



PHYSIOLOGICAL APPROACHES FOR BREEDING DROUGHT TOLERANT BRASSICA GENOTYPES

M. SINGH¹ and V.V. SINGH^{2*}

¹ICAR- Indian Institute of Soybean Research, Indore-452001, Madhya Pradesh, India

²ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur-324001, Rajasthan, India

*Corresponding author's email: singhvijayveer71@gmail.com

Email address of coauthor: ms_drmr@rediffmail.com

SUMMARY

Among various abiotic stresses affecting the crop production, drought is the most serious constraint for global agriculture as it affects more than 75% of the world's land area. It exerts many-fold effects at whole plant level in rapeseed and mustard. Its effects on plant phenology, growth and development, physiological processes, source-sink relations and plant reproduction processes depend on the severity of drought. An understanding of the various physiological traits controlling/ regulating crop responses to drought is required to develop drought resistant varieties of rapeseed and mustard. In this review, we have attempted to amass scattered information at one platform, and suggested as to how these can be integrated towards the ultimate aim of developing drought tolerant varieties in oilseed *Brassica*.

Key words: Drought, reactive oxygen species, antioxidant enzymes, quantitative trait loci, marker assisted selection, molecular breeding, *Brassica juncea*

Key findings: An integrated approach combining advances of plant physiology and molecular genetics and biology is required to increase the precision and efficiency of breeding program for drought tolerance in oilseed Brassica.

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INTRODUCTION

Plants growth under field conditions are subjected to various environmental stresses, such as high

or low temperature, drought and salinity. At any given point of time, plants may face two or more stresses (e.g. drought and salinity). Among these stresses, drought is the most

serious problem for global agriculture, affecting 75% of the world's land area (Ratna Reddy and Syme, 2015). Drought is an extended abnormal dry period that occurs in a region consistently receiving a below-average rainfall. Out of 1474 million hectares (ha) of cultivated land in the world, 86% comes under rain-fed cultivation (Kumar, 2005). The genus *Brassica* comprises some 100 species such as rapeseed (*Brassica napus* L.), Indian mustard (*Brassica juncea* L.), cabbage (*Brassica oleracea* L.) and turnip rape (*Brassica rapa* L.) that are mainly grown for oil, condiments, vegetables or fodder (Hosaini *et al.*, 2009). Rapeseed accounts for most of the oilseed production in Europe and North America, whereas Indian mustard is mainly grown in India and North Africa. The annual production of rapeseed is more than 60 million tons (<http://faostat.fao.org>, 2011). However, drought greatly affects its growth and seed yield. This situation can be alleviated by an approach combining water storage and irrigation, crop management and plant breeding. There has been great interest in breeding stress-tolerant varieties, since significant inter- and intra specific variation for drought tolerance exist within *Brassica* species, which needs to be exploited through selection and breeding. Literature abounds in physiological traits related to drought tolerance and breeding strategies for drought tolerant *Brassica* genotypes. This review highlights the recent advances in physiological mechanisms and parameters and underlying genetical processes imparting resistance/tolerance to drought stress in *Brassica* crops. We have also attempted to integrate molecular, physiological and metabolic aspects to

bring about heritable improvement to help them withstand limited moisture condition.

Physiological traits: response to drought

Drought at the whole plant level brings about many fold changes on plant phenology, growth and development, source-sink relations and plant reproduction processes. An understanding of the various physiological traits controlling/regulating crop responses to drought is required for identifying natural genetic variation for drought tolerance. These traits can be broadly classified as shoot and root-related traits.

Germination, seedling growth and establishment

Drought can be responsible for the inhibition or delayed seed germination, poor seedling growth and establishment. *Brassica* seeds did not germinate up to -13 bars (Channaoui *et al.*, 2017). The relative vigor index of seedlings varied between 0.32 ~ 0.79, with an average of 0.49. Drought stress significantly affected seedling height, fresh weight and survival rate. Compared with water control, 10% PEG-6000 treatment on an average reduced seedling height by 40.68%, fresh weight by 34.2% and survival rate by 18% (Chun-jie *et al.*, 2007). Reduction in germination components by osmotic stress could be attributed to lower infusibility of water through the seed coat and low initial water uptake by the seed under stress condition (Bahrami *et al.*, 2012) and decreased external water potential. Decrease of seed germination under water stress

condition could also be due to metabolic disorders such as slow hydrolysis of storage compounds in endosperm or cotyledons and/or slower transportation of hydrolyzed material to developing embryo axis (Ayaz *et al.*, 2000). The germination process involves two steps, firstly enzymatic hydrolysis of stored material, and secondly the building of new tissue. Under moisture deficit condition, enzymatic activity slows down and consequently the germination percentage decreases under the more negative osmotic potential. As osmotic potential declines, water absorption decreases and as a result, turgidity and cell division decrease and finally, radicle growth and germination get reduced (Zaefizadeh *et al.*, 2011).

According to Ayaz *et al.* (2000), inhibition of seed germination under water deficit condition may be due to the change in metabolic pathways like slower hydrolysis of storage compounds in endosperm or cotyledons and/ or slower transportation of hydrolyzed material to developing embryo axis. Moreover, with the decrease in osmotic potential, water absorption also decreases resulting in reduced cell turgidity and division; all these finally lead to substantial decrease in root length, shoot length and germination percentage (Zaefizadeh *et al.*, 2011).

Phenology

Plant developmental traits such as early vigour or phenology may be particularly significant under water-limited condition (Cairns *et al.*, 2009). Faster phenological development is particularly useful in situations where late season drought is imminent. Compared to small seeds, large seeds

improved shoot dry weight, biomass and seed yield by 13–43, 25–57 and 12 per cents, respectively. Results indicated that seedlings from medium and large seeds were more vigorous and tolerant to flea beetle damage than seedlings from small seeds. Tolerance was due to a higher initial seedling weight rather than higher relative growth rate. Shoot dry weight, biomass and yield of the three cultivars were more strongly correlated with 1000-seed weight than with seed diameter (Elliott *et al.*, 2007).

Root architecture

An increased depth and density of roots is considered a major mechanism for improving water uptake under drought conditions (Turner, 1986). The roots interestingly show a very different (but very logical, from a survival standpoint) response. Root growth is often accelerated by at least moderate drought stresses (perhaps by the action of ABA also) which results in increase in root depth and decrease in root volume. It is obvious where obtaining water is a problem; perhaps more can be obtained by sending out roots to explore a greater soil volume.

Extensive information is available on the value of root traits in relation to drought avoidance in crops (Kashiwagi *et al.*, 2015). Osmotic adjustment is reported to promote root growth in *B. juncea* (Kumar and Singh, 1998). On an average, root zone depth was 118.2 cm in *B. juncea* and 109 cm in *B. napus*. Deeper root depth in *B. juncea* might have led to higher soil moisture extraction from deeper layers than that in *B. napus*. Increased water use in Indian mustard improved plant water status, leaf

water potential (LWP), relative water content (RWC) and photosynthetic activity. Root zone depth was positively correlated to the number of primary and secondary branches and number of silique per plant in *B. juncea*, while no such association was found in *B. napus*. Under drought, positive correlation was found between yield per plant and seedling root traits viz., root length, fresh and dry root weight (Cheema *et al.*, 2004). Association with seedling root traits under drought appeared to be more important than seedling shoot traits; this reflects roots as relatively powerful sink. Well-developed deep root system will have higher potential to absorb water and minerals and transport water to the growing shoots. This might be the reason for strong association of yield per plant with seedling root traits. Therefore, selection under drought should be based on root traits rather than shoot characters.

Plant water relation and osmotic adjustment (OA)

It is generally agreed that water stress brings about osmotic dehydration of the plant tissue, resulting in altered plant water relations. In rapeseed, reduced relative water contents, osmotic potential and potassium contents as well as increased total greenness and proline contents were observed under various levels of water stresses (Alikhan *et al.*, 2010). In *B. juncea*, water deficit decreased leaf water potential (LWP) and relative water content (RWC) of leaf, resulting into greater osmotic adjustment and higher root growth. This helped the plants to explore greater soil volume for water resulting in better yield attributes and ultimately seed yield. *B.*

juncea had greater osmotic adjustment than *B. napus*. This has been supported further by the fact that decreases in WP, RWC and osmotic potential (OP) promoted root growth in *B. juncea*, but not in *B. napus* (Indo-China-Australia Final Report (2011)).

It has been suggested that plant water status, rather than plant function, controls crop performance under drought. Therefore, the genotypes that maintain higher LWP and RWC are drought tolerant simply owing to their superior internal water status (Kamoshita *et al.*, 2008). A positive relationship was observed between grain yield and RWC measured during the reproductive stage in wheat, where the high-yield selections maintained a significantly higher RWC than the low-yield selections (Tahara *et al.*, 1990). However, studies suggest that differences in RWC among cultivars are highly influenced by plant maturity, adaptation and severity of stress, and hence it may be used as a secondary selection trait (Lafitte, 2002).

OA has been shown to maintain stomatal conductance and photosynthesis at lower water potentials, delay leaf senescence and death, reduce flower abortion, improve root growth and increase water extraction from the soil as water deficit develops (Turner *et al.*, 2001). Consistent differences in OA exist among cultivars which can be associated with plant production under drought stress in *Brassica* (Kumar *et al.*, 1984). Beneficial effects of OA on root growth under water deficit conditions clearly show the value of this trait under water limiting conditions. At the juvenile and elongation stages, leaves of both

canola cv. Monty and mustard line EC 397-23-2-3-3 adjusted osmotically after exposure to water deficit (Indo-China-Australia Final Report (2011).

Oxidative damage

Drought stress increased production of reactive oxygen species (ROS) like superoxide radical ($O_2 \bullet^-$), singlet oxygen (1O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH\bullet$) (Hasanuzzaman *et al.*, 2012a; 2012b). Loss of water, decrease of photosynthetic pigments and increase in lipid peroxidation also gets significantly accelerated due to drought stress (Hasanuzzaman and Fujita, 2011). Enzymatic ROS-scavenging mechanisms in plants include superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and catalase (CAT) (Vernoux *et al.*, 2002). The activity of glutathione reductase (GR) increased only slightly at 10% PEG compared to APX and glutathione S-transferase (GST).

Stomatal behavior, gas exchange parameters and water use efficiency

Improving water use efficiency (WUE) would reduce the water requirement for a specific yield potential and thus can help save considerable amount of irrigation water. Genotypic variation in WUE has been reported in mustard (Singh *et al.*, 2007) using carbon isotope discrimination technique. The association of WUE under rainfed condition with total dry matter ($r = 0.632^{**}$) and seed yield ($r = 0.712^{**}$) was positive and significant (Singh *et al.*, 2009). Transpiration showed positive and significant relationship with stomatal conductance ($r =$

0.516^*) and leaf area index ($r = 0.446^*$) under irrigated condition only (Singh *et al.*, 2009). Transpiration efficiency (TE) influences the performance of crop under limited water condition. Genotypes having thicker leaves had greater WUE (Singh *et al.*, 2003b).

Canopy temperature

Canopy temperature and stomatal conductance are directly related. Plants with high stomatal conductance transpire more, and thus maintain a cooler canopy temperature. Use of canopy temperature measurement as a screening technique under drought stress has received attention of the scientists, particularly with the advent of portable infrared thermometers (Ahmed *et al.*, 1998). Canopy measurements in stress condition have been reported as an acceptable criterion in determining the drought stress in rapeseed (Fanaei *et al.*, 2009). Kumar *et al.* (1984) reported close associations of OA with both stomatal conductance and canopy temperature. There was a positive and significant correlation between the amount of ΔT ($T_c - T_a$) in *Brassica* types (mid-day) and stomatal conductance, plant osmoregulation and seed yield (Kumar and Singh, 1998).

Photosynthesis and other physiological parameters

Mustard genotypes with drought tolerance traits provided relatively good yield under water stress condition. Such traits/parameters include OA (Singh *et al.*, 1996), transpirational cooling (Chaudhary *et al.*, 1989), epicuticular wax on leaves, difference between air and canopy

temperature and drought susceptibility index (DSI) (Singh and Choudhary, 2003). Since physiological responses of plants to drought stress may vary at different developmental stages, different indicators may be used for the phenotyping under limited moisture condition. Currently, a number of indicators such as WUE, DSI, RVI and leaf wilting index (LWI) are used in breeding for drought tolerance in rapeseed and mustard. The details of these parameters/indices and their applications in *Brassica* crops are summarized in Table 1.

In Indian mustard (*B. juncea* L.), DSI values for seed yield and component characteristics were calculated to characterize the relative tolerance of genotypes under irrigated and drought conditions (Singh and Choudhary, 2003; Chauhan *et al.*, 2007). Biomass and water potential have also been used as indices to evaluate the relative drought tolerance of different *Brassica* species (Ashraf and Mehmood, 1990). Regression analysis revealed that SLA, photosynthesis and water use efficiency contributed 74.5%, 61.5% and 81.8%, respectively to the variation under irrigated conditions. The corresponding contributions of these characters under rainfed conditions were 61.9%, 63.0% and 59.2%, respectively (Singh *et al.*, 2009). Further, SLA, photosynthesis and water use efficiency also contributed 52.0%, 70.0% and 68.3%, respectively to the total dry matter. The corresponding contributions of these characters under rainfed conditions were 72.0%, 62.0% and 52.9% respectively to the total dry matter. Water stress caused increase in proline content, stomata closure and photosynthesis inhibition.

Also, water stress caused a significant decrease in chlorophyll contents and increase in the accumulation of proline in *Brassica* crops (Gibon *et al.*, 2000). Din *et al.* (2011) reported significant differences among the various canola genotypes for leaf chlorophyll a, chlorophyll b and proline accumulation. The Chla/Chlb ratio in most species increased slightly under moderate stress, while under severe stress it decreased. This is presumably due to faster damage of Chla compared to Chlb under moderate stress condition. The highest value of Chla/Chlb was observed in *B. carinata*, *B. oleracea* (under non-stress condition) and *B. juncea* (under moderate and severe stress condition). The analysis of changes in chlorophyll fluorescence kinetics provides detailed information on the structure and function of the photosynthetic apparatus, especially photosystem II (Strasser *et al.*, 1995).

Breeding for drought tolerance

Breeding oilseed *Brassica* for drought tolerance is necessary to enhance and sustain production under rainfed conditions in many semi-arid parts of India. Substantial heritable variation has been reported in the primary and secondary gene pools, providing ample scope for the improvement of rapeseed and mustard for drought resistance.

Escape mechanism, invariably used in most other crops, may be useful and effective in *Brassica*. Selection for earliness, however, is complex due to continuous podding behavior till maturity. Nevertheless, drought tolerance is associated with early flowering and partitioning of dry matter to reproductive parts.

Table 1. Parameters/indices for drought tolerance in Brassica species.

Parameters/ Indices	Species	Develop- mental stage	Traits	Drought regime	Reference
Biomass and water potential	<i>B.napus</i> , <i>B.juncea</i> , <i>B.campestris</i> , <i>B.carinata</i>	Seedling	Biomass, water potential, osmotic potential	Green house	Ashraf and Mehmood (1990)
DSI	<i>B.juncea</i>	Maturity	Seed yield and component traits	Experimental field	Singh and Choudhary(2003); Chauhan <i>et al.</i> (2007); Singh <i>et al.</i> (2018)
LS, PSS, RVI	<i>B.napus</i>	Germination	Biological and biophysical traits	Petri-dish, 10% PEG-6000 solution	Yang <i>et al.</i> (2007)
Principal component, clustering, subordinate function analysis	<i>B.napus</i>	Flowering	Morphological and agronomic traits	Rain-out shelter	Zhu <i>et al.</i> (2011)
Leaf wilting index (LWI)	<i>B.napus</i>	Seedling	Biological and biophysical traits	Pots in rain-out shelter	Li <i>et al.</i> (2012)
TDM, LAI, RGR, CGR	<i>B.napus</i>	Whole stage	Physiological growth indices	Field	Moaveni <i>et al.</i> (2010)

DSI, drought susceptibility index; RVI, relative vigor index; LS, length of seedlings; PSS, percentage of seedlings surviving after drought stress; LWI, leaf wilting index; TDM, total dry matter; LAI, leaf area index; RGR, relative growth rate; CGR, crop growth rate.

Partitioning differences to reproduction parts may be more in moisture limited than in irrigated condition. However, anthesis, partitioning, harvest index (HI) and maturity are influenced by drought. Drought induced differences, therefore, make it difficult to detect genotypic differences. Thus, plants which bear more mature pods early in the season may be useful while improving *Brassicaceae* for drought situation. Selection based on whole plant maturity may not be useful as maturity period is strongly influenced by the dry environments. The phenomena of drought escape and drought avoidance operate in *Brassicaceae*. Some promising varieties

identified for drought tolerance in India include Aravali, Geeta, GM-1, PBR 97, Pusa Bahar, Pusa Bold, RH 781, RH 819, RGN 48, RB 50, RH 406, RGN 298 and RH 725. DRMR 541- 44 has been registered as a donor for drought tolerance (Singh *et al.*, 2017)

The success of breeding for drought tolerance lies in the screening of high yielding and drought tolerant lines separately, hybridizing them, selecting drought tolerant lines in drought condition (F₁ to F₃), and finally predicting yields in targeted environments (Blum, 2005). In addition to this, time, intensity, duration and frequency of stress as well as plant, soil and climate interactions also affect plant

responses to water stress. In addition, the difficulty to establish well-defined and repeatable water stress conditions makes screening of drought tolerant genotypes even more complex (Ramirez and Kelly, 1998). Therefore, it is imperative to use different indicators for phenotyping of drought tolerance traits. Presently, a number of selection indicators such as stress tolerance index (STI), WUE, DSI, RVI and LWI are widely used to identify genotypes which provide high yield under both stress and non-stress conditions.

Use of physiological and molecular approaches to improve drought tolerance in Brassica

Drought tolerance is a typical quantitative trait; however, monogenic traits such as flowering time, plant height, ear type and OA may have important roles in adaptation to drought-prone environments. Special attention has been given to: (i) genetic variation of the OA (Robin *et al.*, 2003), (ii) genetic bases of phenological trait such as stay green phenotype (Jiang *et al.*, 2004), (iii) the ability of the roots to exploit deep soil moisture to meet evapo-transpirational demand (Nguyen *et al.*, 2004), (iv) the limitation of water-use by reduction of leaf area and shortening of growth period (Anyia and Herzog, 2004), (v) isotope discrimination (Juenger *et al.*, 2005), (vi) the limitation of non-stomatal water loss from leaves, through cuticle, for example (Lafitte and Courtois, 2002), and (vii) the response of leaf elongation rate to soil moisture and evaporative demand (Reymond *et al.*, 2003).

The improvement of crop yield has been possible through the indirect

manipulation of quantitative trait loci (QTLs) that control heritable variability of the traits and physiological mechanisms that determine biomass production and its partitioning. The QTL approach provides an opportunity to dissect out the genetic and physiological components affecting source-sink relationships under abiotic stress (Miralles and Slafer, 2007; Welcker *et al.*, 2007). The QTL for drought tolerance (leaf wilting under drought stress) was identified from seventy two double haploid (DH) lines from a cross between TX9425 (drought and salinity tolerant) and a sensitive variety, Franklin based on a range of developmental and physiological traits in *Brassica*. Composite interval mapping (CIM) analysis revealed that four out of 30 QTLs in F₂ generation under irrigated conditions were identified for drought related physiological traits (Electrolyte leakage). A total of 19 QTLs were identified in F_{2:3} under irrigated conditions for various drought related, yield and other traits in Indian mustard including 4 QTLs for RWC. QTL analysis revealed a total of 7 QTLs accounting for different phenotypic variance in F_{2:3} generation under drought conditions for various drought related, yield and other traits in *Brassica juncea* with one QTL identified for a physiological trait, Electrolyte leakage (Monika, 2015).

A major QTL for days to flowering detected on linkage group 2 was consistent and co-localized with QTL for $\delta^{13}\text{C}$, proportion of aborted siliquae and lateral branch number. QTL was associated with traits such as plant height, root pulling force and $\delta^{13}\text{C}$ in the DHYB canola population in both the treatments across years. However, four QTLs for siliqua length, one for number seeds/siliquae, five for

number of siliquae on the main raceme and one for 1000-seed weight were identified.

Molecular basis of drought tolerance in *Brassica* crops

Out of a total of 1,092 drought-responsive genes, 37 transcription factors were identified. Twenty-eight were involved in signal transduction and 61 were involved in water- and osmo-sensing-responsive pathways. Among these genes, many were involved in response to abscisic acid (ABA) or water stress, indicating that ABA and water-stress-mediated signal transductions are possible mechanisms for root hydrotropic response. Such studies have provided a large number of candidate drought-tolerant genes, which can be manipulated (mainly *via* over expression) to achieve enhanced drought performance in mustard crops. Liang *et al.* (2011) analyzed the top 10 genes predicted by the SVM-RFE to be involved in water tolerance. Among the 41 top-ranked genes, 27 (65.8%) were found to have altered transcript levels under various osmotic stress treatments. In recent years, functions of some drought-related genes have been characterized. Seo *et al.* (2010) reported that over expression of an ethylene-responsive factor (ERF) from *B. rapa* (*BrERF4*) led to improved salt and drought tolerance in *Arabidopsis*. Expression of *BrERF4* was induced by ethylene or methyl jasmonate, and not by ABA or NaCl. Thus, *BrERF4* seems to be activated through a network of signaling pathways in response to salinity and drought.

CONCLUSION

In the last three decades, significant advances have occurred in the area of plant physiology, molecular genetics and molecular biology. Integration of validated methods of screening, physiological parameters, low cost genotyping and phenotyping platforms and novel tools and techniques of molecular biology in breeding programs is needed to improve precision of selection for abiotic stress tolerance. Integrated breeding involving multidisciplinary (genetics, physiology, and biotechnology) approaches needs to be used to enhance efficiency of breeding programs (Choudhary *et al.*, 2017).

Multiple tolerance mechanisms to drought exist in oilseed Brassicas; these need to be integrated to achieve a high level of drought tolerance. To this end, there exists an example. The *cis-acting* dehydration response element (DRE) plays an important role in regulating gene expression in response to abiotic stresses. Under field conditions, plants more often experience multiple stresses. Transgenic approaches should be integrated with conventional breeding if gene(s) (QTLs) of interest are available in tertiary gene pool. It is interesting to note that most drought-tolerant transgenic lines have been developed using a single gene transformation, which may not be as productive as using transformation of many genes. Thus, it is considered to be a more logical approach to enhance crop stress tolerance by transferring a number of target genes.

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