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Molecular and systems approaches towards drought-tolerant canola crops

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Summary

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Modern agriculture is facing multiple challenges including the necessity for a substantial increase in production to meet the needs of a burgeoning human population. Water shortage is a deleterious consequence of both population growth and climate change and is one of the most severe factors limiting global crop productivity. *Brassica* species, particularly canola varieties, are cultivated worldwide for edible oil, animal feed, and biodiesel, and suffer dramatic yield loss upon drought stress. The recent release of the *Brassica napus* genome supplies essential genetic information to facilitate identification of drought-related genes and provides new information for agricultural improvement in this species. Here we summarize current knowledge regarding drought responses of canola, including physiological and -omics effects of drought. We further discuss knowledge gained through translational biology based on discoveries in the closely related reference species *Arabidopsis thaliana* and through genetic strategies such as genome-wide association studies and analysis of natural variation. Knowledge of drought tolerance/resistance responses in canola together with research outcomes arising from new technologies and methodologies will inform novel strategies for improvement of drought tolerance and yield in this and other important crop species.

I. Introduction

Fresh water scarcity is an emerging global problem, and given that the majority of fresh water extracted by humans is used for agriculture (Rosegrant *et al.*, 2009), improving crop production under limited water availability is an important challenge. Although crop production can be enhanced by water conservation through improvements in tillage and irrigation practices, modification of the genetic basis of stress tolerance in crops is an urgently needed complementary strategy for improving productivity under conditions of moisture deficit (Turner, 2001; Pennisi, 2008). It is estimated that crops attain less than half of their potential yield as a result of unfavorable environmental conditions, with water deficit being the most severe stress (Boyer, 1982; Gleick, 1998; Araus *et al.*, 2002). Given climate change scenarios, drought tolerance will be an increasingly necessary agronomic characteristic.

There are over 3000 species within the *Brassicaceae* (mustard family) and they are mainly cultivated in the northern hemisphere. The *Brassicaceae* includes many familiar vegetable crops (e.g. broccoli, cauliflower, Chinese cabbage, and various mustards). Also included in the *Brassicaceae* are the reference plant, *Arabidopsis thaliana*, and the oilseed crops, particularly *Brassica napus* (Al-Shehbaz, 1984). *Brassica* species provide c. 12% of the edible oil worldwide, particularly from the canola varieties (Paterson *et al.*, 2001; Hall *et al.*, 2002). Standing for Canada (Can) oil (ola), the word 'canola' refers to types of rapeseed varieties originally developed in Canada for edible oil, animal feed, and biodiesel, with low glucosinolate and erucic acid content (<http://www.canolacouncil.org/>). Canola quality oil is derived from three species: *B. napus*, *Brassica rapa*, and *Brassica juncea*. Among the canola species, *B. napus*, an amphidiploid species (AC genome, $n=19$), is derived from a recent (presumably < 10 000 yr ago) hybridization of *B. rapa* (A genome, $n=10$) and *Brassica oleracea* (C genome, $n=9$) (Palmer *et al.*, 1983; Wan *et al.*, 2009; Schmidt & Bancroft, 2011; Wang *et al.*, 2011a).

Brassica napus possesses favorable agronomic properties; for example, cultivation under different seasons (annuals and biennials) and rotation with cereals is possible. *B. napus* produces high-quality oil (Ahmadi, 2010) and is currently the third largest source of global vegetable oil supplies, after soybean and palm (<http://faostat3.fao.org>). During the past decade, annual production of *B. napus* increased from 37 million tons in 2003 to 73 million tons in 2014 (<http://faostat3.fao.org>). *B. napus* not only provides vegetable oil with superior nutritional value, its primary commercial use, but also meal for animal feed and a source of biodiesel with excellent flow properties in cold weather as a result of its low saturation.

This review summarizes current knowledge regarding drought responses of canola, with the major focus on *B. napus*. This topic is of interest from both basic and applied science viewpoints, because for most crops drought is the major abiotic stress causing severe reduction in productivity (Jensen *et al.*, 1996b; Angadi *et al.*, 2004; Willenborg *et al.*, 2004; Sinaki *et al.*, 2007). In this article we first review the physiological effects of drought on canola and then describe current knowledge in three areas relevant to modern

strategies to improve drought tolerance: results from translational strategies based on discoveries made in the close relative, *A. thaliana*; large-scale datasets arising from direct -omics analyses in canola itself; and information on canola from contemporary genetic approaches such as genome-wide association studies (GWAS) and analysis of natural variation (Fig. 1). Given the tools and information available, particularly in conjunction with the recent publication of a *B. napus* genome sequence (Chalhoub *et al.*, 2014), we contend that canola is poised to become a crop model system in its own right.

II. Physiological complexity of responses to drought stress in canola crops

Investigations of physiological responses to drought in *B. napus* (Fig. 2) have been conducted under both field and growth chamber conditions (Jensen *et al.*, 1996a,b; Qaderi *et al.*, 2006; Shafiq *et al.*, 2014). Well-known processes influenced by drought stress include photosynthesis, stomatal conductance, transpiration, protein synthesis, and metabolite accumulation, all of which directly or indirectly affect seed yield and quality (Jensen *et al.*, 1996a; Hashem *et al.*, 1998; Sangtarash *et al.*, 2009).

Brassica napus is sensitive to water deficit during all stages of growth, from germination to seed set. Owing to the fact that abscisic acid (ABA) biosynthesis is induced by drought stress, ABA application is often used as a proxy for a drought signal. In *B. napus* seeds, exogenous application of ABA prevented entrance of the embryo into the growth phase (Schopfer & Plachy, 1984). ABA-mediated embryo dormancy was reported to result at least in part from a reversible inhibition of changes in cell wall biophysical

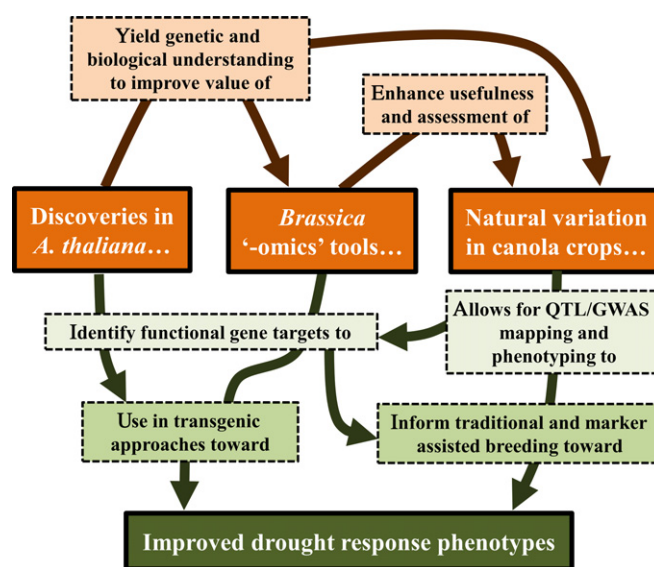


Fig. 1 Discoveries in *Arabidopsis thaliana*, *Brassica* -omics tools, and natural variation in *Brassica* species provide complementary and synergistic research approaches. When combined, these tools can identify the genetic basis for stress response traits that may yield advances in efforts to improve canola drought tolerance by transgenic and breeding strategies. QTL, quantitative trait loci; GWAS, genome-wide association studies.

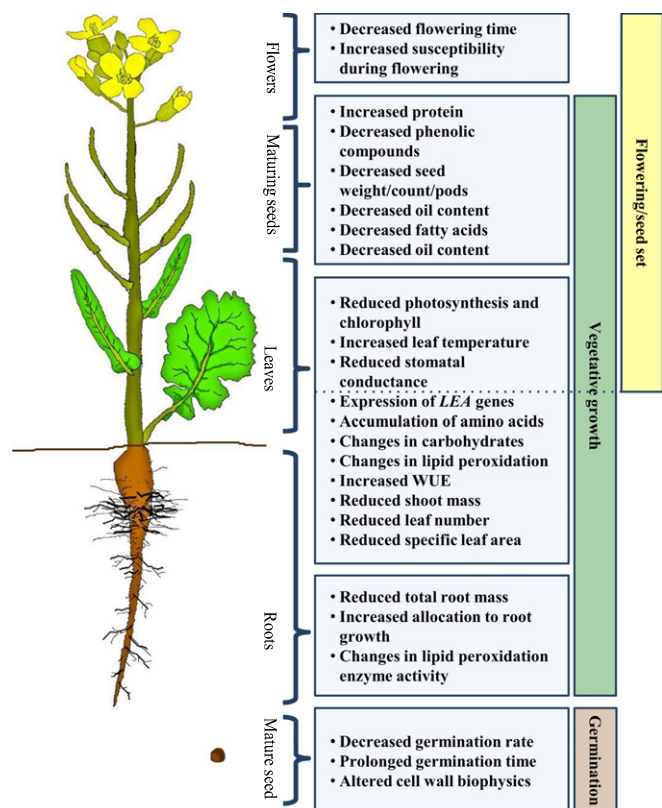


Fig. 2 Physiological and morphological trait responses to water deficit stress in canola crops discussed in this review, organized by organ type. LEA, late embryogenesis abundant; WUE, water-use efficiency.

properties, for example, cell wall extensibility coefficient and minimum turgor required for cell expansion (Schopfer & Plachy, 1985). Prolonged germination time and dramatically decreased germination rate in *B. napus* were also observed upon treatment with polyethylene glycol (PEG) (Willenborg *et al.*, 2004), which simulates the osmotic stress component of drought. Drought stress after seed germination also influences seedling growth: seedling height, fresh weight, and survival rate were negatively affected by PEG-simulated drought stress applied to 14 *B. napus* varieties after seed germination (Yang *et al.*, 2007). Therefore, drought stress during seed germination and initial growth not only impacts seed germination time and rate, but also has adverse effects on vegetative growth, and can ultimately result in yield loss in *B. napus* (Willenborg *et al.*, 2004; Li *et al.*, 2005; Yang *et al.*, 2007).

At the vegetative stage, numerous biochemical changes have been observed when *B. napus* is exposed to drought, including effects on both macromolecules and small molecules (metabolites). As in many other species, increased expression levels of late embryogenesis abundant (LEA) proteins have been observed in *B. napus* leaves under ABA, salt, cold, and osmotic stresses (Dalal *et al.*, 2009). Rapid accumulation of amino acids has been observed in *B. napus* during drought stress until rewatering (Good & Zaplachinski, 1994). Proline, which is involved in osmotic regulation (Ma *et al.*, 2003) and possibly in nitrogen-use efficiency (Albert *et al.*, 2012) under drought stress, accounts for the majority of amino acid accumulation (Good & Zaplachinski, 1994; Ma

et al., 2003; Din *et al.*, 2011). Previous studies revealed that proline content was increased significantly by drought stress in the *B. napus* varieties Okapi, RGS, Rainbow, and Dunkeld, suggesting production of compatible solutes as a mechanism of drought stress tolerance in this species, as is also commonplace in other species (Omidi, 2010; Ullah *et al.*, 2012). Besides proline, carbohydrate dynamics are also regulated by drought stress. For example, drought stress elevated concentrations of trehalose, glucose, fructose, and sucrose and decreased raffinose in *B. napus* var. Titan (Müller *et al.*, 2012).

Lipid peroxidation and antioxidant enzyme activities are also affected by drought stress. PEG simulation of drought treatments increased the content of malondialdehyde (MDA), a product of lipid peroxidation, and enzyme activities of superoxide dismutase, peroxidases, and catalase, in roots and shoots of several *B. napus* cultivars (Abedi & Pakniyat, 2010; Chai *et al.*, 2011; Wang *et al.*, 2011b; Mirzaee *et al.*, 2013). Liu *et al.* (2011) found that aminolevulinic acid (ALA) enhances the drought stress tolerance of *B. napus* seedlings, quantified as shoot biomass and chlorophyll (Chl) content, through enhancing the activities of specific antioxidant enzymes and inducing the expression of specific antioxidant enzyme genes.

Drought stress also causes complex whole-plant physiological and morphological responses. When water deficit occurs, the phytohormone ABA is synthesized and transported to leaf tissue, consequently activating guard cell responses that promote stomatal closure and inhibit stomatal opening to preserve plant hydration. Accumulation of ABA in leaves has been confirmed in drought-stressed *B. napus* seedlings (Qaderi *et al.*, 2006). Stomatal closure induced by exogenous application of ABA has been reported in isolated epidermal peels of *B. napus* (Zhu *et al.*, 2010). Lower stomatal conductance was observed in droughted *B. napus* plants than in well-watered plants, leading to leaf temperatures 1–2°C higher under drought (Hashem *et al.*, 1998). Drought stress decreases net CO₂ assimilation, photosynthetic rate, Chl content, and transpiration in most terrestrial plants, including *B. napus* (Hashem *et al.*, 1998; Din *et al.*, 2011; Qaderi *et al.*, 2012; Shafiq *et al.*, 2014). These responses are associated with the reduced stomatal conductance upon drought stress, which facilitates water conservation (Shaw *et al.*, 2005).

Water deficit results in decreased root and shoot biomass (Hashem *et al.*, 1998; Qaderi *et al.*, 2012; Ashraf *et al.*, 2013; Shafiq *et al.*, 2014). Although the plants are smaller overall, water deficit can increase the relative portion of the biomass allocated to roots, a strategy that is considered to be adaptive. In *B. napus*, a greater reduction in shoot mass is seen with drought at the vegetative stage than at the flowering stage (Ashraf *et al.*, 2013) and shortened shoot height can be accompanied by increased root length in drought-stressed plants (Qaderi *et al.*, 2012; Ashraf *et al.*, 2013). Drought stress also reduces leaf number and area, leaf area ratio (leaf area : plant dry weight (DW) (cm² g⁻¹)), and transpiration, and increases water-use efficiency (WUE), and specific leaf weight (leaf DW : leaf area (g m⁻²)) and leaf weight ratio (leaf DW : plant DW) in *B. napus* seedlings (Hashem *et al.*, 1998; Qaderi *et al.*, 2012). These growth parameters can be employed to assess the severity of drought stress.

Flowering is a critical stage influencing the yield of *B. napus*. Effects arising from drought stress imposed during vegetative growth, such as reduced net photosynthesis and stomatal conductance resulting in increased leaf temperature, were also observed in *B. napus* undergoing drought stress at flowering. Drought stress treatments imposed at flowering reduced seed weight, total seed yield, seed number per pod, and pod number per plant, and resulted in higher yield loss than drought stress applied at the vegetative stage (Champolivier & Merrien, 1996; Hashem *et al.*, 1998; Din *et al.*, 2011).

Yield in oilseed crops is positively correlated with total water availability (Nuttall *et al.*, 1992). It has been reported that after the first 6–8 inches (152–203 mm) of water, canola grain yield can increase by 150–280 kg ha⁻¹ per each additional inch of water (Nielsen, 1997; Si & Walton, 2004). In Europe, for example, yields of winter canola are double those of spring varieties, and this is attributed in part to the fact that winter canola experiences minimal water deficit stress (Wan *et al.*, 2009). Drought stress imposed at the reproductive stage has a more severe impact on yield than drought stress imposed during vegetative growth, as a result of reduced pod number, seed number, and seed weight (Sinaki *et al.*, 2007; Ahmadi & Bahrani, 2009). In one experiment, plants undergoing drought stress during reproduction had *c.* 20–40% reduction in seed yield compared with nonstressed plants (Ahmadi & Bahrani, 2009).

A key agronomic issue for oilseed crops such as canola is not only the effect of drought on yield but the effect of drought on seed quality. Several studies have investigated changes in the biochemical composition of canola seeds produced under drought conditions. Drought stress at any developmental stage decreases seed oil content (Bouchereau *et al.*, 1996; Champolivier & Merrien, 1996) and alters seed oil composition (Enjalbert *et al.*, 2013). In particular, a decrease in fatty acids such as linolenic acid was observed in *B. juncea* under limited water availability (rainfed) conditions (Enjalbert *et al.*, 2013). An increase in total glucosinolate concentration was observed in *B. napus* seeds from plants undergoing drought stress during vegetative and flowering stages; however, application of water stress after flowering caused little to no change in the total glucosinolate concentration of seeds (Bouchereau *et al.*, 1996; Champolivier & Merrien, 1996; Jensen *et al.*, 1996b). Water shortages during either vegetative or flowering stages resulted in significant increases in seed protein concentration (Bouchereau *et al.*, 1996; Champolivier & Merrien, 1996; Jensen *et al.*, 1996b) and inhibited accumulation of phenolic compounds in seeds (Bouchereau *et al.*, 1996). Therefore, water shortage at any stage has potential effects on seed quality and yield in *B. napus*.

III. Translational biology: iterating between *A. thaliana* and *B. napus*

1. *Brassica* genomics and ABA signaling

While a high-density genetic linkage map of *B. napus* was generated in 2011 (Wang *et al.*, 2011a), the first complete *B. napus* genome, that of the *B. napus* European winter cv 'Darmor-bzh', was not reported until 2014. RNA-Seq and expressed sequence tag (EST)

data in combination with *ab initio* gene prediction from the genome sequence led to the identification of *c.* 101 000 gene models, with over 90% confirmed by matching to the *B. rapa* and/or *B. oleracea* predicted proteomes (Chalhoub *et al.*, 2014). Almost half (48%) of the genes were estimated to undergo alternative splicing, mainly from intron retention. Of the assembled genome, 34.8% is composed of transposons, with their positions largely corresponding to those in the progenitor *B. rapa* and *B. oleracea* genomes.

This early Darmor-bzh genome both provides an invaluable resource to *B. napus* researchers and illustrates some of the problems inherent in the assembly of allopolyploid genomes. The polyploid complexity and repeat elements made it difficult to assemble the complete genome. Misassembly can result in specific problems for the downstream design and interpretation of experiments seeking to use the assembled genome to answer specific biological questions. For example, incorrect ordering of genes will introduce errors when inferring the genes involved in a process from an experiment in which quantitative trait loci (QTLs) are identified using linkage disequilibrium between genetic markers. In the future, longer sequencing read lengths will enable reads spanning more repeat regions (Clarke *et al.*, 2009; Eid *et al.*, 2009), leading to more complete and higher quality genomes.

In contrast to the nascent stage of the *B. napus* genome assembly and annotation, the reference plant *A. thaliana* provides a fully sequenced and extensively annotated genome. *A. thaliana* has been used extensively for basic discovery research in plant sciences, especially for gene function characterization. *A. thaliana* is a genetically, evolutionarily, and physiologically close relative of *B. napus* (Noh & Amasino, 1999; Byzova *et al.*, 2004; Rana *et al.*, 2004; Parkin *et al.*, 2005). The ancestral lineages diverged *c.* 16–19 million yr ago. The two species can be crossed and the nucleotide sequence conservation is in the range of 80–90% in exons and 70% in introns (Dixelius & Forsberg, 1999; the Arabidopsis Genome Initiative, 2000; Love *et al.*, 2005). Therefore, knowledge gained from the model plant species *A. thaliana* provides valuable guidance to better understand the drought responses of its close relative *B. napus* (Zhang *et al.*, 2004) and to apply translational biology approaches for development of transgenic *B. napus* with improved drought tolerance. Results from such experiments demonstrate that canola product development based on information transfer between *A. thaliana* and *B. napus* has agronomic relevance.

Absciscic acid biosynthesis can be triggered by drought stress and accumulated ABA is transported from roots to shoots and then stomata through xylem sap. Research using the model plant *A. thaliana* has provided critical insights into the core ABA signaling pathway. 'PYR/PYL/RCAR' family ABA receptors have been identified (Ma *et al.*, 2009; Park *et al.*, 2009). These receptors interact with type 2C protein phosphatases (PP2Cs), and consequently inhibit PP2Cs' function of blocking activity of downstream sucrose nonfermenting (SNF)-related kinase 2 (SnRK2) proteins, particularly OST1 (Li *et al.*, 2000; Mustilli *et al.*, 2002). After activation, OST1 phosphorylation of NADPH oxidase, K⁺ and anion channels, and transcription factors are central processes in ABA signal transduction (Geiger *et al.*, 2009; Sato *et al.*, 2009;

Sirichandra *et al.*, 2009, 2010). The PYR/PYL/RCAR receptors, PP2Cs, and SnRK2 form a key complex referred to as an 'ABA signalosome'. Other important components in ABA signal transduction that have been extensively studied in guard cells include reactive oxygen species (ROS) and nitric oxide production, phosphatidic acid signaling, heterotrimeric G protein-coupled signaling, and cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_{\text{cyt}}$) and pH increases (for reviews on these topics, see Hubbard *et al.*, 2010 and Umezawa *et al.*, 2010).

Because the *B. napus* genome project used syntenic analysis to map *B. napus* genes to the *B. rapa* and *B. oleracea* progenitors and back to *A. thaliana* (Chalhoub *et al.*, 2014), we were able to investigate known ABA signaling pathway genes in *B. napus*. Using the ABA signaling pathway in *A. thaliana* as defined by Hauser *et al.* (Hauser *et al.*, 2011), we succeeded in finding corresponding orthologs of each *A. thaliana* ABA signaling pathway gene in *B. napus*. The distribution of the number of *B. napus* orthologs per *A. thaliana* gene indicates that the ABA pathway in *B. napus* typically retains genes from both the *B. rapa* and *B. oleracea* progenitors. As shown in Fig. 3, most Arabidopsis ABA signaling genes are represented in the *B. napus* genome as one copy from each of the two progenitors, although for a few of these ABA signaling genes *B. napus* has two or three copies from each of the ancestral genomes. There does not seem to be strong evidence for selective deletion of copies of a particular gene from one ancestor as a result of the presence of one or more copies from the other ancestor.

The lack of selective gene deletion from one or the other progenitor genomes in the ABA signalosome of Fig. 3 is perhaps expected given the recent speciation event for *B. napus*, compared with the estimated timescale for loss or mutation of gene copies (Lynch & Conery, 2000; Moore & Purugganan, 2005). Previous work in other species (Adams *et al.*, 2003; Chen, 2007) has found

evidence of rapid epigenetic changes, expression level differentiation, and gene silencing in polyploid plant genomes, as opposed to gene deletion. Future transcriptomic and epigenetic studies on *B. napus* should shed more light on differentiated gene expression profiles and potential silencing of genes from the A and C genomes, potentially revealing crosstalk between the *B. rapa* and *B. oleracea* drought response mechanisms present in *B. napus*. Understanding the extent of this differentiation may also suggest where polyploidy provides the potential for new and intermediate phenotypes via dosage regulation of the multiple copies present for most genes.

The above genomic analysis implicates the existence of a conserved 'ABA signalosome' in *Brassica*. This conclusion is also supported by earlier studies in which specific genes were studied. Transcription factors are important downstream targets of the ABA signaling pathway. Water stress and external ABA application up-regulate the expression of the *BolABI5* transcription factor in *B. oleracea* (Zhou *et al.*, 2013). *BolABI5* is phosphorylated by *BolOST1*, an ortholog of *AtOST1* in *B. oleracea* (Wang *et al.*, 2013). *BolABI1*, a *B. oleracea* ortholog of the Arabidopsis PP2C-type phosphatase, *ABI1*, interacts with the protein kinase *BolOST1* (Wang *et al.*, 2013; Yuan *et al.*, 2013) and dephosphorylates the transcription factor *BolABI5* (Yuan *et al.*, 2013). Other transcription factors have also been found to participate in ABA responses in *Brassica* species. For example, in *Arabidopsis*, *AtMYC2* acts as a transcription factor involved in ABA signaling (Abe *et al.*, 1997) and the *B. napus* ortholog, *BnMYC2*, shows increased accumulation in response to drought in drought-tolerant canola lines (Aliakbari & Razi, 2013). Ying *et al.* (2014) identified a NAC domain transcription factor (*BnNAC485*) from cotyledons and young seedlings that was induced by abiotic stress and ABA treatment. *B. napus* plants overexpressing *BnNAC485* also showed hypersensitivity to exogenous ABA application (Ying *et al.*, 2014), including enhanced stomatal closing and up-regulation of ABA-responsive genes. These phenotypes were comparable to those observed in rice overexpressing the NAC transcription factor *OsSNAC1* (Hu *et al.*, 2006). Saha *et al.* (2015) recently reported that eight MADS-box transcription factors, with known function in floral organ development, were up-regulated by drought treatment in *B. rapa* seedlings (Saha *et al.*, 2015).

Signaling elements in the ABA pathway upstream of gene regulation have been particularly well studied in guard cells. Ca^{2+} elevations are a central process in guard cell ABA signaling (Hetherington *et al.*, 1986; Li *et al.*, 2006). In plants, calcineurin B-like (CBL) proteins serve as one type of calcium sensor. One family member in *A. thaliana*, *CBL1*, positively regulates salt and drought responses but negatively regulates cold responses (Cheong *et al.*, 2007). A variety of stresses, including salt, cold and drought, as well as ABA treatment induce the expression of another CBL family member *CBL9* in young *A. thaliana* seedlings (Pandey *et al.*, 2004). In *B. napus*, a CBL-interacting protein kinase (CIPK), *BnCIPK6*, was isolated; salt and osmotic stresses, phosphorus starvation, and ABA significantly induced the expression of both *BnCBL1* and *BnCIPK6* (Chen *et al.*, 2012). The *Arabidopsis* heterotrimeric G protein α subunit, *GPA1*, also has pivotal roles in multiple signaling events, including ABA-modulated stomatal movement (Wang *et al.*, 2001). The *B. napus* G protein α subunit (*BnGAI*)

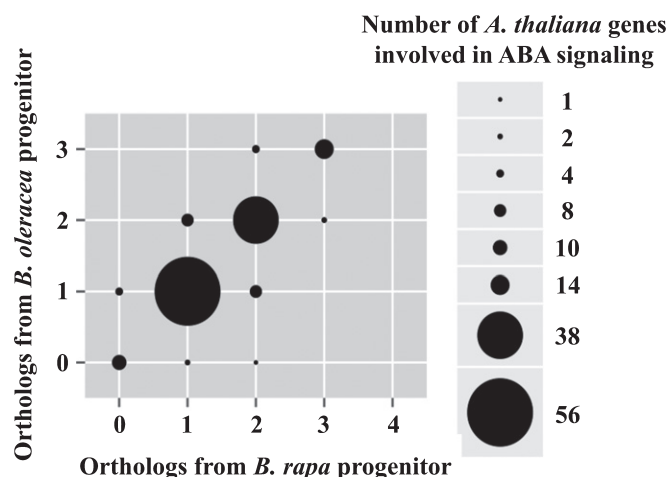


Fig. 3 Orthologs of *Arabidopsis thaliana* abscisic acid (ABA) signaling genes appear in multiple copies in the *Brassica napus* genome as a result of retention of both the *Brassica rapa* and *Brassica oleracea* progenitor genomes. *A. thaliana* ABA signaling genes were summarized by Hauser *et al.* (2011) based on the literature. The size of each circle denotes the number of Arabidopsis genes with the corresponding number of orthologs in *B. napus*, with one ortholog from each progenitor the most common, but two and three orthologs also observed.

gene was found to be strongly inducible by high concentrations of ABA and brassinosteroid (BR). *BnGAI* was also up-regulated by salt and drought stress but down-regulated by heat and cold stresses, indicating that G protein signaling in *B. napus*, as in *Arabidopsis*, plays important roles in both hormone signaling and environmental stress responses (Gao *et al.*, 2010). Studies such as these provide important evidence for the 'translatability' of knowledge obtained in a model species such as *A. thaliana* to its agronomically important relatives. The studies described in the next section show several successful examples of applications of such knowledge to *Brassica* crops.

2. Transgenic manipulations in *B. napus* based on knowledge derived from *A. thaliana*

Orthologs of genes identified in drought responses in *Arabidopsis* are targets for improving physiological responses to drought in *Brassica* (Zhang *et al.*, 2004). Transgenic manipulation of such genes is the most direct avenue for precise engineering of crops using discoveries from *Arabidopsis* (Table 1). In *Arabidopsis*, the β -subunit of farnesyltransferase, *ERA1*, has been shown to regulate ABA sensitivity and drought tolerance. *Arabidopsis* plants with inhibited *ERA1* activity by either gene deletion or chemical inhibitor application were hypersensitive to ABA-induced anion-channel activation in guard cells and stomatal closure (Pei *et al.*, 1998). In addition, transpirational water loss is reduced in *era1* mutants upon drought treatment (Cutler *et al.*, 1996; Pei *et al.*, 1998). Wang *et al.* (2005) evaluated transgenic *B. napus* expressing an antisense *ERA1* construct driven by a drought-inducible *RD29A* promoter. Reduced germination rate and inhibited seedling development following exogenous ABA application were observed in the transgenic *B. napus* compared with nontransgenic plants. However, the transgenic plants also showed reduced stomatal conductance and enhanced ABA sensitivity under water deficit, resulting in increased seed yield under drought conditions in the field as compared with the nontransgenic wild-type plants, with no yield penalty, that is, no loss of yield under well-watered conditions (Wang *et al.*, 2005). Similarly, RNAi knockdown of the farnesyltransferase (FTA) α -subunit in *B. napus* under the shoot-specific promoter *AtHPR1* resulted in higher seed yield under drought conditions in the field than in the nontransgenic wild-type plants (Wang *et al.*, 2009). Similarly, transgenic *B. napus* lines with constitutive expression of *Arabidopsis* C-repeat/dehydration-responsive element binding factor (*CBF1*) showed enhanced drought and freezing tolerance (Jaglo *et al.*, 2001; Zhang *et al.*, 2004).

Several key enzymes in phospholipid metabolism are important components of ABA signaling pathways. For example, phosphatidic acid, a lipid-derived messenger produced by phospholipase D α 1 (PLD α 1), promotes stomatal closure in *A. thaliana* (Jacob *et al.*, 1999; Zhang *et al.*, 2009). Reduced water loss and an increase in biomass accumulation and yield under stress conditions such as drought and salinity were observed in transgenic *B. napus* plants with expression of *Arabidopsis* *PLD α 1* driven by a guard cell-specific promoter (Lu *et al.*, 2013). Another key enzyme, phosphatidylinositol-specific phospholipase C (PtdIns-PLC2), has

demonstrated involvement in ABA signal transduction in *Arabidopsis* (Staxén *et al.*, 1999; Hunt *et al.*, 2003). Transgenic *B. napus* lines with constitutive overexpression of *BnPtdIns-PLC2* driven by the constitutive *CaMV35S* promoter exhibited early flowering and shorter maturation periods, accompanied by reduced transpirational rate and partially closed stomata, and enhanced drought tolerance (Georges *et al.*, 2009).

Poly (ADP-ribose) polymerase (PARP) participates in a number of cellular processes, including programmed cell death. Transgenic *B. napus* with reduced PARP activity showed reduced cell death and improved tolerance to various abiotic stresses, such as high light, drought, and high temperature (de Block *et al.*, 2005). Glycinebetaine (betaine) affords osmoprotection and protects organelles against stress conditions *in vitro*. Choline supplementation to transgenic *B. napus* with constitutive expression of a bacterial choline oxidase gene resulted in enhanced betaine accumulation. Moderate drought tolerance, assessed by measurements of relative shoot growth and net photosynthetic rate, was observed in choline-supplemented transgenic *B. napus* (Huang *et al.*, 2000).

These studies together suggest that initial elucidation of individual genes' roles in response to drought stress in a model plant species can provide fundamental knowledge to improve drought resistance in canola crops (Wan *et al.*, 2009). Commercial crop varieties arising from such *Arabidopsis*-based strategies would provide the definitive confirmation of their usefulness. As described in the next section, there are also a few examples wherein information on drought signaling and response first obtained in *B. napus* has been applied to improve drought tolerance of other species.

3. Transgenic manipulations in *A. thaliana* and other plant species based on knowledge derived from canola crops

Drought tolerance phenotypes observed in other plant species upon transgenic expression of *Brassica* genes also provide insight regarding the drought resistance function of those genes (Table 1). For example, transgenic *Arabidopsis* plants with overexpression of an active (phosphomimic) form of *B. napus* CBL-interacting protein kinase (*BnCIPK6*) showed enhanced tolerance of high-salinity and low-phosphate conditions (Chen *et al.*, 2012). These observations suggest that BnCIPK6 plays a role in responses to high salinity and phosphorus deficiency; the observation of ABA insensitivity of the *Arabidopsis* *cipk6* mutant also suggests a role in ABA and drought signaling (Chen *et al.*, 2012). Transgenic *Arabidopsis* plants overexpressing *B. napus* LEA gene *BnLEA4-1* under control of a constitutive *CaMV35S* or stress-inducible *RD29A* promoter both exhibited better recovery after 15 d of drought stress as compared with wild-type plants (Dalal *et al.*, 2009). Transgenic *B. campestris* overexpressing the *B. napus* group 3 LEA gene *BnLEA* driven by the *CaMV35S* promoter also exhibited enhanced drought tolerance, based on the survival rate after 2 wk of water deprivation, as well as improved salt tolerance as assessed from seed germination and growth performance (Park *et al.*, 2005). An ethylene-responsive factor (ERF) gene from *B. rapa*, *BrERF4*, was found to be induced by treatment with ethylene or methyl jasmonate, but not responsive to ABA or salt

Table 1 A summary of phenotypes related to drought stress in transgenic canola lines and noncanola transgenics with *Brassica* genes

Study	Promoter::Gene	GenBank ID*	Species	Transgenics	Phenotypes
Transgenic canola plants					
Huang <i>et al.</i> (2000)	CaMV35S::COX (choline oxidase gene from <i>Arthrobacter pascens</i>)	Not available	<i>B. napus</i>	Constitutive overexpression	Enhanced betaine accumulation; moderate drought tolerance when supplemented with choline
Jaglo <i>et al.</i> (2001); Zhang <i>et al.</i> (2004)	CaMV35S::AtCBF1 (C-repeat/dehydration-responsive element binding factor)	NM_118681/At4 g25490	<i>B. napus</i>	Constitutive overexpression	Enhanced drought and freezing tolerance
de Block <i>et al.</i> (2005)	CaMV35S::AtPARPs (Poly ADP-ribose polymerase)	Z48243/At4 g02390; AJ131705/At2 g31320	<i>B. napus</i>	Constitutive overexpression	Reduced cell death and improved tolerance to various abiotic stresses, such as high light, drought, and high temperature
Wang <i>et al.</i> (2005)	RD29A promoter (drought inducible)::AtERA1 (β subunit of farnesyltransferase)	BT033079/At3 g59380	<i>B. napus</i>	Antisense	Reduced germination rate and inhibited seedling development upon exogenous ABA application; reduced stomatal conductance, enhanced ABA sensitivity, and increased seed yield under drought
Georges <i>et al.</i> (2009)	CaMV35S::BnPtDlns-PLC2 (phosphatidylinositol-specific phospholipase C)	AF108123	<i>B. napus</i>	Constitutive overexpression	Early flowering and shorter maturation periods; reduced transpirational rate and partially closed stomata; enhanced drought tolerance
Wang <i>et al.</i> (2009)	AtHPR1 promoter (shoot-specific)::BnFTA (α subunit of farnesyltransferase)	XM_013820435	<i>B. napus</i>	RNAi	Higher seed yield under drought in the field
Lu <i>et al.</i> (2013)	AtKAT1 promoter (guard cell-specific)::AtPLDα1 (phospholipase Dα1)	NM_112443/At3 g15730	<i>B. napus</i>	Constitutive expression	Reduced water loss, increase in biomass accumulation and yield under stress conditions such as drought and salinity
Noncanola transgenics with <i>Brassica</i> genes					
Park <i>et al.</i> (2005)	CaMV35S::BnLEA (<i>B. napus</i> group 3 late embryogenesis abundant gene)	NM_001315725	<i>B. campestris</i>	Constitutive overexpression	Enhanced drought tolerance and improved salt tolerance
Yu <i>et al.</i> (2005)	CaMV35S::BnPIP1 (<i>B. napus</i> plasma membrane aquaporin)	AF118382	<i>Nicotiana tabacum</i>	Constitutive overexpression	Reduced wilting after 10 d of water deprivation
Dalal <i>et al.</i> (2009)	CaMV35S or RD29A promoter (stress-inducible)::BnLEA 4-1	AY572958	<i>Arabidopsis thaliana</i>	Constitutive expression or drought-inducible	Better recovery after 15 d of drought stress
Seo <i>et al.</i> (2010)	CaMV35S::BrERF4 (<i>B. rapa</i> ethylene-responsive factor)	XM_009137184	<i>A. thaliana</i>	Constitutive overexpression	Delayed yellowing under salt stress; greater shoot weight and a higher survival rate under drought stress
Yang <i>et al.</i> (2011)	CaMV35S::BnLAS (a <i>B. napus</i> ortholog of the <i>A. thaliana</i> transcriptional regulator LAS)	HQ324233	<i>A. thaliana</i>	Constitutive overexpression	Reduced water loss rates and enhanced drought tolerance; better recovery after dehydration
Chen <i>et al.</i> (2012)	CaMV35S::BnCIPIK6 (CBL-interacting protein kinase 6)	JF751063	<i>A. thaliana</i>	Constitutive overexpression	Enhanced high salinity and low phosphate tolerance
Chen <i>et al.</i> (2012)	CaMV35S::BnCIPIK6M (CIPIK6 phosphomimic form)	†JF751063 (T182D)	<i>A. thaliana</i>	Constitutive overexpression	Enhanced high salinity and low phosphate tolerance
Chen <i>et al.</i> (2012)	CaMV35S::BnCIPIK6	JF751063	<i>A. thaliana</i>	Constitutive overexpression	Complemented the low phosphate-sensitive and ABA-insensitive phenotypes of the mutant
Han <i>et al.</i> (2013)	CaMV35S::BrSAC1 (a <i>B. rapa</i> phosphoinositide phosphatase)	GU434275	<i>cipk6</i> mutant <i>N. tabacum</i>	Constitutive overexpression	Increased germination rate, seedling biomass, and seedling height under cold, dehydration, and salt stresses

*Both GenBank ID and AGI locus number are given for *A. thaliana* genes.

†Sequence that encodes BnCIPIK6 phosphomimic form with Thr182 substituted by Asp, referred to as BnCIPIK6M.

treatment in *B. rapa*. Nevertheless, overexpression of *BrERF4* in *Arabidopsis* led to delayed yellowing under salt stress as compared with the wild-type, and greater shoot weight and a higher survival rate under drought stress (Seo *et al.*, 2010). Additionally, *A. thaliana* plants with constitutive overexpression of *BnLAS*, a *B. napus* ortholog of the *A. thaliana* transcriptional regulator *LATERAL SUPPRESSOR (LAS)*, showed reduced water loss rates and enhanced drought tolerance as well as better recovery after dehydration (Yang *et al.*, 2011).

Transgenic expression of canola genes in non-*Brassicaceae* species can also improve drought tolerance. Transgenic tobacco constitutively overexpressing the *B. napus* plasma membrane aquaporin *BnPIP1* exhibited reduced wilting after 10 d of water deprivation (Yu *et al.*, 2005). A gene encoding a phosphoinositide phosphatase from *B. rapa*, *BrSAC1*, was observed to be induced by different stress conditions, for example, cold, desiccation, salt, submergence, ABA, and heavy metals. Overexpression of *BrSAC1* in tobacco increased germination rate, seedling biomass, and seedling height under cold, dehydration, and salt stresses (Han *et al.*, 2013). All these results indicate the potential of genetic engineering at the transcriptional level for improvement of drought tolerance in crop species.

Direct modification by introduction of a protein-coding transgene (as mainly discussed earlier) is not the only strategy for genetic engineering of crops. Manipulation of gene expression towards desirable traits can also be achieved through small RNA-mediated gene silencing and epigenetic modulation, for example, DNA methylation and histone modifications. Plant microRNAs participate in a wide variety of developmental and stress (both biotic and abiotic) responses. Repression of gene expression using microRNAs has a great potential in crop improvement (please refer to Sunkar *et al.*, 2012 and Kamthan *et al.*, 2015 for reviews on this topic). Small RNAs, especially microRNAs, have been identified in canola crops through sequence-based predictions and deep sequencing (Buhtz *et al.*, 2008; Zhao *et al.*, 2012; Shen *et al.*, 2015). Some of the known canola microRNAs are development-related and stress-responsive (Pant *et al.*, 2009; Körbes *et al.*, 2012; Zhou *et al.*, 2012; Huang *et al.*, 2013; Shamloo-Dashtpaderdi *et al.*, 2015). However, at present there are relatively few canola microRNAs in the registry database (<http://www.mirbase.org>). *B. napus*, for example, has 90 precursors and 92 mature microRNAs, compared with *Arabidopsis* (325 precursors and 427 mature) or other crops (e.g. rice with 592 precursors and 713 mature). This suggests that the microRNA profile of canola crops is far from fully investigated. MicroRNAs particularly responsive to drought stress have been studied in several species, including rice (Jeong & Green, 2013), *Arabidopsis* (Liu *et al.*, 2008), and *Medicago truncatula* (Wang *et al.*, 2011d). The only study in canola to date identified five drought-induced microRNAs and one drought-repressed microRNA, with six transcription factors and a kinase as predicted targets (Shamloo-Dashtpaderdi *et al.*, 2015). These predicted targets are involved in ABA biosynthesis, BR and auxin signaling, and transcription (Shamloo-Dashtpaderdi *et al.*, 2015). Results from this study, together with conserved drought-responsive microRNAs discovered in other species, form an initial inventory of microRNA candidates that could potentially be

manipulated to improve drought tolerance in canola. However, issues within current microRNA screening include lack of functional validation, and lack of spatial and temporal monitoring of the microRNA-induced change (Sunkar *et al.*, 2012). Therefore, investigations on tissue-specific (or even single cell type-specific) drought-responsive microRNAs along a time-course of drought treatment, together with information on expression levels of the corresponding target genes, are essential data for the goal of improved drought tolerance in canola via microRNA-based strategies.

Epigenetic features, for example, DNA methylation and histone modifications, are associated with developmental transitions, responses to abiotic and biotic stresses, as well as numerous quantitative and qualitative traits in crops (e.g. biomass and yield; Hauben *et al.*, 2009; Verkest *et al.*, 2015). Although there is limited knowledge on the epigenome of canola as related to desirable agronomic traits (Lukens *et al.*, 2006; Gaeta *et al.*, 2007), a pioneering study showed that energy-use efficiency (EUE) is epigenetically controlled in *B. napus* (Hauben *et al.*, 2009; Verkest *et al.*, 2015). EUE was defined as the ratio of total NAD(P)H (representing the energy content) vs respiration rate (Hauben *et al.*, 2009). In general, lines with higher EUE showed global hypomethylation in genomic DNA, as well as distinct histone methylation and acetylation patterns, and these were associated with 5% yield increase (Hauben *et al.*, 2009). Furthermore, epilines (lines selected from isogenic lines, i.e. lines and varieties with identical genetic backgrounds, for traits that are epigenetically controlled) selected towards drought tolerance were generated by exposure of hypocotyl explants to 5% PEG (drought stress), and selection for low respiration was repeated over three generations. EUE was determined in the progeny of the last generation and the two epilines with highest EUE showed enhanced drought tolerance, and changes in both the transcriptome and the epigenome, particularly enrichment for regions with histone 3 lysine-4 trimethylation (H3K4me3) (Verkest *et al.*, 2015). These applications suggest significant potential for incorporating epigenetic variation into crop breeding for enhanced stress tolerance.

IV. Systems biology of *Brassica* under drought stress

Diverse physiological processes and gene categories indicate the complexity of drought responses in *B. napus*, as is also true in other species. Systems biology provides a robust tool for comprehensive understanding of drought phenotypes at different levels of biological organization. Given the rapid expansion of genomic databases and the development of -omics tools that can be applied to nonmodel species, -omics-based research on plant stress tolerance can increasingly be performed directly in the species of interest. Different fields of systems biology, for example, transcriptomics, proteomics, and metabolomics, allow simultaneous measurements of thousands of biological molecules, which generate massive datasets toward construction of a comprehensive systems picture (Hsiao & Kuo, 2006; Le Novère, 2007). Large-scale approaches have been successfully employed to understand the drought stress responses of *Brassica* species, and such transcriptomic, proteomic, and metabolomic analyses are summarized here (see Table 2 for summary).

Table 2 A summary of -omics studies on canola crops under water-deficient conditions

Study	Species/tissue	Experimental condition	Platform	Responsive biological processes
Transcriptomics Li <i>et al.</i> (2005)	<i>Brassica napus</i> /seed	PEG- or ABA analog PBI429- inhibited germination	Microarray	Late seed development, carbohydrate metabolism, cell wall loosening, ROS scavenging, lipolysis
Fei <i>et al.</i> (2007)	<i>B. napus</i> /seed	Natural desiccation during seed ripening stage	Microarray	Signal transductions, protein synthesis
Lee <i>et al.</i> (2008)	<i>B. rapa</i> /whole plant	Drought (air-dried)	Oligo microarray	Transcription factors
Niu <i>et al.</i> (2009)	<i>B. napus</i> /seed	Natural desiccation during seed ripening stage	cDNA chip	Fatty acid biosynthesis, auxin and jasmonate signaling
Chen <i>et al.</i> (2010)	<i>B. napus</i> /seedling root	Drought (mannitol simulation)	Macroarray	Metabolism, transcription, signal transduction, hormone and abiotic stress responses, growth and development
Bhardwaj <i>et al.</i> (2015)	<i>B. juncea</i> /seedling	Drought (mannitol simulation)	RNA-Seq	*Stress/defense responses, metabolism, phosphorylation, signal transduction, transcription and translation, cell growth, cell structure, membrane transport, circadian rhythm, catalytic activity
Shamloo-Dashtpagerdi <i>et al.</i> (2015)	<i>B. napus</i> /leaf	Drought (mannitol simulation)	Expressed sequence tag	Transcription factors, kinases, phosphatase, microRNAs
Proteomics Zhu <i>et al.</i> (2010)	<i>B. napus</i> /guard cells	ABA	iTRAQ	Photosynthesis, stress/defense responses, metabolism, protein synthesis, energy production, protein folding/transport and degradation, membrane transport
Mohammadi <i>et al.</i> (2012) Meyer <i>et al.</i> (2012)	<i>B. napus</i> /root <i>B. napus</i> /seed	Drought (irrigation control) Natural desiccation during seed-ripening stage	2D-PAGE Phosphosites mapping	Metabolism, energy, disease/defense, transport Phosphorylation
Zhu <i>et al.</i> (2014)	<i>B. napus</i> /guard cells	ABA	ICAT and saturation DIGE	Thiol-based redox modification
Luo <i>et al.</i> (2015a)	<i>B. napus</i> /leaf	Short-term drought (drying on filter paper)	iTRAQ	*Ion transport, vesicle trafficking, signal perception/transduction, transcription/translation, metabolism, photosynthesis
Koh <i>et al.</i> (2015)	<i>B. napus</i> /leaf	Long-term drought (stop watering)	iTRAQ	Energy production, photosynthesis, protein synthesis, stress/defense response, metabolism, signaling, protein folding and degradation

2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; ABA, abscisic acid; DIGE, two-dimensional difference gel electrophoresis; ICAT, isotope coded affinity tag; iTRAQ, isobaric tags for relative and absolute quantitation; PEG, polyethylene glycol; ROS, reactive oxygen species. *, indicates that drought-responsive biological processes were identified by statistically significant enrichment-based on gene ontology (GO) analysis (e.g. agriGO) in the study. In other studies, biological processes were identified by representation of drought-responsive proteins/genes involved in those processes.

1. Transcriptomics

Before the availability of the genome of *B. napus* (Chalhoub *et al.*, 2014), genomes of other fully sequenced *Brassicaceae* species provided key genomic references for studies in *B. napus*. The complete genome sequence of one ancestor, *B. rapa* (var. Chiifu-401), obtained using next-generation sequencing technologies and *de novo* assembly of sequence scaffolds, was made available in 2011 (The *Brassica rapa* Genome Sequencing Project Consortium, 2011). The genome of the other ancestor, *B. oleracea*, was released in early 2014 (Liu *et al.*, 2014; sequences available at <http://brassicadb.org/brad/>). Additionally, the nucleotide sequence conservation between *A. thaliana* and *B. napus* allows some genomic

platforms developed for *A. thaliana* also to be utilized in research on *B. napus*.

The availability of the *B. rapa* genome made microarray analysis on this species possible. A *B. rapa* oligo microarray, KBGP-24K, was constructed using sequence information from *c.* 24 000 unigenes (about half of the protein-coding genome). This array was used to analyze gene expression changes after 3-wk-old *B. rapa* plants were removed from soil and allowed to air dry in a growth chamber (Lee *et al.*, 2008). Around 3% of the genes on the microarray (738) were identified as responsive genes that were differently expressed fivefold or more at least once during the 48 h time-course of drought treatment (Lee *et al.*, 2008). This work established a useful tool to analyze *Brassica* transcripts and

highlighted a role of transcription factors during drought stress. Another study, on a *B. rapa* DH line, T12-19, used tag sequencing with a Solexa Illumina array and analyzed leaf samples under dehydration treatment for 0, 1, 2 and 3 d (Yu *et al.*, 2012). In total, 1092 genes were found to be significantly altered in response to water deficit. Among these, 37 were transcription factors, 28 were genes involved in signal transduction, and 61 were water- and osmosensing-responsive genes. The results suggested high complexity of changes at the transcriptional level under drought stress (Yu *et al.*, 2012). Taken together, such information from one of the *B. napus* ancestors provides a crucial reference toward understanding drought tolerance in *B. napus*.

The attempt to identify genome-wide drought-responsive genes in *B. napus* itself began a decade ago. Using microarray analysis, a less expensive and less comprehensive microarray variant, a survey of genes induced by drought stresses was performed in *B. napus* (Chen *et al.*, 2010). In total, 288 clones were identified as putative drought-inducible genes, while 189 were candidates for drought-suppressed genes. These drought-responsive genes belonged to gene families participating in metabolism, transcription, signal transduction, hormone (ABA, in particular) and abiotic stress responses, as well as other processes related to growth and development (Chen *et al.*, 2010). This work, although limited owing to the methods available at the time, provided an initial gene list toward understanding drought response in *B. napus* at the transcriptional level. A recent, commercially available *B. napus* 300K microarray designed from 80 696 unigenes clustered from 543 448 ESTs and 780 cDNA provides an opportunity to substantially enhance our knowledge of stress responses in this important economic crop (Roh *et al.*, 2012), but has not yet been used in analyses of *B. napus* transcriptomic responses to drought.

As mentioned earlier, sequence similarity between *B. napus* and *A. thaliana* has allowed the use of *Arabidopsis* microarrays to profile gene expression in *Brassica*, with the caveat that paralogs may cross-hybridize and confound relative expression analyses. For example, *Arabidopsis* AR12K cDNA microarrays have been used to profile *B. napus* seed transcriptomes. In a comparison of transcriptional responses of imbibed vs germination-inhibited seeds of *B. napus*, 40 genes, mainly associated with late seed development, were up-regulated in desiccated nongerminating seeds as compared with imbibed seeds (Li *et al.*, 2005). On the other hand, 36 genes were down-regulated; these transcripts encoded proteins involved in carbohydrate metabolism, cell wall-loosening processes, ROS scavenging, and lipolysis (Li *et al.*, 2005). Specifically, the transcription factor *ABA INSENSITIVE 5* was consistently up-regulated in desiccated seeds and the gibberellic acid (GA)-induced transcription factor *PICKLE* was down-regulated. These results implicated ABA and GA signaling in the regulation of seed desiccation (Li *et al.*, 2005), and application of GA₃ (300 mg l⁻¹) was found to enhance both seed germination and seedling tolerance to drought stress in *B. napus* (Li *et al.*, 2010). Another study using the *Arabidopsis* AR12K cDNA microarrays discovered differentially expressed genes across the full-size embryo, desiccation, and mature stages of seed development in two *B. napus* cultivars (AC Excel and DH12075). Genes associated with signal transductions and protein synthesis were responsive during the desiccation stage

(Fei *et al.*, 2007). In another study, a cDNA chip was generated with over 8000 EST clones from *B. napus* embryos at different stages of seed development (Niu *et al.*, 2009). Using this chip, fatty acid biosynthesis genes were found to be highly expressed in *B. napus* seeds primarily at 21 d after flowering, when seed desiccation starts. Additionally, several auxin- and jasmonate-related genes showed patterns similar to those of the fatty acid synthesis genes. Analysis of *A. thaliana* auxin and jasmonate signaling mutants revealed changes in the fatty acid components of mature seeds, indicating a link between hormone signaling, fatty acid metabolism, and desiccation (Niu *et al.*, 2009). Although desiccation is a normal component of seed development, desiccation tolerance of seeds and drought tolerance of whole plants may share some common mechanisms, because both types of stresses cause cellular dehydration (Nedeva & Nikolova, 1997).

RNA-Seq, another widely used method for genome-wide quantification of gene expression, has also been applied to identify drought-responsive genes in canola. A recent study investigated drought-responsive genes in *B. juncea* seedlings and observed that 132 transcription factors (40 induced and 92 repressed) and 452 kinases (42 induced and 410 repressed) were regulated by drought (Bhardwaj *et al.*, 2015). A similar observation was reported in an analysis of ESTs of *B. napus* under drought treatment (Shamloo-Dashtpajardi *et al.*, 2015). This study found that 17 transcription factors, eight protein kinases, and one protein phosphatase were drought-regulated, including homologs of *Arabidopsis* protein phosphatase 2C *ABI1* and the ABA biosynthesis gene *ABA1*.

Although discovery of drought/desiccation-responsive genes at whole-plant and whole-organ levels provides an overall picture, studies on single cell types can provide insights into unique or cell-specific functions. In *A. thaliana*, several guard cell transcriptomic studies have been carried out. An early microarray study covering around one-third of the genome discovered 69 ABA-inducible genes and 64 ABA-repressed genes specifically in *Arabidopsis* guard cell protoplasts. Transcripts related to drought tolerance and potassium channels were among these ABA-responsive genes (Leonhardt *et al.*, 2004). Later, studies analyzing global transcriptomic responses showed a large number of ABA-regulated genes (Yang *et al.*, 2008; Wang *et al.*, 2011c; Bauer *et al.*, 2013). An analysis was conducted using enriched preparations of *Arabidopsis* guard cells and revealed 696 ABA-induced and 477 repressed genes in this cell type (Wang *et al.*, 2011c). This study also uncovered *c.* 300 genes showing ABA regulation unique to guard cells. Collectively, these transcriptomics studies facilitate understanding of the molecular mechanisms of *Brassicaceae* species in response to drought stresses.

2. Proteomics

While transcriptome analyses constitute a facile approach for candidate gene identification, transcript abundance only indicates a putative functionality of the encoded protein and often does not reflect changes in protein abundance (Bogges *et al.*, 2013). As the final direct macromolecular product of global gene expression, analysis of the proteome is required for a thorough understanding of the cellular processes associated with drought. Early proteomic

analyses were limited both by the wet bench technologies available and by incomplete databases. Proteomics has since developed into a sophisticated research approach (Chen & Harmon, 2006). In general, comparative proteomics approaches include gel-based methods, for example, two-dimensional (2D) difference gel electrophoresis and more recent gel-free methods, for example, isobaric tags for relative and absolute quantitation (iTRAQ). Isotope multiplex labeling strategies such as iTRAQ have become popular because they overcome the limitations of gel-based proteomics methods, for example, poor resolution of membrane proteins and of very acidic or basic proteins (Chen & Harmon, 2006). Gel-based and gel-free proteomics methods complement each other and their combined use can enhance proteome coverage and identify proteins with abundance changes.

Drought-induced changes in protein patterns of *B. napus* var. *oleifera* roots were observed more than two decades ago, which might represent the earliest proteomics analysis of drought-stressed *B. napus* tissue. In the tap roots, 13 2D protein spots with low molecular weight were induced by drought. Twelve of these spots were also present in the short tuberized roots, a specific drought-induced root type. After 3 d of rehydration, the disappearance of these spots suggested their potential roles in drought tolerance (Vartanian *et al.*, 1987). However, the identities of these spots remained unknown. In a more recent study, 2D polyacrylamide gel electrophoresis was employed to investigate the initial response of *B. napus* roots to drought stress (Mohammadi *et al.*, 2012). Protein expression profiles of drought-sensitive (RGS-003) and drought-tolerant lines (SLM-003), and their F₁ hybrid, were analyzed. In the sensitive line, proteins related to metabolism, energy, disease/defense, and transport were decreased under drought stress. In the tolerant line, however, proteins involved in metabolism, disease/defense, and transport were increased, while energy-related proteins were decreased. The identified proteins with abundance changes in these lines suggest that V-type H⁺-ATPase, plasma membrane-associated cation-binding protein, heat shock protein 90, and elongation factor EF-2 have a role in the drought tolerance of *B. napus*. Additionally, decreased levels of heat shock protein 70 and tubulin beta-2 in the drought-sensitive and hybrid F₁ lines might be involved in the reduced growth of these lines in drought conditions (Mohammadi *et al.*, 2012). In a recent proteomics analysis using iTRAQ, proteins responsive to short-term drought stress and salt stress were identified in leaves from 15-d-old *B. napus* seedlings. Within the proteome profile of 5583 proteins, 205 proteins showed expression level changes in response to 4 h of PEG-simulated drought treatment, with 45 common to salt-responsive proteins and 160 specific to the drought stress (Luo *et al.*, 2015a). Functional classification of the drought-responsive proteins suggested that ion transport, vesicle trafficking, and signal perception/transduction (e.g. G-protein related signaling and phosphorylation events) play a role in early drought response in *B. napus* seedlings. Additionally, notable drought-associated changes in proteins involved in transcription, translation, metabolism, and photosynthesis were observed, suggesting drought-regulation of these processes (Luo *et al.*, 2015a). In another study, the proteome response of *B. napus* leaves was studied using iTRAQ over a prolonged time-course of drought (Koh *et al.*, 2015). Respectively,

136, 244, 286, and 213 proteins were significantly altered on the 3rd, 7th, 10th, and 14th days of drought. Drought-induced proteins in *B. napus* leaves were involved in energy production, protein synthesis, and stress and defense responses, whereas drought-repressed proteins were associated with metabolism, signaling, protein folding and degradation (Koh *et al.*, 2015).

Proteomic studies have been conducted not only in *B. napus* using drought-stressed whole plants or organs but also in cell types with specialized roles in drought response. Guard cell protoplasts with high purity can be prepared on a large scale from *B. napus* leaves (Zhu *et al.*, 2009). A total of 431 nonredundant proteins were identified and quantified from untreated and ABA-treated *B. napus* guard cell protoplasts in a comparative proteomics study using iTRAQ (Zhu *et al.*, 2010). ABA up-regulated 66 proteins in *B. napus* guard cells, the majority of which were involved in photosynthesis, stress/defense responses, and metabolism. Proteins involved in photosynthesis and stress/defense responses were also observed to be drought-inducible in *B. napus* leaves (Koh *et al.*, 2015). ABA suppressed 38 proteins in *B. napus* guard cells, particularly in the categories of metabolism, protein synthesis, energy production, protein folding/transport and degradation, and membrane transport (Zhu *et al.*, 2010). The identified ABA-responsive proteins in *B. napus* guard cell protoplasts not only provide molecular details related to known physiological events in the ABA signaling pathway, for example, ROS homeostasis and cytoskeleton reorganization, but also reveal novel components in ABA signal transduction. For example, it is noteworthy that the *Arabidopsis* homolog of an ABA-induced protein, Bet v I allergen family protein, was later identified to be the ABA receptor PYL2 (Melcher *et al.*, 2009).

Proteomics approaches have been developed to identify not only those proteins that change in abundance but also proteins with changes in posttranslational modifications (PTMs), such as phosphorylation, oxidation, and glycosylation (Mann & Jensen, 2003). Posttranslational modifications of proteins are another important component of plant drought responses (Umezawa *et al.*, 2013). For example, the ABA signaling pathway is activated by initial dephosphorylation/phosphorylation events (Hubbard *et al.*, 2010). Enhanced ROS production in different cellular compartments is one of the invariant responses to drought stress (Cruz de Carvalho, 2008), which could potentially change the cellular redox status and result in protein oxidation/reduction (Martínez-Acedo *et al.*, 2012). Zhu and colleagues recently reported 65 redox-responsive proteins from *B. napus* guard cells treated with ABA. Particularly, the *in vitro* activities of an SnRK2 and a 3-isopropylmalate dehydrogenase were confirmed to be regulated by oxidant and reductant treatment (Zhu *et al.*, 2014). This study revealed thiol-based redox modification of proteins as an important regulatory mechanism in guard cell ABA signaling pathways (Zhu *et al.*, 2014). Using iTRAQ methodology, Koh and colleagues observed dynamic changes of protein PTMs (oxidation mostly, and phosphorylation) in *B. napus* leaves during drought stress (Koh *et al.*, 2015).

In a study by Meyer *et al.* (2012), over 400 phosphopeptides were identified within *B. napus* seeds at the late maturation stage. A large fraction (26.0%) of the late maturation unique

phosphopeptides were from proteins annotated as LEA proteins, which are known to play a role in dehydration tolerance (Hundertmark & Hinch, 2008). Another fraction (4.2%) was mapped to other desiccation-related proteins. Accordingly, this work supports a relationship between drought stress and seed desiccation and implicates a regulatory role of phosphorylation in these physiological processes (Meyer *et al.*, 2012).

The recent completion and publication of the *B. napus* genome sequence and anticipated progress in improved gene annotation will also provide an up-to-date database for the predicted proteome, which will allow more accurate identification of proteins in large-scale proteomics datasets generated from this species. Computational and experimentally derived proteomes can then be mined toward elucidating complex networks of protein–protein interactions. For example, protein interactions in *B. rapa* have been inferred using known *A. thaliana* interactions and interspecies homology and synteny (Yang *et al.*, 2012). A number of other methods are also available to infer interactions and regulatory networks using interaction, protein domain, and expression pattern data from related species (Liu *et al.*, 2005; Noor *et al.*, 2013). Such methods, along with availability of an expanded *A. thaliana* protein–protein interaction network (Jones *et al.*, 2014), hold promise for inferring the protein interactome of *B. napus*.

3. Metabolomics

Metabolites are also key components and regulators of biological processes. For example, stomatal closure is induced by extracellular malate and fumarate at millimolar concentrations in tomato (Araújo *et al.*, 2011). Metabolomics has emerged as a high-throughput analytical method to identify pivotal metabolites in biological processes. At present, information on global profiling of metabolites in *B. napus* is lacking, as is also true for most plant species. Two decades ago, however, evidence suggested that accumulation of free amino acids, including proline, alanine, and aspartate, is a direct effect of drought stress in *B. napus* (Good & Zaplachinski, 1994). This might be the earliest identification of key metabolites in *B. napus* drought response. Under drought conditions, considerable changes in chloroplast lipid metabolism were also observed in *B. napus* leaves. Drought stress evoked a decline in leaf polar lipids, mainly as a result of a decrease in monogalactosyldiacylglycerol content (Benhassaine-Kesri *et al.*, 2002). Furthermore, photosynthetic pigments were significantly reduced by drought stress, including Chl*a*, Chl*b*, and carotenoids in two *B. napus* varieties: Rainbow, and Dunkeld (Ullah *et al.*, 2012).

Phytohormones also participate in the regulation of drought stress response. Induction of endogenous ABA synthesis is a universal response to drought in vascular plants, including *B. napus* (Qaderi *et al.*, 2006; Wan *et al.*, 2009). In addition, the application of salicylic acid (10 μ M) can ameliorate some of the adverse effects of drought stress in *B. napus*. After salicylic acid treatment, the relative water content, Chl*a* and *b*, leaf carotenoids, soluble protein, and seed oil contents recovered in drought-stressed plants to values comparable to those in well-watered plants (Ullah *et al.*, 2012). Such observations reveal a role of plant hormone crosstalk in

drought stress tolerance in *B. napus*, as expected from observations on other species.

Improvements in analytical mass spectrometry (MS) have been crucial to the expansion of metabolomics. Not only the mass:charge ratio but also fragmentation information can be provided to aid in deciphering the structure of each metabolite (Dettmer *et al.*, 2007). The coupling of gas chromatography or liquid chromatography with MS allows one to profile (i.e. untargeted metabolomics) or selectively monitor (i.e. targeted metabolomics) many hundreds of compounds within a single injection (Patti *et al.*, 2012). The ionome, defined as the quantified mineral nutrients and trace elements in an organism, can be thought of as the inorganic component of the metabolome (Salt *et al.*, 2008). It is worthwhile performing high-throughput metabolomics/ionomics analysis in drought-stressed canola plants to reveal metabolome/ionome profiles of canola species and associated metabolic and nutrient networks in drought response and tolerance.

Mathematical modeling that incorporates parameters from wet laboratory measurements of metabolites and related enzymatic equations is an emerging approach to quantify and predict complicated metabolic processes at the systems level in plants (Libourel & Shachar-Hill, 2008). Among the modeling approaches, flux balance analysis (FBA) is a constraint-based method aiming to determine the mass balance by optimizing a set of flux values towards an objective function such as maximization of growth (Grafahrend-Belau *et al.*, 2009). FBA of cellular metabolism in *B. napus* has been used to predict the pathways involved in biomass accumulation under different physiological conditions of light and nutrient availability (Hay & Schwender, 2011; Pilalis *et al.*, 2011). A study in rice used FBA to model metabolic changes under drought and flooding (Lakshmanan *et al.*, 2013) and the *B. napus* metabolic model could be adjusted similarly to predict the pathways affected by drought in this species.

4. Phenomics

With advances in sequencing technologies, genomics approaches have generated massive amounts of data on gene sequences and transcriptome profiles in a great number of plant species, which provide directions for crop improvement. However, genomics alone cannot solve all the challenges in developing varieties with desirable traits, as connections between genotype and phenotype, including physiological, morphological, and phenological traits, can be indirect and highly complex. Moreover, even with identical genetic background, interaction with environmental factors results in diversity in phenotypic traits due to gene–environment interactions and the inherent plasticity of plants. Additionally, the plant phenome is itself multidimensional with numerous components, including but not limited to leaf morphology, root architecture, growth parameters, biomass, photosynthetic rate, and other physiological traits related to yield and biotic/abiotic stress responses (Furbank & Tester, 2011). Screening for favorable agronomic traits together with further understanding their underlying genetic basis may be the most promising and efficient avenue to determine gene or QTL candidates for crop improvement.

Phenotyping was manual, time-consuming, and destructive before the emergence of phenomics. Phenomics aims to use automated and reliable platforms for phenotyping in a high-throughput manner and provide traceable and reproducible data. However, owing to the complexity of the phenome, phenomics is currently limited by the availability of methods to measure certain traits. Therefore, advances in phenomics have not yet achieved the capabilities available with genomics techniques. In cereals, infrared thermography has been utilized to quantify responses in different genotypes under drought stress (Munns *et al.*, 2010), but this has yet to be applied to canola species. In canola species, an economic and high-resolution scanner system was developed to quantify root architectural traits in *B. rapa* (Adu *et al.*, 2014). Root phenomics was also reported in *B. napus* with phosphate limitation and the associated genetic loci were identified (Shi *et al.*, 2013). Similarly to Shi *et al.* (2013), phenomics in traits that are related to drought response/tolerance, for example, root elongation and biomass accumulation, could be performed in canola, and QTLs could be identified. Outcomes from such studies, together with genetic understanding, will facilitate marker-assisted selection for enhanced drought tolerance in canola.

The application of -omics and system biology approaches have already provided, and will continue to provide, in-depth knowledge of *B. napus* drought responses. Evidence for regulation of transcription, signaling pathways, protein synthesis, and metabolism, together with other processes, indicate the complexity of drought responses in *B. napus* and, presumably, most plant species. The acquired information provides potential targets for effective genetic engineering strategies towards improved stress tolerance.

V. Natural variation in drought tolerance for informing breeding

Although *B. napus* is a globally important oilseed crop, from a breeding perspective it has received relatively little attention with regard to drought responses. Drought responses as well as their

underlying genetic control represent a particularly complex combination of different phenotypes. As a result, breeding strategies to date have relied largely on direct phenotypic selection for yield. There is an extensive history of using traditional mapping populations to identify QTLs for agronomic and nutritional traits in *Brassica*. Natural variation in WUE among *Brassica* lines has also been well documented (Richards, 1978; Good & MacLagan, 1993). However, despite this, improvements in drought tolerance have been limited (Cowling, 2007).

Incorporating more physiological and phenomics data in studies of drought responses may prove useful for capitalizing on the available natural variation for production of *B. napus* cultivars with improved drought tolerance. Screens of targeted aspects of drought response physiology can lead to selection of lines with altered sensitivity to drought. For example, lines exhibiting natural variation in leaf ABA sensitivity affecting stomatal water loss (Fig. 4a) may also have differences in regulation of ABA concentrations, such as catabolite content, as seen in the field (Fig. 4b). Plant types representing such stomatal dynamics vary in their response to drought conditions (Fig. 4c). Here, reduced ABA sensitivity in guard cells is correlated with decreased leaf water content under drought in the field.

In addition to physiological traits, morphological traits respond to drought stress (Fig. 2). For example, the role of root system architecture in water uptake makes it another candidate for selection. Root systems of canola crops are less dense than those of more drought-tolerant species such as wheat, and they remove less water from the soil (Cutforth *et al.*, 2013). Positive correlations between drought tolerance and increased size and depth of root systems have been found in *B. napus* (Hatzig *et al.*, 2015) and several other crop species (Cortes & Sinclair, 1986; White & Castillo, 1989; Price *et al.*, 2001; Kirkegaard & Lilley, 2007; Lopes & Reynolds, 2010). Semiautomated systems and software have been developed to characterize root architecture, which can aid in rapid phenotyping of large collections as needed for breeding (Farhizadeh *et al.*, 2012; Galkovskyi *et al.*, 2012; Lobet & Draye, 2013; Bucksch *et al.*, 2014; Rellán-Álvarez *et al.*, 2015). However,

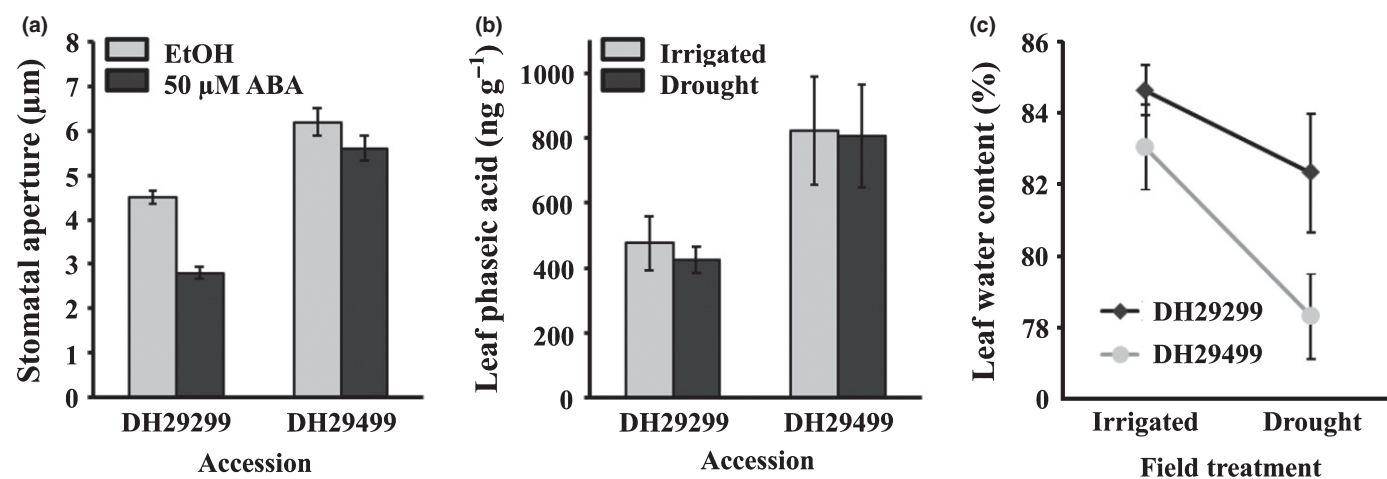


Fig. 4 Natural variation in abscisic acid (ABA)-related phenotypes in *B. napus* plants grown under laboratory and field conditions. (a) ABA sensitivity of stomatal aperture of chamber-grown plants. (b) ABA metabolism in field-grown plants. (c) Drought sensitivity of field-grown lines with different stomatal-regulation types as shown in (a). Data in (a–c) are presented as means $\pm$ standard errors.

the adaptive value of large or deep root systems depends on soil and climatic conditions, so breeding strategies need to be adjusted to match the targeted production region (Araus *et al.*, 2002; Cativelli *et al.*, 2008).

For dealing with drought stress, thus far the most common strategy in crop breeding has been to breed for drought escape, wherein plants have been selected for completing their life cycle quickly, before encountering harsh drought stress. Accordingly, current breeding practices have selected for short flowering times in *B. napus* (Rahman, 2013). As *B. napus* is most sensitive to drought during the transition from flowering to pod development (Cham-polivier & Merrien, 1996), this strategy is beneficial in situations of terminal drought. However, amid a changing climate and as agricultural production moves into more marginal areas and limited irrigation regimes, this strategy may prove insufficient. An alternative strategy to drought escape is dehydration avoidance, the ability to maintain internal water status upon drought stress by reducing water loss and/or enhancing water uptake. In contrast to the drought escape strategy, *B. napus* and *B. rapa* accessions with longer flowering times can have increased WUE and larger root systems for increased water uptake (Mitchell-Olds, 1996; Franks, 2011; Fletcher *et al.*, 2015). Because of the apparent tradeoff that exists between drought escape and dehydration avoidance, breeding for drought escape alone may have reduced the potential for drought tolerance among current varieties. This tradeoff has also been observed in glasshouse studies on *A. thaliana* accessions collected worldwide (McKay *et al.*, 2003; Kenney *et al.*, 2014), suggesting a widespread phenomenon. Some studies, however, have found that the negative relationship between flowering time and WUE is not invariant and there are genotypes of *A. thaliana* with both high WUE and short flowering time (Wolfe & Tonsor, 2014; Kooyers, 2015). Such genotypes with high WUE and short flowering times may exist in *Brassica* as well and could be suitable candidates for simultaneously breeding both drought tolerance strategies. Especially given the earlier mentioned tradeoff, the specific aspects of drought tolerance best suited for improvement depend on the target environment, including local details of climatic and soil moisture conditions along with irrigation practices. As the global climate warms, the co-occurrence of heat stress together with drought will further complicate this effort; for example, evaporative cooling by means of increased stomatal conductance helps to alleviate heat stress, but exacerbates drought stress.

As with many plant species, single nucleotide polymorphism (SNP) discovery in *Brassica* based on next-generation sequencing has improved the prospects for identifying natural variants of interest. Recent GWAS have identified *B. napus* variants associated with desirable agronomic traits such as seed yield and harvest index (seed biomass/vegetative biomass) (Cai *et al.*, 2014; Li *et al.*, 2014; Luo *et al.*, 2015b). These analyses have yet to be extended to drought studies under field conditions. However, a recent report by Yong *et al.* (2015) used GWAS to identify a gene controlling variation in salt tolerance in *B. napus*. This study stands as a model for the power of combining *A. thaliana* biology, *Brassica* -omics data, and natural variation toward crop improvement. Here the authors measured salt tolerance in 85 diverse inbred genotypes of *B. napus*

under salinity stress. Then, using the version 4 *B. napus* genome pseudomolecules (Harper *et al.*, 2012) as a guide, they identified a set of 24 834 SNP markers in this population. A subsequent GWAS for salt tolerance revealed several QTLs. Finally, they chose candidate genes under those QTLs based on gene ontology of *A. thaliana* orthologs, and upon sequencing those genes in the *B. napus* genotypes, they identified polymorphisms in a *TSN1* (RNA-binding protein Tudor-SN) ortholog as highly explanatory of variation in salt tolerance of *B. napus*. *TSN1* is therefore a promising target for transgenic or traditional breeding for improved salt tolerance in *B. napus*. These results demonstrate the efficacy of exploring natural variation, in concert with the use of -omics and *A. thaliana* tools toward improving abiotic stress tolerance in *Brassica* crops (Fig. 1).

Genetic diversity is necessary for successful breeding of desirable traits. A number of groups have measured genetic diversity in *B. napus* (Batley *et al.*, 2003; Delourme *et al.*, 2013) and the C genome appears to have lower genetic diversity than the A genome (Wu *et al.*, 2014). There also appears to have been a loss of overall genetic diversity within at least some breeding pools, such as those in Australia (Cowling, 2007) and Canada (Fu & Gugel, 2010). Furthermore, the genetic diversity available for selective breeding within *B. napus* does not fully represent that of its parental species (Becker *et al.*, 1995; Seyis *et al.*, 2003). Therefore, in addition to the diversity within *B. napus*, the larger phenotypic diversity of other *Brassica* species could also be a source of favorable drought-related phenotypes, such as increased osmotic adjustment (Gunasekera *et al.*, 2009). Accordingly, there have been attempts to increase genetic diversity by resynthesizing *B. napus* from *B. rapa* and *B. oleracea* (Bennett *et al.*, 2012; Wu *et al.*, 2014). Additionally, there has been increased interest in introgressing loci controlling phenotypic variation using hybrid bridges and the generation of new type *B. napus*, wherein the entire A or C genome is replaced by a wild *B. rapa* or *B. oleracea* genome (Qian *et al.*, 2006; Chen *et al.*, 2011; Mei *et al.*, 2011). Indeed, Mei *et al.* (2015) demonstrated that the hybrid bridge approach successfully transferred a pathogen resistance QTL from wild *B. oleracea* into *B. napus*. This may be a powerful approach if applied to introgressing drought tolerance traits into canola crops by tapping into the vast diversity in drought responses of different wild and cultivated *Brassica* species (Richards & Thurling, 1978a,b; Kumar & Singh, 1998; Enjalbert *et al.*, 2013). Therefore, it appears that, together, the diversity of the *B. napus* gene pool and those of close relatives provide a promising resource for future selective breeding toward favorable drought tolerance traits in canola crops. The challenge will be to select for domestication traits and adaptation to agronomic management, without imposing the strong bottleneck that occurred in the original breeding of *B. napus*. Emerging methods in field-based phenomics (Andrade-Sanchez *et al.*, 2013) might allow breeding programs to work with much larger populations, and minimize the effect of drift and fixation of deleterious mutations.

VI. Conclusions/hurdles/perspectives

The availability of the *B. napus* genome has opened the door to computational as well as reverse genetic approaches that can inform

strategies to improve drought tolerance, such as analysis of promoter motifs of drought-regulated genes, studies documenting effects of copy number variation on drought tolerance, and target prediction for stress-regulated microRNAs (Xie *et al.*, 2007). Such studies were not possible with the limited genomic resources previously available in this species. The more complete picture of gene models in *B. napus* also provides an opportunity for cross-species inference of drought-regulated protein interactomes (Yang *et al.*, 2012). Knowledge of the mechanisms of drought response and resistance and their participating genes in other well-studied model species such as *A. thaliana* or crop species such as rice, maize, wheat and soybean can be used to infer parallel mechanisms in canola crops, especially when orthologous gene models are present in the canola species. Conversely, as the availability and quality of genome sequences and gene models of canola species improve, identification and subsequent manipulation of potential canola-specific genes and stress tolerance mechanisms can be accelerated.

In parallel with the genomics breakthrough in canola crops, linking phenome and genome has become urgent and indispensable to discover genes and traits contributing to canola drought tolerance. Genes related to drought tolerance in canola have been discussed earlier. Favorable traits for enhanced drought tolerance include but are not limited to: traits to enhance the plant's ability to obtain water, such as rooting depth, root architecture, water extraction capability, and ability to withstand deleterious (pathogenic) aspects, and capitalize on favorable aspects, of the microbiome; traits for improved water conservation under drought conditions, including osmoprotectant accumulation and optimized control of guard cell density, drought/ABA sensitivity and stomatal response kinetics; and traits that allow optimal plasticity in flowering time in response to varying water availability (Mullet, 2009; Ashraf, 2010).

Progress in phenomics and genomics, together with outcomes from other systems biology studies, as well as knowledge gained from other species, will deepen understanding of the mechanisms involved in drought response, adaptation, and tolerance, forming the basis and direction for canola improvement through traditional breeding or genetic engineering. Nowadays, genome manipulation is not limited to overexpressing a gene or repressing a gene through RNA interference technology (Table 1). Epigenetic modifications and the CRISPR/Cas9 system for targeted genome editing can also be applied in genetic engineering of canola crops (Belhaj *et al.*, 2015). Because *B. napus* is allopolyploid, homeologs within *B. napus* (homologs from *B. rapa* and *B. oleracea*) can share high sequence identity. CRISPR/Cas9 genome editing has been successfully applied to target two loci simultaneously in the Arabidopsis genome (Mao *et al.*, 2013). Therefore, this system has great potential for editing multiple homeologs in the *B. napus* genome. Additionally, doubled haploid (DH) lines as potential canola varieties have been developed to reduce genetic complexity and shorten breeding time for this crop (Kučera *et al.*, 2002). In combination with the earlier-mentioned genetic modification strategies, obtaining DH lines (varieties) with enhanced drought tolerance can be accelerated.

It has been argued that, particularly for the phenomenon of drought tolerance, the number of successful examples wherein

translation of knowledge from laboratory studies (primarily on *A. thaliana*) has resulted in adoption of a new transgenic crop cultivar, relative to the total number of studies on drought tolerance in, for example, *A. thaliana*, is disproportionately small (Passioura, 2007; Blum, 2014). Reasons that have been proffered for the low success rate include the complex, polygenic nature of plant water relations, imposition of unrealistic drought scenarios in *A. thaliana* growth chamber and glasshouse experiments, and the need to identify transgenes that will result in optimal plant performance in nonstressed as well as stressed field conditions, that is, the need to avoid yield drag (Blum, 2014 and reference therein). While these arguments have validity, it should also be noted that when the crop to be manipulated is more closely related to *A. thaliana*, as is the case for *B. napus*, the success rate is likely to be proportionately much higher. In addition, the value of the model plant *A. thaliana* as a reference genome cannot be overstated, as perfectly exemplified by its use in the initial annotation of the *B. napus* genome (Chalhoub *et al.*, 2014).

Nevertheless, drought adaptation is highly polygenic and new large-scale approaches that can be conducted directly in the crop species of interest, including both -omics analyses and large-scale genetic studies of natural variation and genome-wide association, signal a new era in drought research, with great potential for implementation via targeted molecular breeding. As illustrated in this review, current development of -omics and genetic tools and datasets for *B. napus* is allowing its development as a model crop species in its own right. This knowledge is enabling direct (intraspecies) approaches to improve drought tolerance in *B. napus*, as will become increasingly necessary for all major crop species if we are to successfully combat the vagaries of climate change and provide food, fuel, and shelter for over nine billion people by 2050.

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