REVIEW PAPER

Impact of salt, drought, heat and frost stresses on morpho-biochemical and physiological properties of *Brassica* species: An updated review

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ABSTRACT Abiotic stresses seriously impact crop productivity and agro-morphological and biochemical properties of all *Brassica* species. It also decreases the yield of many important *Brassica* species by disturbing their normal growth and development. In this review, we have highlighted the latest reports about the impact of different abiotic stresses on different growth stages and other morpho-physiological processes of important *Brassica* species such as canola/rapeseed (*Brassica napus*), indian mustard (*Brassica juncea*), *Brassica oleracea* and *Brassica rapa*. Several researchers reported that abiotic stresses affect the important morpho-biochemical processes such as shoot and root length, shoot fresh and dry weight, proline and relative water contents, chlorophyll amount, antioxidant enzymes activity of important Brassica species. These stresses also disturb normal oxidative processes that lead to cell injury. The genetic modification approaches for the development of transgenic plants against these environmental extremes have been described. The present study will be useful to identify the best abiotic stress tolerant *Brassica* genotypes for further genetic engineering program and crop improvement programs.

Keywords: Abiotic stresses, *Brassica* species, Morpho-biochemical, Transgenic *Brassica* species

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INTRODUCTION

Abiotic stress affects the growth, development, yield and other physiological characters of different Brassica species. The effect on these characters is directly correlated with economically yield loss of crop plants. Various researchers conducted studies to screen the abiotic stress tolerant *Brassica* genotypes for further study in environmental stress affected areas. The detailed information about the effect of all sort of abiotic stresses on important *Brassica* species is given below:

Effect of salt stress on important properties of *Brassica* species

Salt stress is one of the major abiotic stresses that affects plant growth and its production (Allakhverdiev et al., 2000; Hasanuzzaman et al., 2017; Nejat & Mantri, 2017). About 20% of our cultivated lands and 50% of crop land are highly affected by salt stress (Lakhdar et al., 2009). It is estimated that more than 50% of our arable lands will be affected by this type of stress by the year 2050 (Wang et al., 2003). This stress can be determined at time when plant death occurs or when it badly affects its morpho-physiological process. However, the abiotic stress tolerance varies with plant species/sub-species. Therefore, development of abiotic stress tolerant varieties is so important (Zheng et al., 2009). Salt stress affects both the qualitative and quantitative characters of important *Brassica* species such as canola (*Brassica napus* L.), Indian mustard (*Brassica juncea* L.), cabbage (*Brassica oleracea* L.) and turnip rape (*Brassica rapa* L.). The diploid nature of *Brassica rapa* is more sensitive to salt stress as compared to other polyploid species i.e. *B. napus* (Farhoudi et al., 2015; Kumar, 1995). The high salt concentration in soil and irrigation water decreases the germination rate of almost all *Brassica* species. Sometime plant germination can occur but shows stunted growth and poor development (Zamani et al., 2010). So, it is important to identify and characterize improved genotypes against these environmental extremes (Almodares et al., 2007; Islam & Karim, 2010).

Salt stress affects the N (nitrogen) uptake and its assimilation process in many plant species. The high level of salt affects various enzymes of *B. juncea* such as nitrite reductase (NiR), glutamine synthetase (GS), glutamate dehydrogenase (GDH) and asparagines synthetase (ASN) (Siddiqui et al., 2009). It also disturbs plant biomass, root and shoots length, CO_2 assimilation rate of *B. juncea* (Ahmad et al., 2012). The other morpho-biochemical and physiological processes such as growth rate, chlorophyll contents, leaf area index, flower abortion and N, K and P contents are also significantly affected in many *Brassica* species (Hayat et al., 2009).

Canola shows moderate level of resistance to salinity. But, its growth and productivity are highly affected by different salt concentraions (Lomonte et al., 2010). Salt affects the photosynthetic rate, growth and sodium (Na^+) ion accumulation and distribution in leaf area of two important canola genotypes (NYY 1 and BZY 1). The plant dry biomass, overall photosynthesis, Na^+ level and net water potential rates in leaf area were higher in genotype NYY 1 as compared to other genotype (BZY 1). However, the %Na^+ content in leaf symplast remained higher in genotype NYY 1. The moderate salt level (3 g/kg) has slight effects on the stomatal conductance. While high salt levels (6 and 9 g/kg NaCl) significantly affects the assimilation rate due to stomatal and non-stomatal limitations and leads leaf necrosis and stunted growth. The high salt resistant potential in genotype NYY 1 is due to low accumulation of Na^+ in shoot area confined Na^+ to the apoplast area thus lowering leaf toxicity. This is one reason that genotype NYY 1 shows more tolerance than that of BZY 1. Hence salt tolerance response varies with the type of genotype (Yang et al., 2012). The shoot, root fresh and dry weights of canola plants decreased with all salt stress levels (50, 100, 150 and 150 mmol) (Sergeeva et al., 2006). Similarly, the percent relative water contents 24 hours also decline with the increasing NaCl levels (0, 100, 150, 200, 250 and 300 mM) in many important canola cultivars. It means that water loss from leaves occur at high rate at elevated NaCl concentration (Dai et al., 2009).

Elevated salt concentrations (12 and 15 dSm^{-1}) significantly decreased the germination rate, root shoot length and seedling dry weight many folds of kohlrabi (*Brassica oleracea* var. *gongylodes*). While it showed good morpho-physiological performance at up to 9 dSm^{-1} NaCl (Biswas et al., 2016). Kandil et al. (2016) reported that salinity stress affects all the ten tested canola genotypes. However, the salt tolerance level varied among genotypes. The cultivar Screw 6 showed excellent morphogenic response for all quantitative traits like root/shoot length and root/shoot fresh and dry weights. While cultivar Screw 51 gave better seedling height, total chlorophyll contents and relative dry weight. However, these characters were highly affected at high NaCl concentration (1.8%). Umar et al. (2011) also observed different type of responses at different salinity levels of many imported *B. rapa* genotypes. The abiotic stress decreased the amounts of chlorophyll a, b and a+b up to several folds of *B. napus, B. juncea* and *B. rapa* genotypes (Alam et al., 2014).

Salt stress also affects the growth and total fatty acid (TFA) contents of many important *Brassica napus* genotypes. The plant biomass was decreased by 25 and 35% at high NaCl levels (100 and 150 mmol). The overall decrease in biomass occurred by 55% at very high salt stress (200 mmol) in all canola genotypes. The overall TFA value was decreased by 25% with increasing of salt stress from 0 to 200 mmol. It might be due to membrane lipid degradation at high stress level. More interestingly, the poly-unsaturated fatty acids decreased, while the mono-unsaturated fatty increased with the rise of salt stress. The oleic acid, palmatic acid, linoleic acid and linolenic acid optimum concentrations were significantly disturbed by saline condition (Bybordi et al., 2010). The increase level of degradation of important secondary metabolites (glucosinolates) occur with the increase level of salt stress, due to membrane damage/high relative electrolyte leakage. The high glucosinolates content was observed in *Brassica oleracea* L. var. *italica* after NaCl (40 and 80 mM) stress for two weeks. The same trends were also recorded for *B. rapa* after subjecting NaCl levels (20, 40 and 60 mM) for five days (Lopez-Berenguer et al., 2008). These abiotic stresses may change the defense mechanism of many important *Brassica rapa* germplasm (Steinbrenner et al., 2012). Salt stress affected the growth and other important enzymatic activity of three important canola genotypes (Consul, Zarfam and Okapi). The growth decreased by several folds but the rate of Catalase (CAT) and Peroxidase (POD) increased many folds with the increase of salt concentration from 0 to 120 mmol. The cultivar Opaki showed more salt tolerance and maximum enzymatic activities; CAT (14.2 mgH2O2/g.pro/min)/POD (63.4 mgH2O2/g.pro/min) as compared to other two cultivars at 120 mmol NaCl (Farhoudi et al., 2015).

Salts stress severely affects different plant species at early germination and seedling growth stages by disturbing their various agro-morphological and physiological processes (Su et al., 2013). Torabi & Ardestani (2013) reported that salinity and drought stress affected the morpho-biochemical processes of important canola genotype Opaki at germination stages. The seeds were germinated at 0, -0.1, -0.2, -0.3, -0.4, -0.6 and -0.8 MPa NaCl and PEG-6000 concentrations. The maximum germination frequency (72%) was obtained at controlled condition (0 MPa) and it decreased up to 26.1% at -0.8MPa for NaCl and PEG. The 50% maximum germination value was estimated for 50.4 h at 0 MPa and increased to 62.5 and 123.7 h at -0.8 MPa concentrations. Salinity causes reduction in average yield, oil contents and other growth performance of many important *Brassica* species. It has adverse effect on plant morphology and other morpho-physiological processes (Su et al., 2013; Jan et al., 2016).

Impact of heat stress on morpho-physiological properties of *Brassica* species

High temperature stress disturbs normal plant growth and development, especially at the early stages of plant growth, which is one of the major problems in many cultivated areas of the world. The high heat stress retards the normal agronomical, morphological, biochemical and physiological processes of many different plant species and causes severe yield loss. It also affects many *Brassica* species including important canola oilseed crop at early growth stage. The increase in levels of ascorbate peroxidase and gene expression in canola hypocotyl occurs at high temperature. However, ascorbate peroxidase levels increased for a short period upon high temperature stress. The up-regulation of these proteins play key role in energy and metabolic processes and can help to provide maximum nutrients to early seedling at high temperature (Ismaili et al., 2015). The optimum temperature for *Brassica napus* germination is 28 °C and any temperature above this level retard its growth and development (Kaya et al., 2006). The effect of low to high temperature gradient on plants generally depends on some important factors like anti-oxidant enzyme concentrations, plant species/ cultivar used, type of organs, time period of exposure, magnitude of stress and growth stages (Lu et al., 2008; Zhang et al., 2015). Similarly, the proline content increases with rise of heat stress. Proline protects the proper protein structure from denaturing, stabilizes the cell membrane by interacting with phospolipid bilayer and maintains the osmotic pressure between cytoplasm and environment (Claussen, 2005). On the other hand, the decrease in chlorophyll content was recorded at unfavorable temperature (Gupta et al., 2013; Shah et al., 2015).

Temperature above 27 °C leads to floral sterility and yield loss of many economically important *Brassica napus* cultivars. The high heat stress at vegetative growth stage leads to low flower number in all three important *Brassica* species (*B. rapa, B. juncea and B. napus*). The yield significantly increased with the increase of flower number. The loss of yield was due to reduced seed size per flower. Therefore, heat tolerance genotypes are important to achieve maximum flower numbers and healthy seed size (Morrison & Stewart, 2002). The *B. carinata* shows poor germination and early seedling growth at high heat stress. Therefore, proper inter/intra-specific hybridization methods are important to develop new heat tolerant *Brassica* species. New alien genes introgression can be used for the improvement of many important *Brassica* species (Deol et al., 2003).

The flower and grain filling stage are more sensitive for temperature stress. It affects pollen viability, grain development, anthesis time and fertilization process. The high thermal stress at terminal growth stage affected normal photosynthesis process, transpiration rate, stomatal conductance, mean productivity and geometric mean productivity, and important yield characters of 43 important rapeseed germplasm. A 20% reduction in plant yield was recorded in many genotypes. The rapeseed mustard genotypes, BPR-549-9, BPR-540-6 and BPR-349-9 showed more heat tolerance at terminal growth stage and gave better yield and other morpho-physiological response than that of other accessions (Singh et al., 2014). The elevated level of temperature increased the transpiration rate and stomatal conductance, and decreased the water use efficiency and chlorophyll a content of Chinese cabbage (*Brassica campestris* subsp. napus var. pekinensis cv. Detong) (Oh et al., 2014).

Effect of frost stress on physiological properties of *Brassica* species

The frost stress is one of the key environmental extremes that affects the yield and other agronomic important characters of many crop plant species (Singh et al., 2008; Shah et al., 2016). The canola crop is very sensitive to frost stress especially at reproductive stages. The spring and winter temperature affect some of the important steps during the reproductive period like gametogenesis, pollination, fertilization and embryogenesiss (Angadi et al., 2000). The low temperature leads to few mature seeds formation due to poor pollen formation (Jinling, 1997). Frost stress at early seedling stages causes death of the whole canola plant. The damage of frost stress mainly depends on many important factors such as duration and extent of cold stress, different plant growth stages and moisture content. The seedling growth is significantly affected by high frost stress (−16 °C). The frost stress leads to wilting of leaves, bleaching, or in extreme cases can cause plant death. A significant level of difference in growth was found among spring, hybrid and winter types to cold stress. However, the response of hybrid and winter types remained the same (Fiebelkorn and Rahman, 2016). The wilting symptoms can cause loss of maximum water from cells. The blackened cotyledons and/or leaves of canola genotypes serve as indicator to frost damage. The canola is more sensitive at cotyledons stage than at three- to four-leaf stage. The slow growing seedling shows less susceptibility than that of rapid growing seeding canola genotypes (Sovero, 1993).

Effect of drought stress on morpho-physiological characters of *Brassica* species

Drought is one of the most drastic abiotic stresses that damages agricultural crops affecting its development, growth and production (Micheletto et al., 2007). Drought stress in plants may result some physiological disorders such as reduction in photosynthesis and transpiration (Sarker et al., 2005). The drought decreases the average production rate of different crop species (Robertson & Holland, 2004). Plants react to water stress through a number of developmental, functional and biochemical changes. The tolerant canola genotypes have more ability for adapting themselves under drought condition. The genetic diversity of cultivated *Brassica napus* relatives provides valuable genes for improving this tolerance (Hosseini & Hassibi, 2011).

Nasri et al. (2008) studied drought stress that caused a significant reduction in the number of seeds per siliqua, number of siliquae per plant, 1000-seed weight, seed yield, seed oil content, and oil yield of five rapeseed cultivars. Sinaki et al. (2007) observed that low water stress at flowering stage decreased seed yield, the biological yield, and the number of siliquae per plant of important rapeseed cultivars. Stroeher et al. (1995) reported that phenology of rapeseed affected seed quality characteristics such as protein and oil percentage and the quantity of glucosinolates under water stress condition. The main qualitative properties of rapeseed plants strongly affected by water deficit are oil and protein contents (Istanbulluoglu et al., 2010). Tesfamariam et al. (2010) found that the seed oil content of rapeseed plants was low due to water deficit at flower budding stage. Richards & Thurling (1978) observed variation in response to drought stress between and within species such as *B. rapa* and *B. napus*. They found that seed production and its components in different cultivars of *B. rapa* and, *B. napus* are significantly influenced by low water stress. Variation in drought patience cultivars or species has frequently attributed to differences in their time of ripening. Thurling (1974) suggested that *B. napus* may be more resistant to drought stress than that of *B. rapa* since it flowers later and stores less of its dry matter after flowering. Drought stress negatively affected many biological processes in plants including the reduction in photosynthesis, accumulation of dry matter, stomatal opening, and protein synthesis (Larcher, 2003; Ohashi et al., 2006). Drought causes disorganization of thylakoid membranes resulting in reduction in chlorophyll contents and the pigments (Ashraf & Harris, 2013).

Development of transgenic abiotic stress tolerant *Brassica* species

In nature, plants produced tolerance to both types of biotic and abiotic stresses but it is a very slow process and some time these extreme stresses affect plants negatively. The transgenic plants expressing transgene show more abiotic stress tolerance as compared to non-transgenic plants (Shinwari et al., 1998; Kasuga et al., 1999; Maqbool et al., 2002; Ali et al., 2016). Transgenic plants against biotic and abiotic stress can be produced through several gene transfer approaches using *Agrobacterium*-mediated transfer; this will enhance tolerance against drought, salinity and low temperature in *Arabidopsis thaliana* (Kasuga et al., 1999). Agarwal et al. (2006) described that with recent advances in molecular biology have shown that several genes are induced under abiotic stress condition. These genes have been isolated, characterized and transformed to plants under the presence of specific induced or constitutive promoters. The resulted transgenic plants showed tolerance to these extreme environmental conditions and played important role in the improvement of sustainable agriculture. Many efficient, quick, direct and indirect transformation protocols have been developed to wide range of plant species (Kumar et al., 2014; Shah et al., 2015; Jan et al., 2016). Various transgenic *Brassica* species has been produced against these environmental extreme that shows tolerance as compared to non-transgenic plant. The detailed information of these transgenic *Brassica* species against abiotic stresses is shown in Table 1.

Table 1 Transgenic *Brassica* species developed against abiotic stress through genetic modification

Transgenic Plant	Transgene	Against	References
Brassica napus	*BnSIP1-1*	Salt and Osmotic stress	Luo et al. (2017)
Brassica napus	Differentially expressed genes (DEGs)	Drought	Wang et al. (2017)
Brassica juncea	Glyoxalase I	Drought and salt stress	Rajwanshi et al. (2016)
Brassica juncea cv. Varuna	Lectin	Drought and salt stress	Kumar et al. (2015)
Brassica napus	*AtDWF4*	Drought and heat stress	Sahni et al. (2016)
Brassica napus Var. Wester	*DREB*	Salt stress	Qamarunnisa (2015)
Brassica oleracea var. botrytis	APX, SOD	Salt stress	Metwali et al. (2012)
Brassica napus	Vacuolar Na+/H+ antiporter*BnNHX1*	Salt stress	Wang et al. (2004)
Brassica napus	Vacuolar Na+/H+ antiporter*AtNHX1*	Salt stress	Zhang et al. (2001)
Brassica napus	*AtCBF1*	Frost stress	Jaglo et al. (2001)
Brassica napus	*BNCBF5/BNCBF17*	Frost stress	Savitch et al. (2005)
Brassica napus	*Coda*	Salt stress	Huang et al. (2000)
B. juncea	*Coda*	Salt stress	Prasad et al. (2000)
B. oleracea	*Beta*	Salt stress	Bhattacharya et al. (2004)
B. campestris	*Lea*	Drought and salt stress	Park et al. (2005)

CONCLUSION

The drought, salt, frost and high temperature stresses significantly affect the morpho-physiological processes of some important *Brassica* species. Development and identification of abiotic stress tolerant cultivars are important economic goals for our globe. The morphological and agronomical study of *Brassica* species performing under environmental extremes could lead the research and development of new stress-tolerant cultivars. The genetic engineering approaches play a key role for the development of improved transgenic

Brassica species against wide range of abiotic stresses. The present study provided updated information about the toxic effects of abiotic stress on important *Brassica* species, detailed information of abiotic stress tolerant and non-tolerant *Brassica* species/genotypes and transgenic approaches against these stresses.

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