions in situ, thereby revealing root-specific tolerance mechanisms to abiotic stress (Section 3), and (3) discuss how RSA can be used to direct breeding programs and guide the adoption of agronomic technologies to support sustainable rapeseed/canola production now and under future climate change scenarios (Section 4).

2. HEAT AND DROUGHT AS ENVIRONMENT-INDUCED ABIOTIC STRESSORS

2.1 Definition of Abiotic Stress

Abiotic stress is defined as the negative impact of nonliving factors on organisms in a specific environment. In the agronomic sciences, this generally refers to the environment conditions that adversely affect cellular homeostasis

and therefore suppress plant growth and productivity (Mickelbart et al., 2015). Abiotic stress is unavoidable for field crop plants because their growth depends on fitness and adaptation to variable environmental conditions, which may be in the favorable range for the crop at certain growth stages or suboptimal for crop performance (Lavirf, 1972). The fluctuations in weather and atmospheric environments that control temperature, moisture, solar radiation, wind, and other abiotic stressors must be considered in relationship to the developmental growth stage of the plant because phenology determines the plant's susceptibility to the stress.

In general, plants exposed to a specific abiotic stress during early growth stages will suffer from slow growth because the stress restricts cell division and expansion; however, early stressors may cause a negligible reduction in seed yield (Lavirf, 1972). When an abiotic stress occurs during the reproductive stages, it can reduce the crop productivity tremendously (Barnabás et al., 2008). Meanwhile, plants can overcome a variety of abiotic stresses through their plasticity in cellular metabolism and physiological adaptations (Zandalinas et al., 2017). For instance, plants will reestablish cellular and organismal homeostasis or reduce episodic shock effects in response to stress recognition (Mickelbart et al., 2015). As a cool-season crop (Ma et al., 2016a), canola, or oilseed rape encounters numerous abiotic stresses, such as cold and hot temperatures, drought and flooding, soil compaction, nutrient deficiencies, salinity, acidity, and so on. Among these, heat and drought stresses are considered the two major environment-induced abiotic stresses that have the greatest impact on crop production.

2.1.1 Heat Stress

Globally, the mean temperature is projected to increase by 1−4°C by the end of the 21st century (IPCC, 2007; Lobell and Gourdji, 2012). Thus, heat stress is increasingly a concern for crop production, particularly due to the fact that heat stress will create additional variation in regional and seasonal growing conditions, and is expected to cause more pronounced temperature alteration at higher latitudes (IPCC, 2007). In Canada, the rising global temperature is expected to have a significant, positive effect on canola crop productivity. Predicted temperature scenarios would alleviate low-temperature growth inhibition, especially at the seedling and early growth stages. It may extend the growing season or increase the number of cropping cycles per year (Jing et al., 2017). Overall, higher temperatures could benefit crop production in Canada because it could permit the expansion of soybean and corn production in Manitoba and extend canola

production into the northern Prairie and other regions where it is not traditionally grown. Globally, increasing temperature is expected to expand the land area suitable for rapeseed/canola production in the Russian Federation, North America and northern Europe, and East Asia, where sowing dates and seedling establishment are currently limited by too-cold spring temperatures (Lotze–Campen and Schellnhuber, 2009). However, these possibilities depend on other climate conditions besides temperature, and future climate scenarios suggest that uneven distribution of precipitation coupled with more frequent drought and flooding events may negate the potential benefits for crops on regional or national scales.

A number of recent reviews discuss how heat stress affects crop plants in terms of their phenology, physiology, genetics, and molecular biology (Bita and Gerats, 2013; Bokszczanin et al., 2013; Jha et al., 2014; Mickelbart et al., 2015; Guo et al., 2016; Wang et al., 2016a). In summary, heat stress induces changes in photosynthesis and other metabolic pathways that reduce the cumulative solar radiation interception and carbon assimilation during the plant's life cycle. In addition, it may cause significant oxidative damage to cell structures and reduce overall metabolic efficiency (caused by heat-induced imbalance of photosynthesis, respiration, and carbon assimilation processes), due to the excessive production of ROS in stressed plants and lower antioxidant enzyme activity (Stone, 2001; Reddy et al., 2004; Barnabás et al., 2008). More importantly, heat stress affects TFs and heat-shock proteins (HSPs), and the regulation of other stress-related proteins/genes involved in protein biosynthesis, signaling, and metabolism (Schramm et al., 2008; Liu et al., 2013; Guan et al., 2014; Sato et al., 2014). We will discuss recent developments on these plant responses to heat stress later in this section.

Plants rely on a variety of life cycle, biological, and physiological strategies to cope with heat stress as part of their natural acclimation process (Pillai et al., 2012). Crops can escape or resist heat stress through two mechanisms: stress avoidance and stress tolerance (Li et al., 2017). Some of the escape mechanism are leaf rolling, leaf glaucousness (Li et al., 2014), and early flowering (Lou et al., 2007; Mei et al., 2009; Fletcher et al., 2015; Mo et al., 2016). For instance, annual crops could flower and set seed before the onset of heat stress to avoid pollen sterility at critical flowering stage (Young et al., 2004; Morrison et al., 2016). The consequence of early flowering and seed formation is a shorter life cycle, which typically results in lower yield potential of the stressed crop compared with the one that flowered at the normal growth stage in a favorable environment. The resistance mechanism may

involve avoidance or tolerance of the heat stress. Rapeseed/canola plants are able to avoid stress because their indeterminate growth habit and long duration of flowering. This permits the plants to abort some flowers when exposed to heat stress and maintain pollen viability and dehiscence synchronized with stigma receptivity for successful fertilization when heat stress diminished during reproductive growth stages (Bueckert and Clarke, 2013; McVetty and Duncan, 2015). Tolerance to stress is another form of resistance, such as when plants increase transpirational cooling to reduce leaf surface temperature, but this requires greater water consumption (Borrell et al., 2014a). Another tolerance strategy is the physiological response to heat stress, such as the energetically demanding alteration of metabolic pathways or increase in membrane stability (Al−Whaibi, 2011; Yu et al., 2014; Guo et al., 2016). Although the avoidance and tolerance strategies will ensure plant survival when faced with heat stress, it is certain that heat stress events of longer duration or that occur during reproductive growth stages will reduce yield substantially (Stone, 2001; Ortiz et al., 2008).

2.1.2 Drought Stress

Drought is the most important abiotic stress that impairs crop growth and limits yield in rain-fed agricultural system or arid and semiarid regions (Barnabás et al., 2008). Rapeseed/canola is highly sensitive to drought stress, and global climate changes that cause severe and prolonged drought in some parts of the world are expected to reduce rapeseed/canola productivity (Tesfamariam et al., 2010). Plant response to drought stress is complex and involves a myriad of physiological, biochemical, cellular, and molecular changes aimed at ensuring plant survival and reproduction (Sakuma et al., 2006a). At the physiological level, drought response leads to changes in leaf turgor, respiration, transpiration, chlorophyll fluorescence, and osmotic adjustment, which together reduce the stomatal conductance and photosynthesis (Lawlor and Tezara, 2009; Zandalinas et al., 2017). At the biochemical level, accumulation of stress metabolites and protein synthesis could preserve cell homeostasis, such as through regulating ribulose-1,5-bisphosphate carboxylase/oxygenase and ABA, or other metabolites, including glutathione, betaine, proline, raffinose, and galactinol (Barnabás et al., 2008; Chen et al., 2016c). In addition, cellular integrity is protected when ROS is scavenged by defense- and detoxification-related enzymes such as ascorbate peroxidase (APX), glutathione reductase, and peroxidase (Cruz de Carvalho, 2008). On a molecular level, several drought-inducible proteins are responsible for regulating cellular activities that underlie adaptive

responses, including functional proteins such as chaperones, HSPs, and mRNA-binding proteins, regulatory proteins such as TFs, protein kinases, and other signal-related proteins (Al−Whaibi, 2011; Augustine et al., 2015; Huang et al., 2015; Wang et al., 2016b; Phukan et al., 2016).

Drought-survival mechanisms of plants are similar to those used to combat heat stress, described above, and are categorized as drought stress escape, avoidance, and tolerance (Chaves et al., 2003; Reddy et al., 2004; Li et al., 2014; Kooyers et al., 2015). The most effective strategy for an annual crop to cope with water deficiency is to escape from drought, through shortening the growth period, increasing the growth rate or storing more reserves for seed production before the onset of stress (Chaves et al., 2003; Fletcher et al., 2015). The alternative strategy is stress avoidance by maintaining the plant's water status or conserving water during the drought. Plants exhibit a number of adaptations that reduce water loss (e.g., stomatal closure, leaf rolling, and leaf area reduction) and increase water uptake, such as by promoting deep root growth (Reddy et al., 2004; Aroca et al., 2012; Borrell et al., 2014b). When drought stress is mild to moderate, the escape and avoidance strategies may be the most effective to ensure survival and reproduction, depending on the seasonal context and life history strategy of the plant (Kooyers et al., 2015). Once drought stress becomes severe, the crop must rely on tolerance strategies to avoid desiccation, which involve energetically demanding processes such as osmotic adjustment and ROS scavenging (Cruz de Carvalho, 2008). These processes depend on the plant's ability to modulate certain genes, microRNAs, phytohormones, and proteins (Kwon et al., 2016; Shriram et al., 2016; Ohama et al., 2017), which will be discussed in the following section.

2.1.3 Combined Heat and Drought Stresses

Heat and drought stresses often occur in combination or in succession (for example, drought followed by heat stress), resulting in severe damage to plants (Suzuki et al., 2014). Combined heat and drought stress is becoming common in many agricultural regions around the world, with more catastrophic impact on crop productivity than heat stress or drought stress alone (Wang and Huang, 2004). Unfortunately, plant responses to combined heat and drought stress cannot be predicted directly by studying plant performance when heat and drought stresses were applied separately (Rizhsky et al., 2002; Suzuki et al., 2014). For example, plants are unable to reduce leaf temperature through transpiration, to alleviate heat stress, because stomata must remain closed to conserve water when combined heat and

drought stresses occur (Rizhsky et al., 2002; Barnabás et al., 2008). Plant molecular responses to combined heat and drought stresses also differ from single stresses (Suzuki, 2016; Zandalinas et al., 2017). For instance, Rasmussen et al. (2013) suggested that approximate 61% of the transcriptome changes in response to combined heat and drought stresses could not be predicted from the responses to one of these stressors. One of the TFs, DREB2A, appears to control the crosstalk between heat- and drought stress—response pathways, which implies that a cascade of TFs may enable plants to tolerate heat and drought stresses simultaneously (Sakuma et al., 2006b). However, the specific molecular mechanisms responsible for plant tolerance to the combined heat and drought stresses remain unclear and deserve further investigation (Suzuki et al., 2014). In summary, crops with enhanced tolerance to combined heat and drought stresses are needed to maintain yield in the more stressful growing conditions that are predicted in future climate change scenarios (Rizhsky et al., 2004; Barnabás et al., 2008; Zandalinas et al., 2017).

2.2 Critical Growth Stages and Intensity of Abiotic Stress

During the life cycle of a rapeseed/canola crop, the timing, intensity, and duration of heat and drought stresses will determine the impact on crop growth and productivity (Canola Council of Canada, 2016). Heat and drought stresses during the early vegetative stages can suppress leaf area because leaf expansion slows, photosynthetic ability is reduced, and leaf senescence accelerates (Wu et al., 2017). The reduction in leaf area will further impede photosynthesis and consequently there is less carbon assimilated for tissue production at meristems and pod filling. In the end, stressed rapeseed/canola has less branching and fewer pods set (Morrison and Stewart, 2002). However, stress-induced damage such as leaf area suppression in the early vegetative stages can be recovered in rapeseed if the plant has the opportunity to produce new leaves, without any yield penalty (Tesfamariam et al., 2010). The period from flowering to the early and middle phases of pod filling is the most critical and sensitive growth stage for a rapeseed/canola plant exposed to heat and drought stresses (Ahmadi and Bahrani, 2009; Pillai et al., 2012). It is impossible for rapeseed/canola to escape or recover when heat or drought stress occurs during flowering stage because drought and heat (above 28°C) stress interferes with sexual reproduction and subsequent grain filling, often resulting in a sink limitation with pod abortion (Morrison, 1993; Young et al., 2004; Ghobadi et al., 2006). Yield loss from heat or drought stresses that occurred at flowering

or shortly after flowering were affected by the intensity and duration of the stresses and other yield-limiting factors such as disease and pest pressure. Consequently, up to 70% of the yield loss in rapeseed/canola was attributed to heat and drought stresses (Wu and Ma, 2018).

In general, the cool-season rapeseed/canola crop grown in temperate zones is more sensitive to heat stress than warm-season crops grown in tropical regions. It appears that canola is most sensitive to heat stress from late bud development to early seed formation. Temperatures higher than 29.5°C during these crop development stages resulted in significant yield loss in *B. napus, B. rapa, and B. juncea* (Morrison and Stewart, 2002). In a growth chamber study, Angadi et al. (2000) showed that exposing three *Brassica* species to a high-temperature regime of 35/15°C (day/night) for 1 week during the flowering stage reduced seed yield by 89% on the main stem and by 52% on the whole plant, on average. Gan et al. (2004) evaluated seed yield of *B. juncea* and *B. napus* that were exposed to short periods (10 days) of high temperature (35/18°C) and moderate temperature (28/18°C) at different developmental stages. They reported yield losses of 15%, 58%, and 77% when severe stress was imposed at bud formation, flowering, and pod development, respectively.

Similar to heat stress, severe drought during flowering or reproduction stages could shorten the reproductive growth period and result in large yield penalty (Chaves et al., 2003). Ghobadi et al. (2006) observed a 30% yield reduction due to water stress that occurred at flowering, compared with a 21% yield loss for drought at the silique developmental stage. These yield losses were attributed to a reduction in the silique number per plant and seed weight. Similarly, a yield penalty of about 30% was observed for a drought event that lasted from the flowering to pod formation stages (Ahmadi and Bahrani, 2009).

Heat and drought stresses occurring during the critical reproductive growth stages could influence seed oil and protein contents as well. For instance, drought stress at flowering stage was responsible for decreasing oil concentration by 0.39%–2.16%, equivalent to a 20%–36% reduction in oil yield, but gave a significant increase in protein content (Ghobadi et al., 2006; Ahmadi and Bahrani, 2009; Ma and Herath, 2016). A high-temperature stress regime (28/23°C, day/night, with a peak of 38°C for 5 h at midday) of short duration (5 days, starting 25 days after flowering, DAF) can significantly reduce yield of the main branches by more than 52%, with concurrent reductions in seed weight and oil/protein ratio, although fatty acid composition was not

affected (Aksouh–Harradj et al., 2006). In contrast, a moderate heat stress of 28/23°C for a longer duration (10 days) starting at 20 DAF had less impact on yield performance but altered the fatty acid profile significantly, with increased oleic acid and reduced linoleic and linolenic acid concentrations (Aksouh–Harradj et al., 2006). This suggests that high temperatures, even for short periods of time after flowering, can be more damaging to canola yield and oil content than moderate heat stress over a longer period of time during this critical developmental stage.

2.3 Pollen Longevity, Pollination, and Fertilization

As documented in the previous section, high-temperature stress has more impact on reproductive development than on vegetative growth, and the substantial decline in yield caused by heat stress is mainly associated with pollen abortion, and failure of pollination or fertilization (Young et al., 2004; Zinn et al., 2010). For instance, two *B. napus* varieties were found to be almost completely sterile when grown under high-temperature conditions (27:17°C, light/dark) during flowering (Morrison, 1993). It is of great interest to understand how heat stress affects pollen viability, pollen thermos-tolerance, and pollination (Grover et al., 2016; Morrison et al., 2016). In rapeseed/canola, heat stress reduces the number of floret and subsequent seed set per plant (Young et al., 2004; Prasad et al., 2006). Within a floret, anthers and pollen are more susceptible to heat stress than ovules. For instance, pollen fertility was reduced by 40% under high temperature (29/24°C, light/dark) than the control temperature (23/17°C) in a greenhouse study (Wu and Ma, unpublished data). Fahad et al. (2016) also found that heat stress significantly reduced pollen fertility, but plant growth regulators (PGRs) such as ascorbic acid, alpha-tocopherol, brassinosteroids, methyl jasmonates, and triazoles can increase pollen fertility under heat stress conditions.

Pollination ability is influenced by the anther dehiscence, shedding of pollens, germination of pollen grains on the stigma, and elongation of pollen tubes (Prasad et al., 2006; Jagadish et al., 2010; Fahad et al., 2016). Even if the pollen is fertile and able to germinate on the stigma, it may fail to form the fertilized ovule if pollen tubes fail to elongate enough that pollen nuclei reach the ovary. These steps of pollination are sensitive to heat stress (Chen and Fang, 2016). The delivery of pollen nuclei to the ovule is influenced by several factors, such as receptivity of the pistil, pollen deposition, immature ovules, abnormal anatomical development, or any combination of these

(Barnabás et al., 2008). Pollen tube elongation is affected indirectly by carbohydrate metabolism because starch is hydrolyzed into osmotically active sugars on pollen germination as a source of energy for pollination, and carbohydrate metabolism is influenced significantly by the plant's water balance (Stanley and Linskens, 1974; Müller and Rieu, 2016). This is another way that drought stress could impair pollination process and reduces seed set.

Fertilization efficiency, development of the endosperm, and embryo formation are also vulnerable to high-temperature stress (Stanley and Linskens, 1974; Müller and Rieu, 2016). Zahid et al. (2016) reported that extreme heat stress can lead to fertilization failure, due to the disturbed carbohydrate metabolism and reduced calcium levels. Although maternal tissues of the pistil and the female gametophyte are considered to be more thermostolerant than male organs, malformation of the female tissues can occur under extreme heat stress (Zinn et al., 2010). Final seed set or filled seed number is the true indicator of whether pollination and fertilization are successful. Yet, Labra et al. (2017) illustrated the trade-off between seed number and seed weight, where lower seed set was apparently compensated by an increase in seed weight, without compromising oil content, in oilseed rape subject to shading during flowering. Further investigation into the molecular mechanisms underlying the stress response exhibited during pollination and fertilization, particularly in the male reproductive organs (Jagadish et al., 2010), will be critical to select stress-tolerant varieties for future breeding programs.

2.4 Transcription Factor

Plants trigger a cascade of genes expression and TFs that provide molecular-level protection against abiotic stresses via transcriptional and/or translational regulation (Wang et al., 2016a, 2017; Shriram et al., 2016; Ohama et al., 2017). The TFs are of particular interest, since up to 10% of the genome potentially encodes TFs (Franco−Zorrilla et al., 2014), classified into several gene families: AREB/ABF, AP2/ERF, MYB, WRKY, NAC, bZIP (see Table 1 for recently identified TFs). Many TFs are identified to be involved in heat and drought stress responses, along with their underlying mechanisms (Joshi et al., 2016; Ohama et al., 2017). As TFs provide direct regulation of gene expression by serving as molecular switches, several recent reviews discussed the complex transcriptional regulation mechanisms involved in heat and drought stresses and explained how genetic engineering approaches could be integrated into plant breeding program (Hu and Xiong, 2014; Joshi et al., 2016; Guo et al., 2016; Ohama et al., 2017).

The TF profile in response to heat and drought stresses was investigated for *Arabidopsis thaliana* (Rizhsky et al., 2004; Sakuma et al., 2006a; Rasmussen et al., 2013; Gong et al., 2014), rice (Joo et al., 2013; Saad et al., 2013; Ravikumar et al., 2014), and maize (Ying et al., 2012; Lu et al., 2012). In rapeseed/canola, Chalhoub et al. (2014) reported the complete sequence of the polyploid *B. napus* that originated from recombination of two distinct genomes. Subsequently, several studies investigated drought-/heat-resistance genes and TFs in *Brassica* species (Yu et al., 2014; Zhang et al., 2015a; Chen et al., 2016a; He et al., 2016b; Hwang et al., 2016; Niu et al., 2016; Phukan et al., 2016). By combining differentially expressed genes (DEGs) detected by RNA sequencing with associated loci from genome-wide association studies, Zhang et al. (2015a) identified 79 candidate genes associated with drought response in *B. napus*, of which 8 genes are putatively related to drought tolerance based on the gene ontology of *A. thaliana*. Wang et al. (2017) also explored the transcriptomic basis for drought tolerance in *B. napus*. Among the 6440 reported DEGs, 169 crucial drought-responsive DEGs were found to be uniformly distributed among the 19 chromosome pairs. Crucial DEGs were downregulated in drought-resistant varieties but upregulated in drought-sensitive varieties under drought-prone conditions. He et al. (2016b) evaluated the expression of 26 BnaWRKY genes in *B. napus* under multiple stresses and noted that 3 of the BanWRKY genes (BnaWRKY147, BnaWRKY166, and BnaWRKY210) responded strongly to multiple stresses, indicating the multifunctional role of these genes in heat and drought tolerance. They suggested that the BanWRKY genes will be advantageous when breeding new varieties of *B. napus*. Niu et al. (2016) illustrated the similarity of BnaNAC55 in *B. napus* to ANAC055 in *Arabidopsis* and further characterized its involvement in ROS scavenging and defense-related gene expression in oilseed rape protoplasts. The TFs described above were involved in key physiological processes, such as biosynthesis of secondary metabolites, starch and sucrose metabolism, phenylpropanoid and zeatin biosynthesis, plant hormone signal transduction, ATP—binding cassette transporters, and oxidative phosphorylation pathways.

Heat stress transcription factors (HSFs) regulate a subset of heat—responsive genes including HSPs and other heat stress-induced transcripts that are responsible for basal thermotolerance (Al—Whaibi, 2011). All organisms possess HSFs that induce the transcription of these HSPs. The HSPs in plants are grouped into five classes, according to their approximate

molecular weights: (1) Hsp100, (2) Hsp90, (3) Hsp70, (4) Hsp60, and (5) small HSPs (sHsps). First described in *Arabidopsis*, HSPs are well known to regulate protein quality because they can renature many proteins that were damaged by heat stress (Schramm et al., 2008; Liu et al., 2013). For instance, Zhong et al. (2013) showed that a small HSP, HSP21, was involved in repair to the plastid nucleoid protein (pTAC5 in *Arabidopsis*) responsible for chloroplast development under heat stress conditions. Under heat stress conditions, 7-day old seedlings of *B. rapa* showed significant accumulation of HSPs with time and an upregulation of cell wall—modification genes afterward, based on comparative transcriptional profiling analysis (Yang et al., 2006). During the reproductive stage of *B. napus*, Young et al. (2004) identified several HSPs transcripts that were induced primarily in the pollen and pistil tissues, rather than in leaves. This implies that modification of developing gametophytes could be an alternative strategy to improve heat stress tolerance in rapeseed/canola plants.

Recently, HsfA1 was suggested to be a "master regulator" for the activation of transcriptional networks in response to heat stress (Ohama et al., 2017). Knockdown or multiple knockout mutation of HsfA1 genes in *Arabidopsis* and tomato exhibited a suppression of many heat-responsive genes in sensitive phenotypes (Yoshida et al., 2011). Furthermore, HsfA1 may directly regulate the expression level of genes encoding heat-responsive TFs, such as DREB2A (Sato et al., 2014), HsfA3 (Schramm et al., 2008), HsfA2 (Liu et al., 2013), HSFB1/HSFB2b (Ikeda et al., 2011), and MBF1C (Suzuki et al., 2011). Among those heat-responsive TFs, the best known is DREB2A, which is induced by the AREB/ABF TF families (e.g., AREB1, AREB2, ABF1, and ABF3I) and involved in posttranslational regulation (Sato et al., 2014; Ohama et al., 2017). DREB2A regulates a candidate protein that binds to the DRE sequence and therefore facilitates the crosstalk between heat and drought stress signaling (Ohama et al., 2017). Overexpression of DREB2A could enhance drought tolerance and regulate expression of many drought inducible genes in transgenic *Arabidopsis* plants, as demonstrated by microarray and RNA gel blot analyses (Sakuma et al., 2006b). Using a *Brassica* 95k EST microarray profiling technique, Yu et al. (2014) identified genes responsive to heat stress during grain filling stage and reported upregulation of many HSF/HSP transcripts and heat-related marker genes (ROF2, HSA32, DREB2A, MBF1C).

2.5 MicroRNA

A microRNA (abbreviated miRNA) is a small, noncoding, single-stranded RNA molecule involved in the posttranscriptional regulation of gene expression, critically important for plants to sustain and reestablish the cellular homeostasis when exposed to abiotic stresses (Sunkar et al., 2012). The functions of miRNAs have been investigated through miRNA cleavage, translational repression, chromatin remodeling, and DNA methylation techniques (Sunkar et al., 2012).

There is clear evidence for the involvement of miRNAs in heat and drought stress responses in plants (Zhang et al., 2011; Zhou et al., 2013; Bhardwaj et al., 2014). A number of stress-related miRNA are considered good candidates for genetic manipulation to enhance abiotic stress tolerance in plant species such as cotton, corn, wheat, rice, potato, strawberry, and rapeseed (Xie et al., 2007; Shriram et al., 2016). For instance, miR169 plays a significant role in regulating drought stress in *Arabidopsis* plants, whereas miR394 is involved in the regulation of leaf curling responsiveness and its target gene encodes an F-box containing protein that responds to drought stress (Song et al., 2013). Another well-studied miRNA, miR172, appears to regulate the drought-induced transcriptional factor AP2 (Lauter et al., 2005). Similarly, miR319 and miR398 are involved in hormone regulation and signal transduction processes that provide thermotolerance in *Arabidopsis* (Guan et al., 2013) and protection against drought stress in tomato and transgenic creeping bentgrass (Zhang et al., 2011; Zhou et al., 2013). Heat stress responses also involve miRNAs responsible for the regulation of several TFs (Guan et al., 2013; Stief et al., 2014; Ohama et al., 2017). For example, Guan et al. (2013) found that miR398 downregulated several ROS-scavenging enzymes, leading to ROS accumulation and subsequent activation of the HsfA1 gene. In contrast, miR156 upregulated the expression of abiotic stress-inducible genes when plants were exposed to heat stress (Stief et al., 2014).

Most of the evidence of TFs and other molecular-level genes involvement in the abiotic stress response of plants comes from studies on aboveground tissues (leaves, stems, flowers). However, roots are an early-detection system of drought stress in particular because roots are responsible for water acquisition and in direct contact to the water-limited environment (de Dorlodot et al., 2007). We now know that several miRNAs control root development and have a role in plant tolerance to heat and drought stresses. For instance, overexpression of miR160 can downregulate the expression of auxin

response factor (ARF) genes, ARF10 and ARF16, which control cell division and differentiation in the root distal region, leading to root tip defect and root abnormality in *Arabidopsis* plants (Wang et al., 2005). Mallory et al. (2005) also found that miR160 targeted at least three ARF genes and therefore it directly influenced the primary root growth and development. Similarly, overexpressing miR390 during the lateral root initiation stage can negatively regulate the expression of ARF2, ARF3 and ARF4, three well-known repressors for lateral root growth (Marin et al., 2010; Yoon et al., 2010). In another study, miR160/167 was shown to trigger on or off posttranscriptional regulation genes, including ARF6, ARF8, and ARF17, and subsequently control adventitious root initiation and development in *Arabidopsis* (Gutierrez et al., 2009). Meanwhile, proteins produced by these three ARF genes also exerted a feedback on the expression of miR160 and miR167. Recently, MiR1514a was identified in root segments of common bean after drought stress (Sosa–Valencia et al., 2016). These authors also demonstrated the participation of miR1514a in the regulation of two NAC TFs (Phvul.010g121000 and Phvul.010g120700), and the production of small interfering RNAs (phasiRNAs) in response to drought stress.

Most knowledge of how miRNAs respond to abiotic stress comes from research in *Arabidopsis*. Computational methods indicate the presence of 21 miRNAs in *Brassica* species, representing most of the miRNAs known to be involved in plant growth and abiotic stress responses (Xie et al., 2007). With the help of UEA small RNA workbench software, Bhardwaj et al. (2014) identified 51 conserved and 126 novel miRNAs in *B. juncea*. This new information about miRNA sequences, their expression, and putative targets is highly relevant to develop crop improvement strategies in breeding programs for *B. juncea* and related species (Bhardwaj et al., 2014). These advanced bioinformatics tools, coupled with next-generation sequencing (NGS) technology, are sure to bring new opportunities for functional characterization of individual miRNA with potential for improving heat and drought tolerance in *Brassica* species.

2.6 Proteome Analysis

Proteomics is a useful approach to identify stress-responsive and tolerance genes and their pathways, through a comparative analysis of protein abundance between unstressed and stressed plants or between tolerance and susceptible genotypes (Koh et al., 2015; Wang et al., 2016b). Protein abundance is likely to change in plants under drought stress or experiencing

combined heat and drought stresses because the production of ROS and other toxic compounds cause damage to essential proteins and reduce the activity of enzymes (Li et al., 2015). To counteract these deleterious effects, plants will trigger a cascade of protective molecular responses that encode for proteins that help the plant tolerate stress and maintain cellular homeostasis (Zadraznik et al., 2013). A number of stress-inducible proteins, such as mRNA-binding proteins, detoxification enzymes, and plastid proteins, are of particular importance for stress tolerance in multiple plant species, including *Arabidopsis*, rice, and barley (Ashoub et al., 2013; Singh and Jwa, 2013).

As heat stress affects the abundance of HSPs, which is detected in all cells that undergo proteomic profiling (Feder and Hofmann, 1999; Wang et al., 2004; Zhong et al., 2013). HSPs have chaperonic activities and act as intracellular reservoirs for intermediates of denatured proteins undergoing repair to ensure normal folding or by helping to refold proteins that were damaged by stress (Feder and Hofmann, 1999). HSPs also protect cellular metabolism through protein aggregation (Wang et al., 2004). A combined increase in HSPs with Ca^{2+} signaling proteins enhanced heat tolerance in rice, through efficient protein modification and repair (Shi et al., 2013). The importance of HSPs for maintaining protein integrity and functions with cells of rapeseed/canola under heat stress is poorly documented and requires further investigation.

Roots are sensitive to heat stress and have a low optimal growth temperature, relative to shoots (Trinidad et al., 2016). Significant declines in root growth and associated physiological functions are often noted in root tissues of plants with a low root/shoot ratio under temperature stress (Wu et al., 2017). Root-specific expressed proteins respond to heat stress, such as an A20/AN10 type zinc finger protein ZFP177 that was found at the cytoplast of the root segment (Huang et al., 2008). Overexpression of ZFP177 protein in transgenic plants provides further evidence that this protein is involved in plant tolerance to heat stress. Tissue-specific protein expression patterns were also reported in soybean (*Glycine max*) seedlings, with a total of 54, 35, and 61 specifically expressed proteins in leaves, stems, and roots after heat treatment (Ahsan et al., 2010). Root-specific proteins, particularly those associated with secondary metabolism, were mostly downregulated, whereas aboveground-specific proteins, such as chaperonin 60 and ChsHSPs, were mostly upregulated in leaves and stems. Other functions of heat-responsive proteins in roots are root elongation, signal transduction, cell growth, and ROS detoxification (Trinidad et al., 2016).

Using shotgun proteomic analysis, 3009 nonredundant proteins were identified in *B. rapa* (Kwon et al., 2016). Of these, 440 proteins were differentially expressed following drought stress. Kwon et al. (2016) noted that drought stress increased the level of proteins involved in catabolic processes, osmotic stress responses, and antioxidant activities but reduced the level of proteins associated with photosynthesis. Genes that encode for five differentially expressed proteins are recommended targets for future efforts to enhance drought tolerance in *B. rapa* and include phospholipase D delta, annexin, sDNA-binding transcriptional regulator, TRAF-like family protein, and the auxin-responsive GH3 family protein. A study testing the response of rapeseed to salinity stress displayed 75 proteins that were differentially expressed and most were involved in photosynthesis, oxidative stress responses, and energy production (Bandehagh et al., 2011). Wang et al. (2016b) also identified 138 differentially abundant proteins (DAPs) in *B. napus*, and 9 of the DAPs were considered drought-responsive phosphoproteins involved in signal transduction, photosynthesis and glutathione—ascorbate metabolism. The Tyr207 phosphorylated site of beta carbonic anhydrase 1 (βCA1) was the most likely phosphorylation target that could regulate enzyme activity; therefore phosphorylation of βCA1 may be a critical regulator of photosynthesis in canola under drought stress (Wang et al., 2016b). Similarly, Koh et al. (2015) indicate that drought stress increases protein diversity and function due to posttranslational modifications. Further research on the crosstalk between protein expression and posttranslational modifications may lead to a deeper understanding of the proteomic-level influence on rapeseed/canola tolerance to abiotic stresses.

Roots are the most sensitive tissues that first perceive dehydration once the plant is in a water deficit (Comstock, 2002). This is confirmed by the root-specific expression of drought-responsive gene encoding proteins (such as OsNAC10; Jeong et al., 2010), which are common in maize (22 root-specific expressed proteins; Hu et al., 2011) and wheat (34 root-specific differentially expressed proteins; Peng et al., 2009) grown under drought conditions. In rice, a novel protein RSOsPR10 was identified to be root-specific expression via the jasmonic acid signaling pathway (Hashimoto et al., 2004). In a study examining drought stress on *B. rapa* plants, Mohammadi et al. (2012) extracted proteins from roots and suggested that HSP90, a V-type H^+ ATPase and cation-binding protein, played an important role in drought tolerance. Understanding the functions of proteins that respond to abiotic stresses, as well as the tissue-specific protein expression in response to heat and drought stresses, should point to

adaptive mechanisms and tissue-specific defense strategies at the proteome level that could lead to the selection of stress-tolerant phenotypes in breeding programs (Salekdeh et al., 2007).

2.7 Regulation of Phytohormones

Stress-induced processes at the cell, tissue, and whole-plant levels are regulated by PGRs, also known as phytohormones (Maestri et al., 2002). Recent studies showed that phytohormones, such as ABA, salicylic acid (SA), and ethylene, may be important metabolic engineering targets for designing abiotic stress-tolerant crop plants , as they are directly related to stress tolerance (Wani et al., 2016), whereas gibberellins increase susceptibility to stress (Maestri et al., 2002). ABA has two important functions in plants under heat and drought stresses: regulating water status to protect cell systems and inducing genes that encode dehydration tolerant proteins or HSPs (Clarke et al., 2004). As well ABA is linked to ROS signaling and related crosstalk affecting several signal transduction pathways that trigger the expression of stress-responsive genes related to accumulation of osmoprotectants, antioxidants, and ROS scavengers. These metabolic processes may also protect the plants from being damaged by the abiotic stress (Cruz de Carvalho, 2008; Hu and Xiong, 2014). The importance of ABA was confirmed in *Arabidopsis*, where survival of ABA signaling mutant (*abi1/abi2*) was reduced significantly after heat stress treatment (Larkindale et al., 2005).

Several phytohormones were confirmed to be associated with root initiation, elongation, and development (Osmont et al., 2007; Yamada and Sawa, 2013). Auxin controls almost all of the cellular processes that occur during root apical cell differentiation and elongation (Vieten et al., 2007). This is because auxin regulates the interaction of transport inhibitor response I/auxin F box protein and Aux/IAA proteins (Dharmasiri et al., 2005). In addition, auxin is responsible for the degradation of the Aux/IAA proteins by proteasome (Khan et al., 2011). This is thought to release the auxin response transcription factors (ARFs) that regulate the expression of auxin-responsive genes that control root development by determining the length and angle of the lateral roots (Guilfoyle and Hagen, 2007). As a key phytohormone in roots (Zhang and Wang, 2015), auxin also participates in crosstalk with cytokine and gibberellins to modulate other aspects of root development (Yamada and Sawa, 2013). For instance, IAA can be transported to the root tip with the help of ABA modulation to activate the plasma membrane H^+-ATPase (Xu et al., 2013). The coordination between

IAA and ABA has a significant impact on root elongation and root hair development in response to abiotic stress, especially heat stress (Xu et al., 2013; Wang et al., 2016c).

2.8 Reactive Oxygen Species

Although plants have developed strategies to adapt to heat, drought, and other abiotic stresses, overaccumulation of ROS under stressful conditions will inevitably result in oxidative damage to the cell (Suzuki et al., 2012). ROS, such as superoxide radical (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), and oxygen-containing molecules, are commonly produced by cells under heat and drought stresses (Cruz de Carvalho, 2008; Sharma et al., 2012). ROS oxidizes multiple cellular components such as proteins, RNA, DNA, and membrane lipids. Consequently, plants with excess ROS show suppressed crop growth, reduced leaf photosynthesis, increased electrolyte leakage, and accelerated cell death and plant senescence (Mittler, 2002). Therefore, ROS production and scavenging processes need to be understood in the context of stress adaption in crops.

First, we need to understand the dual effects of ROS in plants under abiotic stress (Apel and Hirt, 2004). At relatively low levels, ROS act as important stress-signaling molecules that, in association with the stress acclimation pathway, trigger a series of defense responses (Suzuki et al., 2012; Ohama et al., 2017). Several enzymatic systems contribute to the overproduction of ROS, such as plasma membrane–associated respiratory burst oxidase homologs (Rboh) and NADPH oxidase. Meanwhile, some antioxidant enzymes, such as SOD, catalase, APX, glutathione peroxidases, and glutathione S-transferase can scavenge ROS, thereby controlling ROS levels and maintaining ROS homeostasis (Apel and Hirt, 2004; Sharma et al., 2012). This is important because high levels of ROS cause damage to cell membranes and other cellular components and eventually lead to cell death (Sharma et al., 2012). Recent studies suggest that ROS signaling transduction is associated with protein phosphorylation involving the mitogen-activated protein kinase (MAPK) cascade (Li et al., 2015; Raja et al., 2017). The MAPK pathways are probably the most important signaling components, downstream of second messengers and phytohormones (Raja et al., 2017). Several MAPK components were found to participate in ROS signaling and modulate homeostasis, such as MKK1–MPK6 and other MAPkinases (MAPKKKs)–ANP1 (Pitzschke and Hirt, 2009). In canola (*B. napus*), Sun et al. (2014) reported that 28 BnaMAPPKKK genes (including 2 novel kinases, MAPKKK18/19) were involved in

ROS signaling and cell death, whereas a follow-up study isolated a novel MAPKKK4 gene that elicits ROS accumulation and cause cell death (Li et al., 2015). Stress-adapted plants must tightly regulate the ROS level, allowing enough ROS production to turn on signaling pathways activated by ROS-responsive regulatory genes, while maintaining a threshold ROS level by inducing ROS-scavenging enzymes and antioxidant molecules (Apel and Hirt, 2004; Sharma et al., 2012).

2.9 Quantitative Trait Loci

QTL analysis is a statistical method that links a phenotypic trait with molecular markers. It aims to explain the genetic basis of variation in phenotypes (Miles and Wayne, 2008). QTL analysis is useful to bridge the gap between gene expression and phenotypic traits in plant breeding programs. Grain yields and the tolerance of crop plants to heat and drought stresses will likely be improved by the manipulation of QTLs that control heritable variability of key morphological traits and physiological processes (Collins et al., 2008). Genetic dissection of QTLs controlling the adaptive response of crops to heat and drought stresses is a promising and cost-effective application of genomic-based approaches to achieve a stable grain or oilseed yield under adverse conditions (Collins et al., 2008). To date, QTL applications have proven effective in *Arabidopsis*, as well as in cereals, and other crops (Hu and Xiong, 2014). Hundreds of QTLs were identified and shown to consistently mitigate the negative effects of heat and drought stresses (Hu and Xiong, 2014), and the QTLs that were identified for *Brassica* species subjected to these stresses, are summarized in Table 2.

Flowering is one of the most important agronomic traits, with wide variation, not only among *Brassica* genus but also within canola species (Mei et al., 2009). As a stress escape strategy, crops that can adjust their flowering time have a strategy to cope with heat and drought (Dechaine et al., 2014; Mo et al., 2016). In *Brassica* species, QTLs that control flowering time and therefore influence the crop productivity under heat or drought stress have been identified. For example, VFR1, VFR2, and VFR3 in *B. rapa*, DTF1 and DTF2 in *B. napus*, were reported to control flowering time (Lou et al., 2007; ArifUzZaman et al., 2017). ArifUzZaman et al. (2017) suggest that light-regulated WD1 and flowering BHLH 1 (FBH1) were two putative candidate genes for the expression of QTL DTF 1, located on chromosome C08 in *B. napus*. Other studies also suggested that CO, COL 1, and FLC were candidate flowering QTLs in *B. juncea*, *B. nigra*, *B. rapa*, and *B. oleracea* (Schranz et al., 2002).

Table 2 Major Quantitative Trait Loci (QTL) Identified in *Brassica* Species That Are Involved in Heat and Drought Stress Responses

QTL	Chr./ Genomic Regions	Species	QTL Genetic Position	Genetic Position	R^2 (%)	Candidate Genes	Gene Functions	References
NRV	A01	*Brassica napus*	130 cM	127.7–143.1 cM	16.3	GIP 1; SAUG-like family protein	Control root vigor; root system architecture	ArifUzZaman et al. (2017)
DTF1	C08	*B. napus*	24 cM	21.1–26.8 cM	21.7	LWD1; FBH1	Flowering time; might relate to heat and drought stress escape	ArifUzZaman et al. (2017)
DTF2	C04	*B. napus*	70 cM	69.8–71.5 cM	15	TPR; PAB3; ATC	Flowering time; might relate to heat and drought stress escape	ArifUzZaman et al. (2017)
qTRT1/qTRT2/ qTRT3/ qTRT4a/ qTRT4b/ qTRT6/ qTRT9	LG1–4; LG6/9	*B. rapa*	3.5–19 cM		83.5		Control taproot thickness	Lu et al. (2008)
qTRL2/qTRL3/ qTRL4/ qTRL7/qTRL9	LG2–4; LG7/9	*B. rapa*	3.0–9.0 cM		55.7		Control taproot length	Lu et al. (2008)

(*Continued*)

Table 2 Major Quantitative Trait Loci (QTL) Identified in *Brassica* Species That Are Involved in Heat and Drought Stress Responses—cont'd

QTL	Chr./ Genomic Regions	Species	QTL Genetic Position	Genetic Position	R^2 (%)	Candidate Genes	Gene Functions	References
qTRW1/ qTRW2/ qTRW4/ qTRW5a/ qTRW5b/ qTRW9	LG1/2/4/ 5/9	*B. rapa*	4.0—12.0 cM		69.2		Control taproot weight	Lu et al. (2008)
YIdSens/Yid.dry/ YID.wet/ RFP.dry/ RPF.wet/ DTF.dry/ DTF.wet	A02/A03/ A10/ C02/C07	*B. napus*	refer to Fig. 3 of cited ref.			Flowering time gene CO, FY, FLC	Related to root pulling force (taproot mass), flowering time and yield, as well as drought sensitivity	Fletcher et al. (2015)
FLC1/2/3/5, Fta, Ftbc, FCA, VRN2, CO, MAF, LFY	R01—03/ R06 —08/ R10	*B. rapa*	Refer to Fig. 4 of cited ref.	Refer to Fig. 4		BrFLC2; BrFLC5; ATLFY; BrFLC1	Flowering time; might relate to the abiotic stress escape	Lou et al. (2007)
rrllb.2/rrl8.2/ rsdw9.1/ rrdw5.2 with total number of 19 QTLs	LG1—20	*B. napus*	Refer to Tables 3—4 of cited ref.	Refer to Tables 3 —4 of cited ref.	5.92— 17.44		Control root length, root dry weight, and shoot dry weight	Li et al. (2014)
ft13; ft17	LG13/17	*B. napus*	Refer to Fig. 2 of cited ref.		14.6— 30.4		Flowering time	Mei et al. (2009)

FQTL3−1/ GHQTL3−1/ GHQTL10−1	A 10	*B. rapa*	Refer to Table 2 of cited ref.		10.72/ 5.26/ 26.39	brFLC1	Related to days to bolting, seed germination and yield formation	Dechaine et al. (2014)
L_IPRL1/2/3/4	A1/A5/C4	*B. napus*	101−111/44.6 −48.5/52.3 −54/58 −61.4		8.3/6.2/ 13.4/ 12.9		Control primary root length and might be involved in drought stress under low boron level	Shi et al. (2012)
L_RDW1/2	C4/C7	*B. napus*	34−42.6/56.5 −71.8		7.3/7.6		Control root dry weight and might be involved in drought stress under low boron level	Shi et al. (2012)
22 major QTLs in relation with root and shoot traits	A3/C6	*B. napus*	≥10	Fig. 5 of cited ref.	≥10		Alter root morphology and architecture and enhance phosphorus use efficiency	Zhang et al. (2016)
uq.A1/up.A3a/ up.A3b/ up.A3c/ up.A3d/up.C2/ up.C3a/up.C3b	A1/A3/C2/ C3	*B. napus*	Table 3 of cited ref.		9.36− 17.1	AtGPT1/ AT4	Related to root morphology and tolerance to phosphorus deficiency	Yang et al. (2010)

In several studies, QTLs were identified for RSA traits, which affects yield performance of *Brassica* species under abiotic stress (Lu et al., 2008; Shi et al., 2012; Fletcher et al., 2015; Zhang et al., 2016; ArifUzZaman et al., 2017). For example, the NRV controlling root vigor, located on chromosome A01, was shown to explain 16.3% of the phenotypic variation in root vigor in *B. napus* (Arifuzzaman et al., 2017). In that study, the researchers also illustrated that G-box binding factor interacting protein 1 (GIP1) and SAUR-like family proteins are candidate genes for RSA. Lu et al. (2008) detected seven QTLs for taproot thickness, five QTLs for taproot length, and six QTLs for taproot weight in *B. rapa*. These QTLs accounted for 8.4%−27.4% of the phenotypic variation, a clear indication of substantial genetic variation in taproot traits within *B. rapa* and of beneficial alleles that could improve tolerance to heat and drought stresses and yield performance. Li et al. (2014) found several QTLs were associated with waterlogging tolerance and drought resistance at the seedling stage in a double haploid rapeseed population. Their study also indicated overlap of the QTLs for waterlogging tolerance and QTLs for drought tolerance, which implies a genetic association of these two tolerance traits. Similarly, several QTLs for root traits that responded to soil nutrient concentrations (e.g., boron and phosphorus) were closely linked with the QTLs for drought tolerance (Yang et al., 2010; Li et al., 2014; Zhang et al., 2016).

An important strategy to maintain tolerance to heat and drought stress is to avoid leaf senescence, such as by upregulating the "stay-green trait" and thereby increasing the photoassimilates accumulated over the crop life cycle. The stay-green trait refers to heritably delayed foliar senescence characteristics that reflect a delayed chlorophyll catabolism (Thomas and Ougham, 2014). In some studies, QTLs involved in stay-green functions coincided with those that conferred crop tolerance to heat and drought (Harris et al., 2007; Borrell et al., 2014b; Christopher et al., 2016). Therefore, improvement in stress tolerance can be achieved by selecting stay-green as a preferred ideotypic trait in rapeseed/canola (Thomas and Ougham, 2014). For instance, retention of green leaf area of selected lines with major stay-green Stg QTLs improved the shoot carbohydrate content, grain filling process, and grain weight under heat and drought conditions (Harris et al., 2007). Several major stay-green QTLs, Stg1, Stg 2, Stg 3, and Stg 4, identified in *Sorghum bicolor* were reported to enhance crop performance when exposed to abiotic stress (Harris et al., 2007). Borrell et al. (2014a) showed that Stg QTLs can alter crop canopy development and therefore optimize crop water consumption and grain yield under severe drought

stress. This modification and optimization of stay-green QTLs appear to be associated with beneficial RSA traits (larger root angle and deep roots) that increase water uptake by facilitating water extraction from a larger volume of soil (Manschadi et al., 2006). Therefore, the expression of stay-green might allow the plant to optimize its RSA. In general, stay-green trait is associated with hormone regulation and signaling (particularly involving in cytokinins and ethylene), TFs (WRKY and NAC families), and ROS (Thomas and Ougham, 2014). Although there are few studies to examine the stay-green trait in rapeseed/canola, this trait appears to be a valuable phenological metric that merits further consideration when designing rapeseed/canola varieties that are adapted to future climate change (Collins et al., 2008; Thomas and Ougham, 2014).

Since hundreds of QTLs were linked to crop tolerance of abiotic stresses, it is desirable to introgress those candidate QTLs into elite lines or high-yielding varieties through marker-assisted selection (MAS). Unfortunately, MAS has not yet helped to generate rapeseed/canola varieties with greater tolerance to heat and drought stresses (Collins et al., 2008; Hu and Xiong, 2014). As tolerance of abiotic stress is generally controlled by several epistatic QTLs (Bita and Gerats, 2013), the lack of progress in this area is likely due to the anticipated interactions between multiple stresses or diverse environments. Alternatively, Tester and Langridge (2010) suggested that the combination of reliable phenotyping and improved MAS can be adopted in transferring desirable alleles into elite germplasm. The rapid development in genome-wide selection and molecular breeding technologies is promising, but it is still challenging to explore large data sets from "OMICS" and determine the key functional units for efficiently genetic engineering and breeding of varieties with high tolerance to abiotic stress.

3. ROOT SYSTEM ARCHITECTURE

RSA refers to the spatial and temporal configuration and structure of the plant's root system (de Dorlodot et al., 2007). On a macroscale, RSA describes the organization of the primary and lateral roots (Smith and Smet, 2012). At the microscale, RSA extends to the root microstructure including fine root hairs and root tips, and their interactions with soil and soil microorganisms that are responsible for the uptake of water and nutrients.

Rapeseed has a taproot system with a central, dominant taproot, and lateral roots that support the fine roots (Fig. 4A). A taproot is generally

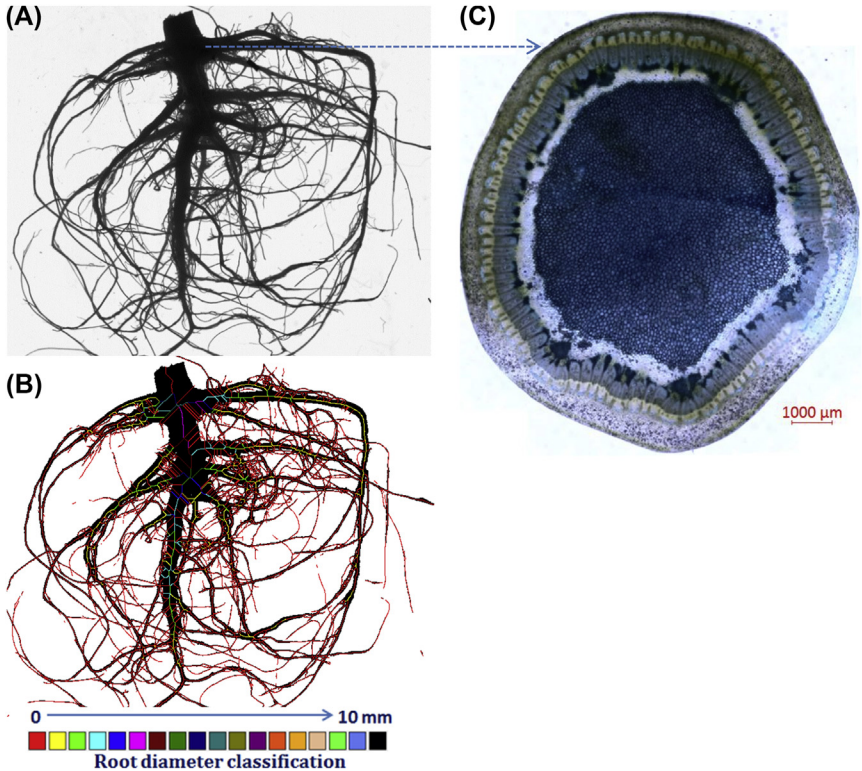

Figure 4 Images showing (A) original canola root system, (B) root structure image from the WinRHIZO method, with root diameter classifications in color superimposed on the root system, (C) light microscopy image of a lateral cross section taken near the root crown of the canola taproot. *Figures are adapted from Wu and Ma (2018).*

straight and very thick at the root crown, tapering to a small root tip, and grows downward (Wu and Ma, 2016). Rapeseed root systems have a typical taproot architecture shaped like an inverted cone (Fig. 4B). Rapeseed has an extensive root system and root hairs with large surface area that take up nutrients and water from the soil. The cross section near the root crown (collar) of canola is shown in Fig. 4C. The vascular bundles are arranged in an open concentric ring, separating central pith from an outer cortex. In each bundle, separating the xylem and phloem that transports water and nutrients internally, is a layer of meristem or active formative tissue known as cambium that gives rise to thickened cells to enhanced mechanical strength and reduce water loss. The rooting depth for winter and spring canola was approximately 2.2 and 1.5 m, respectively, under drought conditions (Johnston et al., 2002).

The taproot system serves a variety of functions including water and nutrient uptake, anchoring and mechanical support, storage of photosynthates and other reserves, and signal transduction (Ryan et al., 2016; Wu and Ma, 2016). The major importance of RSA lies in the fact that most resources are heterogeneously distributed in the soil, so that the spatial and temporal deployment of roots determines the ability of a crop to be aware of soil conditions and exploit edaphic resources (Brussaard et al., 2007). Better understanding of RSA functions, from the genetic to farming system levels, will lead to predictions about how the crop will be impacted by environmental conditions and management practices, of crucial importance for narrowing the gap between the actual average yields and genetic potential (Garnett et al., 2009; Arai–Sanoh et al., 2014; Judd et al., 2015; Ryan et al., 2016).

Several simulation studies based on the genetic and molecular-level functions of rapeseed and cereal crops suggest that RSA manipulation will increase yield potential by improving the resource use efficiencies (de Dorlodot et al., 2007). For instance, Lilley and Kirkegaard (2011) illustrated that a narrow root angle and large number of seminal roots could increase water uptake and improve plant tolerance to drought in dryland cropping regions. In addition, roots serve as the major sensory interface between the plant and soil, able to detect abiotic stresses and respond to environmental cues, which helps the plant to adapt to diverse environments by altering RSA for maximal growth (De Smet et al., 2012; Jiang and Hartung, 2008). More importantly, root system has an important role in plant–soil–microbe interactions and mediates crosstalk with beneficial soil microorganisms in the rhizosphere, an emerging hot topic for plant biology (Ryan et al., 2016).

3.1 Methods for Measuring Root System Architecture

Precise evaluation of RSA is critical to understand plant growth and response to environmental stresses (Fang et al., 2012) and is viewed as essential to developing advanced *Brassica* varieties that possess great potential yields (Fetcher et al., 2015) and exhibit high tolerance to abiotic stresses (Lilley and Kirkegaard, 2011). For instance, Svačina et al. (2014) identified specific indicators of robust RSA detected by an electrical measurement technique that can be implemented in barley breeding programs. This approach may overcome the difficulties in characterizing, quantifying, and interpreting belowground growth and RSA, in terms of its morphology and functionality, without destroying the native architecture. This section examines traditional and modern methodologies for root quantification and summarizes the pros and cons of each method in Table 3.

Table 3 Summary of Advantages and Disadvantages Associated With Methods to Quantify or Describe Plant Root Systems

Class	Advantages	Disadvantages	References
Traditional Methods			
Root–soil cores sampling	No specific instrument required; simple process; inexpensive if done by hand;	Sampling is difficult in dry clay soils; time–consuming and labor intensive; finest roots may be missed; destructive sampling procedure; tedious work; extra cost and possible impact on plots if cores are collected by mechanized techniques	Schuurman and Goedewaagen (1971), Böhm (1979)
Ingrowth core method	No specific instrument required; simple process; inexpensive; easy to collect the mesh bag; root decomposition can be studied under field condition	Root–free soil is not identical to the undisturbed soil; disturbance of soil condition when introducing the mesh bags; alternation of soil aggregate structure, soil aeration and moisture	Hansson et al. (1991)
Pinboard method	Obtain the entire root system; visualize branching architecture;	More skill required; limited number of samples can be prepared; fine root loss;	Schuurman and Goedewaagen (1971), Floris and Van Noordwijk (1984)
2D Quantification Methods			
WinRHIZO	Automatic image acquisition and analysis; provides information on topology, architecture, color analysis and root diameter classification; inexpensive; widely used	Original root architecture is altered; root structure not fully described by 2D analysis; root overlapping;	Wu et al. (2017)

EZ-RHIZO	Nodestructive; track root system development over time; semiautomatic software; original root architecture unchanged; open source; nondestructive	Plant should be grown on a solid support agar medium; only in 2D analysis	Armengaud et al. (2009)
RootTrace	High-throughput; nodestructive; track root system development over time; automatic method; open source; nondestructive	Plant should be grown on a solid support agar medium; only in 2D analysis	French et al. (2009), Iyer—Pascuzzi et. al. (2010);
Other: Delta—T—Scan; WR—RIPL; RMS; DART	More complex root system can be recorded such as root branching, angles, root order or even lateral root emergence	Only in 2D analysis; require artificial growing conditions	Ingram and Leers 2001, Le Bot et al. (2010); www.delta—t.co.uk; (http://rootimage.msu.edu
3D Quantification Methods			
Minirhizotrons	3D structure measurement; more root characteristics, such as root longevity, or even mycorrhization and parasitism; root can be monitored in time during whole growth stage	Difficult of tube installation, and the associated distribution and compaction of soil condition	Bates (1937), Vamerali et al. (2012), Johnson et al. (2001)
X—ray computed tomography	More applicable; noninvasive 3D imaging in the field; higher spatial resolution	Same attenuation coefficient between root tissue and other nonspecific soil organic matter and high soil moisture make it difficult to distinguish fine roots; expensive	Tracy et al., 2010, Gregory et al. (2003); Mooney et al. (2012), Perret et al. (2007)

(*Continued*)

Table 3 Summary of Advantages and Disadvantages Associated With Methods to Quantify or Describe Plant Root Systems—cont'd

Class	Advantages	Disadvantages	References
Magnetic resonance imaging	High-resolution cross-section images; nondestructive 3D imaging in the soil; high fractions of the root systems	Sensitive to soil moisture; expensive	Callaghan (1993), Borisjuk et al. (2012); van Dusschoten et al. (2016)
Ground—penetrating radar	Noninvasive 3D imaging in the field; allow relatively rapid and repeated measurement; applicable for entire root system over time;	Its accuracy is only sufficient for large tree root; results also be influenced by the changes in the soil context, soil moisture and other environmental factors;	Butnor et al. (2001), Metzner et al. (2015)
3D digitizer; 3D laser scanning; digital camera	3D structure measurement and model; more 3D root architecture parameters could be achieved	Only use in laboratory condition; requirement of excavation of the entire root system; time intensive;	Lang (1973), Petzold et al. (1999), Fang et al. (2009), Zhu et al. (2006)
Others			
Electrical measurement	Convenient, fast, nondestructive method; high efficiency; no requirement of excavation of the root system	Not in 2D or 3D analysis; accuracy will be influenced by soil moisture, current frequency, position of the positive plant electrode and negative soil electrode, and soil/root temperature	Dalton (1995), Chloupek (1972), Ellis et al. (2012), Wu et al. (2017)
Carbon isotopic method	Accurate estimation of root turnover and C budget; without alteration or interruption of soil and root growth	Requirement of isotope labeling C incorporated into structural tissue; only root biomass value instead of root architecture	Liang et al. (2002), Subedi et al. (2006), Milchunas (2012), Studer et al. (2014)

3.1.1 Traditional Methods

The traditional method for evaluating the root system involves the separation of roots from the soil in which they grew and tend to disrupt the soil environment while destructively sampling the root system (Taylor et al., 1991). Such so-called shovelomics approach describes as the low-cost root excavations using handheld tools (Thomas et al., 2016) and is still considered the standard method to evaluate RSA details. Three variations of this method, the root–soil core sampling, ingrowth core, and pinboard methods (Oliveira et al., 2000), are described below.

Root–soil cores. Root–soil core method is a convenient sampling technique that requires no sophisticated equipment of special skills to obtain volumetric-based soil samples. With this method, root–soil cores are taken from the field with hand-operated or mechanical apparatus, and then washed carefully to separate fine roots from soil (Schuurman and Goedewaagen, 1971). A hand-driven auger is often recommended to collect the root–soil core. The apparatus contains a cylindrical tube of approximately 15–30 cm long with an inside diameter of 5–10 cm, and a rotating T-handle at the top of the auger shaft to aid the auger penetration into and removal from the soil (Oliveira et al., 2000; Frasier et al., 2016). When hundreds of root–soil core samples are to be collected, the sampling depth exceeds 30 cm of the soil is difficult to penetrate, a mechanized instrument can be employed (Böhm, 1979). The root–soil core samplers can be mounted on a handheld motorized drop–hammer or on a tractor with a drawbar that can provide hydraulic power to move the corer upward and downward (Oliveira et al., 2000). The mechanized technique can reduce sample collection time and labor but is more invasive and possibly damaging to the plot area. For plants with a large root system, a combination of surface excavation and the root–soil core method was used successfully to describe the temporal root mass distribution of maize growing in plots under conservation and conventional tillage (Dwyer et al., 1996).

Ingrowth core method. This method uses measurements of fine root growth into a root-free medium to estimate root production. A mesh bag filled with root-free soil is buried in the root zone for a fixed length of time, and root growth is quantified after washing to separate roots from the root-free soil (Hansson et al., 1991). The detailed procedure to prepare and install mesh bags for the root ingrowth cores is described in Oliveira et al. (2000). Retrieval is facilitated by attaching one end of a rope to the bottom of the mesh bag, and the other end of the rope is labeled

and placed on the soil surface. The mesh bag can be pulled out at the desired sampling time, which saves labor and time. The main drawback is that the root-free soil is not identical to the undisturbed soil and likely affects the root growth because the process of sieving and repacking the root-free soil will modify soil conditions, such as soil aggregate structure, aeration, and moisture. The effect of soil disturbance on root growth must be considered when interpreting the data (Mackie—Dawson and Atkinson, 1991).

Pinboard method. Pinboard method refers to the procedure of using double pins to hold the root system in a suitable position and represent a 2D root monolith. The procedure, construction of the monolith, and other requirements of this method are found in Schuurman and Goedewaagen (1971). Pinboard samples contain the main visible structures in the root system, which can be washed on a coarse mesh screen. This saves time for sample cleaning, as there is less organic debris attached. In addition, the pinboard method exhibits the branching architecture that enables the user to trace individual roots to their origin and conduct analysis of root functions (Floris and Van Noordwijk, 1984). The entire root system can be photographed, providing a holistic image for visual assessment, comparison, or conservation. However, this method requires more skill and labor than the root—soil core or ingrowth core methods, due to large soil volume that must be excavated to obtain each pinboard sample (Oliveira et al., 2000). In addition, more of the fine roots are likely lost with the pinboard method than other traditional methods.

As these traditional methods require collection of most or all of the root system, soil surrounding the root must be removed carefully before the entire or partial root system is pulled from the ground and measured. All those methods are destructive, and the topology or geometry of the root architecture is often destroyed by excavation or during washing when roots are separated from the soil. Loss of the fine roots is inevitable during the sampling and cleaning processes (Mackie—Dawson and Atkinson, 1991) and affects the quality of the data. Furthermore, the traditional methods are time-consuming and labor intensive, although the introduction of mechanical coring devices has eased the labor requirement. Nevertheless, these methods provide a direct approach to evaluate root system and serve to calibrating other nondestructive techniques (Fang et al., 2012). Some modern methods for examining root systems with automated or nondestructive techniques are provided below.

3.1.2 2D Quantification Systems

WinRHIZO. Root imaging acquisition with computer software (WinRHIZO) was developed by Regent Instrument Inc. (Quebec, Canada) to obtain root measurements under laboratory conditions (Metzner et al., 2015). It allows quantitative measurements of root morphological traits, including root length, surface area, volume, and root number that may be linked to root functions (Judd et al., 2015). The software also facilitates the investigation of root topology (e.g., the pattern of root branching; Berntson, 1997), root health by color analysis to identify specific root types, diseased roots, and mycorrhizae. During the measurement, the root is spread out on an image scanner surface in a manner that minimizes root overlap. The major limitation of WinRHIZO is that the laboratory manipulation inevitably changes of original RSA, which may cause inaccurate estimation of the architectural traits, such as root angle, root depth, root number, etc.

EZ-Rhizo. To address the limitations of WinRHIZO, Armengaud et al. (2009) adapted the method to analyze root images on a solid support medium such as agar plates. Multiple root traits relevant to the root architecture are measured with a Windows-integrated, semiautomated software with data storage and data analysis capacity. The EZ-Rhizo system is convenient and suitable to monitor root development and provide phenotypic descriptions of individual plants during their growth. The system allows examination of root growth and differentiation as influenced by plant genotypes and environmental factors. With EZ-Rhizo, natural variations in RSA were identified in 23 *A. thaliana* accessions (Armengaud et al., 2009) and the identified RSA traits will serve as a baseline for future QTL analysis.

RootTrace. Similarly, RootTrace is a software tool developed at the Centre for Plant Integrative Biology, University of Nottingham. It has the advantage of automation for high-throughput quantification of root length, tip, and root curvature, as well as determination of root responses such as onset of gravitropism. Once the starting point is set by the user, the software could trace primary branches to the tips, in every image captured during the time series, and analyze the results (French et al., 2009). The original software was developed to quantify traits in simple dicot root system and was later updated to accommodate a greater variety of root systems (Iyer–Pascuzzi et al., 2010). A digital camera takes root images at every 18° in a 360° container, and plants are grown in transparent gel system. Analysis of 16 acquired phenotypic root traits collected from 2297 images of 118 individual plants demonstrated the feasibility of this imaging and analysis

platform for phenotyping and quantifying root system automatically (Le Bot et al., 2010; Iyer–Pascuzzi et al., 2010).

3.1.3 3D Quantification Systems

As a root system has 3D structure, techniques that represent RSA as a 2D measurement are overly simplistic. Advanced instruments and techniques are now available for high-throughput 3D root image analysis to complement other phenotyping analyses. The 3D structure of a root system describes two characteristics: topological arrangement and geometric formation of the entire root system (Danjon and Reubens, 2008). Topological arrangement describes the physical connections between each root branch and the biological sequences (embedded in axes) and may be considered the basis for internal fluxes of energy, mass, and information (Böhm, 1979). Geometric formation represents the orientation, shape, size, and spatial structure of root branches responsible for plant–soil interactions, anchorage, and nutrient acquisition (Fang et al., 2012).

Minirhizotrons. The first minirhizotron developed by Bates (1937) consisted of a clear glass or plastic tube that was inserted into soil near the root as a noninvasive, nondestructive viewing platform. The root system was observed using a mirror with an electric bulb mounted at one side. Currently, minirhizotrons are transparent tubes inserted into the soil permanently and specialized cameras are installed at selected positions to take images of roots growing outside the tube (Vamerali et al., 2012). This technique is used in field studies to identifying root development process, root behavior, root longevity, or even mycorrhizal colonization and parasitism (Fang et al., 2012; Vamerali et al., 2012). High-resolution cameras coupled with a high-precise digital sensor allow images to be stored in digital form. The image data, transferred to computer memory directly without any loss of quality, make the method accurate and convenient (Faget et al., 2010). Several software programs were developed to analyze minirhizotron root images, such as Root Tracker, Rootfly, and RootView (Fang et al., 2012). One of the advantages of minirhizotron method is that specific root segments can be monitored in time during the growing period, without any impact on the root system (Johnson et al., 2001). It means that root images can be taken for continuous root observation without disturbing the plant or the surrounding soil. However, tube installation is a critical step because soil compaction and gaps between the tube and the adjacent soil must be avoided. Johnson et al. (2001) suggested that waiting about 6 months between tube installation and image collection would ensure

that soil conditions returned to predisturbance levels, but this consideration would significantly limit the use of minirhizotrons in agronomic studies on annual crops.

X-ray computed tomography. X-ray computed tomography (CT) refers to a computerized X-ray imaging procedure in which a narrow beam of X-ray is aimed at specific areas of a scanned object (such as plant roots) to produce several cross-sectional (tomographic) images at different angles (Tracy et al., 2010). CT imaging was originally developed for medical uses, but it has become a promising tool for noninvasive 3D imaging of plant roots and demonstrated in various species such as *Arabidopsis*, rice, maize, wheat, and chickpea (Gregory et al., 2003; Lontoc−Roy et al., 2006; Perret et al., 2007; Mooney et al., 2012; Rogers et al., 2016). Digital geometry processing is used to generate a nondestructive visualization of the 3D root system from large number of 2D radiographic images. X-ray CT is an effective tool to visualize root systems growing in soil (Zhu et al., 2011). For instance, Rogers et al. (2016) provided a framework for using X-ray CT technique to study RSA in different growth environments, for the purpose of selecting genotypes with desirable root traits. Limitations of the CT technique arise from the reliance on X-ray beams passing through the root tissue since part of the X−ray beam is absorbed by the root tissue, thereby reducing its intensity, via a process called attenuation (Metzner et al., 2015). The similar attenuation coefficient between root tissue and soil organic matter is a major limitation of using the X-ray CT technique in a soil medium (Fang et al., 2012). This makes difficult to distinguish smaller root structures from soil, particularly when soil has a high organic matter (Rogers et al., 2016), or under high soil moisture (Lontoc−Roy et al., 2006). In addition, X-ray CT can be expensive but it generates high-quality images of the root system (Zhu et al., 2011). In the future, multiple energy CT techniques that resolve the problems associated with attenuation differentiation need to be developed and adopted to improve discrimination of components in the plant−soil system and reduce costs as well.

Magnetic resonance imaging. The magnetic resonance imaging (MRI) technique delivers structural information in a nondestructive manner. With a powerful nuclear magnetic resonance and pulses of radio wave energy, the MRI system creates detailed, high-resolution cross-section images (Callaghan, 1993). MRI was originally developed for medical purposes and does not use radiation, distinguishing it from X-ray CT scanning (Metzner et al., 2015), and provides an alternative approach to visualize plant root systems (Gruwel, 2014; van Dusschoten et al., 2016).

For instance, a pipeline for root analysis relying on MRI for advanced image visualization and the analysis software toolbox named "NMRooting" was developed to examine the root growth of barley and maize crops (van Dusschoten et al., 2016). The major shortcoming of the MRI technique is its sensitivity to soil moisture because root tissue is surrounded by water filled pores and impure soil medium that can exacerbate image distortion (Fang et al., 2012; Gruwel, 2014). Combined image analysis using positron emission tomography (PET) with MRI may solve this problem (Jahnke et al., 2009), although extraction and analysis of MRI−PET data is still a challenge (Cho et al., 2008). Metzner et al. (2015) compared MRI with the X-ray CT technique and found that both techniques performed equally well for imaging a 3D root system. MRI is more suitable for larger root systems, as it can analyze larger root volumes than the CT technique, which is better suited to provide finer spatial resolution of a specific root segment.

Ground-penetrating radar. Ground-penetrating radar (GPR) is a geophysical technique that uses radar pulses to image the subsurface of root structure (Metzner et al., 2015). In GPR, short pulses of electromagnetic energy penetrate into the ground, and the energy is either absorbed by the surveyed material (e.g., roots) or reflected back to the antenna when materials of dissimilar dielectric constant are encountered (Butnor et al., 2001). The reflected energy is then collected on a portable computer for further analysis to create a 3D root architecture image (Stokes et al., 2002). GPR is a noninvasive technique that allows relatively rapid, repeated measurements of the entire root system over time, especially for large tree species. However, its accuracy is only sufficient to resolve coarse roots with diameters of at least 1 cm or larger. The accuracy of GPR results may also be influenced by the soil conditions and other environmental factors, as suggested by Miller et al. (2004) and confirmed by Butnor et al. (2001), who showed that GPR was ineffective in clay soil or soils having high moisture content. Further work is needed to improve the accuracy and extend the use of GPR for crop root quantification.

3D digitizer and laser scanning techniques. Modern techniques such as 3D digitizer and 3D laser scanning were developed to study root architecture (Danjon and Reubens, 2008; Fang et al., 2012). Lang (1973) introduced the first 3D digitizer for plants. This apparatus consisted of four rotationally articulated arms that pivot, and the angles between the arms are recorded by a precision potentiometer. The apparatus must be in contact with some points of the plant to record its 3D spatial coordinates.

Using a 3D digitizer (3Space Fastrak, Polhemus, USA), Wu et al. (2015) quantified the spatial distribution of the root system and suggested that the 3D digitizer was suitable to take accurate measurements of maize root 3D architecture under field conditions. The 3D laser scanner was originally introduced to generate digital terrain models in the geosciences field (Petzold et al., 1999). This technique was then adapted for root studies with a revised model. With a 3D laser scanner, Fang et al. (2009) quantified the root architecture in situ of soybean and rice plants within a transparent gel-based growth system. Both methods have a similar disadvantage that they do not work in opaque growth media and require excavation of the entire root system to describe its 3D structure.

3.1.4 Other Root Quantification Methods

Electrical measurement method. Electrical capacitance (EC) and electrical impedance (EI), at a single frequency, or at multifrequencies, were used to characterize plant root systems (Dalton, 1995; Postic and Claude, 2016; Wu et al., 2017). This method uses EI spectroscopy (Repo et al., 2012) or a simple handheld capacitance meter to estimate root parameters nondestructively (Chloupek, 1972; Dalton, 1995; Ellis et al., 2012). Dalton (1995) described the electrochemical interpretation of the EC measurement as follows: fine roots behave as cylindrical capacitors and their capacitances can be added together and wired in parallel mode. Therefore, the capacitance (in farads) is a measure of the amount of electrical charge stored in the root membranes, and quantity is proportional to the active root surface area. EI is another feasible method to estimate the absorptive root area (Čermák et al., 2006; Repo et al., 2012), based on the theory of polarization and relaxation phenomena of biological membranes involved in geometric and dielectric properties, which was illustrated in Aubrecht et al. (2006) and Repo et al. (2012).

EC and EI were significantly correlated with root length, surface area and volume, as well as root fresh/dry weights when plants were grown under hydroponic, sand-based medium, and field conditions (Dalton, 1995; Svačina et al., 2014; Wu et al., 2017). Furthermore, EC and EI can be used as potential indicators of stress tolerance in a crop breeding program or in appraising agronomic management technologies for adaptation to a changing climate (Wu and Ma, 2016; Wu et al., 2017). In general, EC and EI give a better reflection of root properties in hydroponic or sand-based growth medium than under field conditions because these measurements are significantly affected by soil moisture (Wu et al., 2017). Fig. 5 illustrates

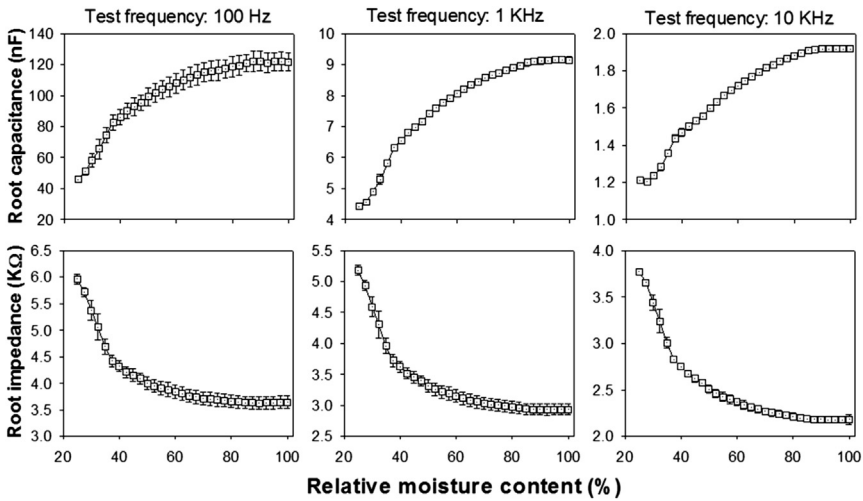

Figure 5 Effect of relative moisture content (%) on root capacitance and impedance measured at several electrical frequencies (100 Hz, 1 KHz, and 10 KHz; unpublished data).

the gradual increase in EC with increasing relative moisture content (RMC, %) that plateaus when RMC is at ≥80%, regardless of the test frequency, which may be caused by incomplete contact between root tissue and the soil medium as discussed by Wu et al. (2017). EC and EI are affected by other independent factors, such as current frequency, position of the positive electrode on the plant and relative to the position of the negative soil electrode, and soil/root temperature (Aubrecht et al., 2006; Postic and Claude, 2016; Wu et al., 2017). For instance, correlations between EC and root morphological traits were stronger under 1 KHz or 10 KHz, than under 100 Hz, leading Wu et al. (2017) to recommend 1 KHz and 10 KHz as the current frequencies for EC measurements in root studies.

Carbon isotopic method. Soil and crop roots are differentially enriched in carbon (C) and N staple isotopes (Subedi and Ma, 2010). Carbon isotopic method involves isotope pulse labeling and then traces the stable isotope ^{13}C (or the radioactive ^{14}C) in the organic matter (plant roots or soil and root mixture), considering the isotopic dilution from the ^{12}C isotope fixed through photosynthesis (Subedi and Ma, 2010; Studer et al., 2014). The C stable isotope is suitable to study soil C dynamics, while the use of radioactive ^{14}C is limited due to radiation safety and disposal issues (Milchunas, 2012; Studer et al., 2014). Liang et al. (2002) illustrated the feasibility of the ^{13}C isotopic method to quantify soil root residues and

calculate the C budget, whereas ^{13}C isotopes were used to evaluate the effect of elevated CO_2 on aboveground and root biomass (van Vuuren et al., 2000). Root-derived carbon in soil as a proxy for root biomass from a wheat crop was estimated using the ^{13}C isotopic method (Subedi et al., 2006). The C isotopic method could provide more accurate quantification of root biomass because it accounts for a substantial amount of root-derived C (i.e., 26% of root-derived C according to Subedi et al., 2006) that was lost or underestimated from root excavation and washing procedures. Overall, ^{13}C isotopic method offers an alternative to study root system without disturbing soil and root growth, although root architecture cannot be explored.

3.2 Impact of Stress on Roots and Its Anchorage

Although root development and spatial distribution is influenced by abiotic stress (Van Noordwijk et al., 2015), root biomass is usually less affected by drought stress than aboveground components. Consequently, the root/shoot ratio increase under drought stress for rapeseed (Wu et al., 2017) and most cereal crops (Dwyer et al., 1996). Meanwhile, other root morphological and anatomical characteristics (root length, angle, fresh/dry weight, surface area, diameter, deep rooting, cortex thickness, xylem vessels, root hairs, and suberin deposition) and dynamic behavior (root turnover, lignification and suberization, hardening, hydraulic conductivity) are often influenced significantly by drought stress (Jeong et al., 2010; Bengough et al., 2011, 2016). In general, rapeseed is likely to develop a deeper root system in soils under drought condition, with less root growth in the surface layer of soil profile (Johnston et al., 2002). Both taproot and lateral roots extend into deeper soil layers for extracting water (Bengough et al., 2011), resulting in a significantly larger root length/weight ratio under drought conditions, especially at the deep soil profile.

Penetration resistance and limited water are the major limitations to root elongation under drought stress (Bengough et al., 2011). This is mainly due to the increase in resistance to water flow through the soil to the plant root under drought conditions (Osonubi, 1984). Penetration resistance occurs because of shrinkage of soil away from the roots and contraction of the stressed roots in drier soil. Both factors create vapor gaps between the root surface and the surrounding soil (Bengough et al., 2011, 2016) that significantly affect water flow resistance at the root—soil interface. To counteract this effect, Bengough et al. (2011) suggested that root tips and root hairs can be beneficial to root penetration and improve root growth significantly.

Soil moisture content also influences root anchorage because it alters soil shear strength, which declines with increasing soil moisture content (Crook and Ennos, 1993). This mathematical expression describing soil shear strength (proposed by Crook and Ennos, 1993 and confirmed by van Delden et al., 2010) establishes the link between root anchorage strength (S_r, N), soil shear strength (τ, N m^{-2}), and root cone diameter (D, cm), as follows: $S_r = k \times \tau D^3$, where k is a dimensionless constant. In general, agricultural soils display plastic behavior in shear strength, meaning that the soil is at high initial stiffness but fails at very low strains (Ennos, 2000). Under these conditions, the anchorage strength of plant roots in wetter soil is significantly weaker than in drier soil (Ennos, 1990, 2000). The effect of soil moisture on soil shear strength is expected to be greater in fine-textured clay soils than in coarse-textured sand soils due to their different cohesion strength (Ennos, 2000).

High temperature tends to alter the partitioning of photoassimilate to roots, suppresses root growth/elongation, and changes RSA (Wang et al., 2015; Wu et al., 2017), which may reduce the elliptical root–soil cone volume (Tripathi et al., 2016) and likely reduces root anchorage strength. These phenomena can be explained by cellular-level responses because high temperature accelerates cell growth and cell proliferation, which may produce larger cells with thinner cell walls and smaller vascular bundles (Fig. 6). Consequently, stem bending strength or root rigidity may be weakened. Wu et al. (2017) observed a significant reduction in lateral roots, but no effect on taproots of canola under high temperature or combined heat and drought stresses. In canola, lateral roots represented the greatest proportion of the total root system, whereas the taproot only accounted for $\leq$1% of root length, about 2% of surface area and less than 15% of root volume under greenhouse environment (Wu et al., 2017). This leads us to propose that lateral roots contribute significantly to root anchorage, considering their overall contribution to the root system and structural arrangement (Wu and Ma, 2018). Some lateral roots grow at the stem base, pointing radially outward and tapering downward (Wu and Ma, 2016, Fig. 4A). In addition, lateral roots originating from the taproot can also branch to produce tertiary roots with larger angles and extending horizontally to create an inverted crown shape, similar to in the root system of rice and wheat (Crook and Ennos, 1993; Ennos, 2000). Our further investigations (Wu and Ma, 2018) clearly showed that canola plants are more prone to anchorage failure than stem buckling under high-temperature condition (Fig. 7), which was mainly due to suppression of lateral root growth

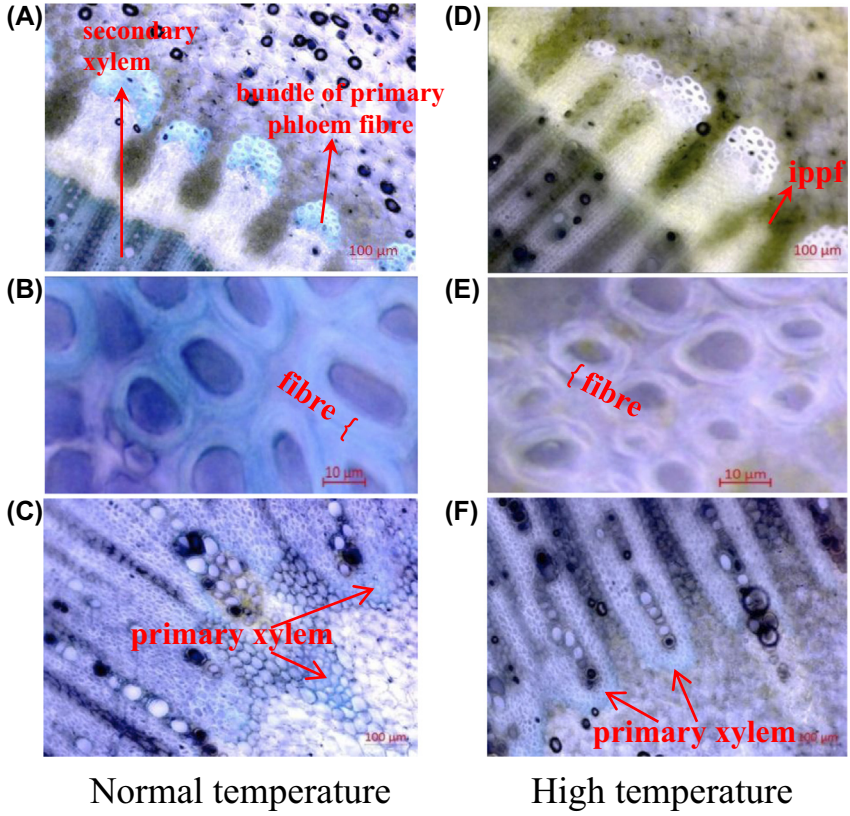

Figure 6 Light microscopy images showing rapeseed/canola responses in the root crown of the canola taproot to normal temperature (A–C; 23.0/17.0°C) and high-temperature treatment (D–F; 27.01/24.33°C). Incomplete primary phloem fibers (ippf) were observed in high-temperature condition (D) and absent at normal temperature (A); thinner fiber walls are seen under high-temperature condition (E) in comparison with normal temperature (B); the conspicuously thickened primary xylem found in normal temperature (C) was reduced at high temperature (F). *Figures are adapted from Wu and Ma (2018).*

(32%), and thereby reduction in root bending resistance (33%), root–soil cone dimension (13%), and its shear strength (33%). Therefore, root lodging risk will become a bigger problem as temperatures continue to increase due to global warming. We recommend that root lodging resistance should be targeted as the priority trait in canola breeding for a highly productive crop with a rigid root system that is well adapted to climate change.

Root hairs are also extremely sensitive to heat and drought stresses. Root hairs were long recognized for their contribution to increase root surface

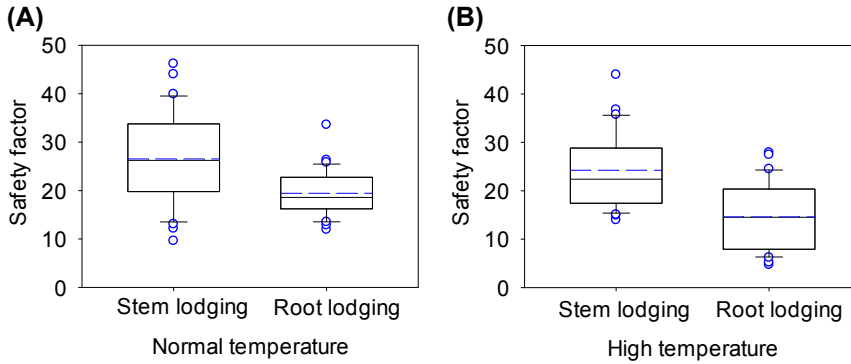

Figure 7 Relative susceptibility to lodging of stems and roots of rapeseed/canola under normal temperature (A; 23.0/17.0°C) and high-temperature (B; 27.01/24.33°C) conditions. Safety factor refers to lodging resistance against stem buckling or anchorage failure. *Figures are adapted from Wu and Ma (2018).*

area and root—soil contact area that facilitates nutrient and water uptake (Bengough et al., 2011) and are now known to have a physical role in anchorage. Bengough et al. (2016) demonstrated that root hairs helped anchor the root tip in loose seed beds above more compact soil layers and could also assist root tips to penetrate the compacted bulk soil or drying soils. This observation is consistent with Stolzy and Barley (1968), who found that the force required to pull a pea root out of the soil increased to 0.2 N mm^{-1} length of hairy roots, compared with 0.04 N mm^{-1} of root tip before root hair emergence. In contrast, Ennos (2000) suggested that root hairs were more important for anchorage during seedling establishment but had a minor role in the anchorage of mature plants. This was based on the argument that root hairs generally appear near the root tip of elongation roots where the cells are dividing and are far away from majority of the root volume during later growth.

In summary, root functions include more than simply water and nutrient uptake, based on work that examined root anchorage and lodging in response to abiotic stresses. RSA is strongly correlated to root physical strength and therefore is of critical importance in determining susceptibility of root lodging (van Delden et al., 2010; Wu and Ma, 2018). Crops grown in the field can be expected to encounter weather conditions such as wind gust and rain storms, and these external forces can increase the bending moment at the base of the stem, which causes root lodging (Ennos, 1990, 2000; Wu and Ma, 2016). More importantly, root lodging needs to be considered when selecting varieties with RSA that is altered for better

adaptation to heat and drought stresses (Stolzy and Barley, 1968; Wu and Ma, 2018). We recommend more study into the mechanisms that explain RSA in relationship to crop lodging.

4. IMPLICATIONS AND FUTURE CHALLENGE

4.1 Modifying Root System Architecture for Stress Adaptation

Increasing temperature and water scarcity are predicted to be the major limitations of crop production in the future, so agronomists are developing adaptive strategies to combat heat and drought stresses. Past efforts to improve crops for better stress tolerance generally focused on aboveground plant properties, with relatively few studies examining the roots (Mittler, 2002; Thomas and Ougham, 2014; Zahid et al., 2016). RSA is the key determinant of water acquisition and nutrient uptake under heat or drought stress and must be optimized for stress tolerance. The most common negative impact of heat and drought stresses is tissue dehydration (Lu et al., 2012), due to the imbalance between the smaller reserve of soil water that can be supplied to roots and increasing demand for water from the shoots (Aroca et al., 2012). Development of RSA that achieves a well distributed and deeply penetrating root system should compensate for stressful conditions in the surface soil layers and improve root-mediated regulation of the aboveground plant function (de Dorlodot et al., 2007). However, accurate quantification of the root system under field conditions still remains challenging, due to the inherent nature of RSA as the "hidden half" of the plant and the plasticity of root systems. Technological innovations for root biomass quantification and visualization (summarized in "Methods for measuring RSA" section) should be helpful in identifying key indicators of root functions that can be helpful in this regard.

A deep understanding of root biology is of critical importance in breeding programs to develop stress-tolerant rapeseed/canola genotypes. One possibility is to develop cultivars with the deeper rooting 1 (DRO1) gene (Uga et al., 2013). The DRO1 was identified in rice from a breeding population derived from the cross of shallow-rooting IR64 and the deep-rooting landrace Kinandang Patong. This gene provides drought tolerance and therefore improves yield stability in rice. DRO1 is a QTL that regulates RSA by altering root growth angle and stimulating cell elongation in the root tip so that more, deeper roots are produced (Uga et al., 2011).

In shallow-rooting rice, the variety possessing the introduced DRO1 gene exhibited deeper rooting and therefore maintained a high yield performance under drought-prone conditions, relative to the wild type. The deep-rooting variety also had improved nutrient uptake and greater yield under nondrought conditions in the field (Lynch, 2013; Arai–Sanoh et al., 2014). Beneficial genes and loci for root traits that could enhance heat and drought tolerance are also found in rapeseed or other field crops such as wheat, maize, sorghum, and chickpea (Placido et al., 2013; Burton et al., 2014).

4.2 Agronomic Management for Stress Adaptation

Selection of stress-tolerant varieties with high yield potential remains one of the most important agronomic strategies, although cultivation technologies and management practices could help crops cope with heat and drought stresses. Rapeseed growers may avoid drought stress by sowing earlier during the season (Ma et al., 2016a). Rapeseed/canola is a cool-season crop with a long flowering duration and early planting increases the probability that the crop will encounter favorable weather conditions for a longer period of reproductive growth (Ma et al., 2016a). Earlier planting dates could also help rapeseed/canola avoid periodic heat waves that usually occur in early summer when the crop reaches the flowering stage (Wu and Ma, 2016). Optimum planting dates are a successful strategy for maize and spring wheat production in the US Midwest (Reilly et al., 2003) and North Central Plains of China (Mo et al., 2016).

Improved water management, such as subsurface drip irrigation, is another option to cope with drought stress and enhance water use efficiency and yield (Wu and Ma, 2016). This agronomic practice also alleviates heat stress in rapeseed, as the crop transpires water to regulate leaf temperature. Therefore, subsurface drip irrigation and rain water harvesting with straw or plastic mulching have become popular practices in rain-fed agricultural systems throughout the semiarid regions in the world (Wang et al., 2011; Li et al., 2016). In addition, adjusting the timing of irrigation could ensure a crop's water supply at critical, stress-sensitive growth stages (Lotze–Campen and Schellnhuber, 2009). This strategy is not feasible for regions where irrigation water is scarce or insufficient rain water is collected (Wang et al., 2011). Other water management strategies, such as regulated deficit irrigation, partial-root drying, alternate drying and wetting techniques, are reported to improve water-use efficiency in various crop species under drought conditions (Kang and Zhang, 2004).

Several innovative fertilization techniques, such as slow release or controlled release fertilizers with deep placement, ensure a gradual nutrient supply during the growing season (Roger et al., 1980). Controlling the nutrient supply avoids overluxuriant growth during the vegetative stage and reduces water consumption by leaf transpiration, while conserving nutrients for uptake later in the growing season, such as to achieve full canopy size during reproductive stage and meet yield goals. Deep placement of fertilizer encourages deeper root growth and preserves nutrients because deeply placed fertilizer is less susceptible to loss through erosion and volatilization in soil surface layers (Johnson et al., 2016). Improved nutrient management is likely to improve the shoot and root growth, providing greater crop tolerance to heat and drought stresses.

Chemical priming is another option to increase crop tolerance to abiotic stresses (Subedi and Ma, 2005; Martinez–Medina et al., 2016). Analogous to vaccination, chemical priming involves exposing seeds or plant tissues to small doses of stress-inducing chemical compounds so that the plant begins preparing defense mechanisms. Thus, when abiotic stress occurs, the plant has already acquired defense mechanisms that can be activated faster and to resist the stressor. A recent review by Savvides et al. (2016) addressed the challenges and opportunities of using various chemical priming agents (e.g., sodium nitroprusside, hydrogen peroxide, melatonin, polyamines, etc.) for crop stress management.

4.3 Breeding Selection for Stress Adaptation

There have been numerous plant breeding achievements in rapeseed/canola in the last 50 years. For instance, Canadian breeders successfully developed a series of herbicide-tolerant hybrids with better seed quality and better tolerance to abiotic stress (Bueckert and Clarke, 2013; McVetty and Duncan, 2015). As heat stress and drought becomes more common worldwide, there is an opportunity to use molecular technologies and tools, such as MAS, genetic transformation, or other genetic engineering approaches, to augment conventional plant breeding efforts aimed at improving yield and ensuring food security (Fischer and Edmeades, 2010; Tester and Langridge, 2010; Hu and Xiong, 2014). MAS was used successfully in the *Brassica* oilseed breeding program and will continue to be helpful in breeding new varieties with improved tolerance to abiotic stress (He et al., 2014). Introduction of NGS will accelerate the genome sequencing and targeted resequencing capabilities for all plant genomes, leading to the identification of new molecular markers (Varshney et al., 2009). These markers may

include the important candidate genes, TFs, microRNA, and QTLs reviewed in section 2 of this paper, as they are already associated with crop responses to heat and drought stresses.

Genetic improvement of rapeseed is essential for abiotic stress adaptation (Hu and Xiong, 2014; Jha et al., 2014). Given that RSA was not included as a desirable trait in traditional breeding programs, there is huge potential to integrate root-based data into the selection process since RSA is proven to improve tolerance to heat and drought stresses and support greater resource acquisition and yield. However, the process of accurately identifying relevant QTLs or major genes remains a bottleneck. This is because (1) RSA traits usually display low heritability values due to root plasticity in heterogeneous soil environments; (2) there are no highly precise and high-throughput phenotyping techniques or platforms for root systems; and (3) root tolerance mechanisms are often environment-specific due to the unpredictable nature of the timing and intensity of abiotic stresses (Collins et al., 2008; Miles and Wayne, 2008; Bueckert and Clarke, 2013; Hu and Xiong, 2014). Lack of high-throughput phenotyping may be overcome by designing a robust phenotyping platform or new techniques that provide accurate estimates of the heritability and repeatability of RSA. Combining extensive phenotyping information with the modern genomic resources will lead to an exploration of alleles that govern RSA and relate to enhanced productivity in *Brassica* species (Chalhoub et al., 2014). It will also provide breeders with a unique opportunity to enhance genetic gain based on root traits (Fig. 3).

Although this task may appear daunting, many of the molecular mechanisms for stress acclimation were evaluated in *Arabidopsis*, a relative of rapeseed/canola. Hence, much is already known about the key genes/loci, and the associated physiological processes and molecular mechanisms that will be important for rapeseed/canola crop performance. The applications are not limited to Brassicas because there are common genetic, molecular, biochemical, cellular, and physiological processes occurring among all crops (Ying et al., 2012; Qin et al., 2013; Huang et al., 2015). However, the gene targets from model species must be exposed or expeditiously introgressed into popular high-yielding varieties in *Brassica* oilseed crop for new variety development to be successful. Further efforts and improvements are required to integrate genetic engineering and MAS applications in breeding programs for the rapeseed/canola crop. To make genomic selection a more cost-effective approach, a two-part genomic selection strategy, a population improvement component, and a product development

component, in a breeding program, has been illustrated to generate twice the genetic gain of breeding programs with equal budgets and time frames (Gaynor et al., 2017).

5. CONCLUDING REMARKS

Roots, as the "hidden half" of plants (Eshel and Beeckman, 2013) are now recognized as an "early warning system" that detects and responds to abiotic stresses. Roots have significant roles in plant anchorage and resource acquisition, but we are now recognizing their value as a signaling network that relies on genetic, molecular, cellular, and physiological processes to avoid or tolerate heat and drought stresses. Modern methods for quantification of RSA will be important to understand how molecular cues and biochemical reactions affect the phenotype of stressed plants, although it remains challenging to perform root observations in situ. Ongoing research on Brassica root systems is absolutely essential for selecting varieties that are well adapted to the heat and drought stresses, which are likely to intensify due to ongoing global climate change. Rapeseed/canola is an economically important crop that is susceptible to these abiotic stressors, and the information presented here will be of considerable value in breeding programs and for practical agronomic improvements to sustain oilseed production for future food security.

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