Review Paper

Effect of Abiotic Stresses on Brassica Species and Role of Transgenic Breeding for Adaptation

ABSTRACT

Abiotic stresses extremely affect crop productivity and agro-morphological and biochemical properties of all Brassica species. Due to these stresses, yield of many important Brassica species decreases by disturbing their normal growth rate. 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*. Many researchers reported that abiotic stresses influence the important morpho-biochemical characteristics such as root and shoot length, shoot fresh and dry weight, proline and relative water contents, chlorophyll amount, antioxidant enzymes activity of important Brassica species. Cell injury also occurs by disturbance in normal oxidative processes due to these stresses. Against these stresses genetic modification have been described for the development of transgenic plant. The present study will be useful to identify the best abiotic stress tolerant Brassica genotypes for further genetic engineering program and crop improvement programs.

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1. INTRODUCTION

Rapeseed (*Brassica napus* L.) is the essential oil seed plant source. After soybean (*Glycine max*) and palm oil (*Elaeis guineensis* L.) it is the third most important plant oil in the world. Plants are exposed to many environmental stresses when they are growing under field conditions [1]. Abiotic stress disturbs the growth, development, yield and other physiological properties of different Brassica species. Drought can severely affect seed traits like seed germination, seed yield and seed quality as well as plant growth and flowering. Irrigation stop at flowering and grain filling stages of canola causes 35 and 18 percent yield loss [2]. Significant loss in the number of seeds per siliquae number of siliquae per plant, 1000-seed weight, seed oil content, seed production and oil production is mainly caused by drought stress. Polyploidy species of ~~brassica~~ Brassica are less sensitive to salt stress as compared to diploid species of ~~brassica~~ Brassica (*Brassica rapa*). In all, when canola genotypes are at very high salt stress, it results in reduction of plant biomass and TFA value by 55 and 25% respectively from 0 to 200 mmol. Membrane lipid degradation affects the overall decrease in biomass and TFA at high salt stress level [3]. During reproduction stage spring and winter temperature affects some of the important stages like gametogenesis, pollination, fertilization and embryogenesis~~'s~~. The ideal temperature for the germination of Brassica *napus* is 28 °C and plant growth and development starts to ~~be~~ reduce~~d~~ when temperature rises above 28°C. The effect on these characters is directly linked with economically yield loss of crop plants [4]. Various researchers have conducted studies to screen the abiotic stress tolerant *Brassica* genotypes for further study in environmental stress affected areas. The transgenic plants expressing transgene show more abiotic stress tolerance as compared to non-transgenic plants [5]. The detailed information about the effect of all type of abiotic stresses on important *Brassica* species is given below

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

Drought can severely affect seed traits like seed germination, seed yield and seed quality as well as plant growth and flowering. The ability of the plant to adapt to drought stress and plant biochemical, physiological and molecular processes also causes variation in the degree of these influences [6]. Champolivier [7] reported that decrease in chlorophyll contents is estimated due to the extreme loss of pigments as well as inefficiency of thylakoid membranes mainly caused by drought. Ali [8] observed that drought decreases seed oil content and yield at a rate of 2.6 and 25%, respectively. Shekari [9] concluded that the most sensitive stage for drought injury was flowering resulting in an extreme loss in seed as well as oil yield by 29.5% and 31.7%, respectively. Richards [10] estimated that rapeseed and oil yield were more sensitive to water stress at flowering and less sensitive during the seed-filling and vegetative stages. Nasri [11] concluded that at both growth stages chlorophyll **a** and **b** content of all the Napus genotypes decreased due to the drought stress. Sinaki [12] showed that decline in oil yield during reproductive stage is due to drought stress. Irrigation stop at flowering and grain filling stages of canola causes 35 and 18 percent yield loss. Drought stress enhance the activity of antioxidant enzymes catalase and peroxidase in canola genotypes, but the increase in enzyme activity among genotypes was not the same for genetic diversity, and it was observed that the genotypes that have higher yields under drought stress had higher levels of enzyme activity [13]. Drought stress significantly reduces the seed protein content of plants. Rapeseed plants with maximum water availability had the highest protein content. Reduction in water availability resulted in loss of protein content [14]. Hasanuzzaman [15] reported 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. Nejat [16] observed differences in response to drought stress between and within species such as *B. rapa* and *B. napus*. They observed that in different cultivars of B. rapa and B. napus seed yield are significantly affected by low water stress. These differences are estimated at the time of ripening. Significant loss in the number of seeds per siliquae number of siliquae per plant, 1000-seed weight, seed oil content, seed production and oil production mainly caused by drought stress [17]. Martinez [18] estimated that at flowering stage of important rapeseed cultivars drought reduced the seed and biological yield and the number of siliquae per plant. Ashraf [19] observed that for improving this tolerance genetic diversity of cultivated *Brassica napus* relatives provides valuable genes.

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

One of the major abiotic stresses that alter plant growth and its production is salt stress [20]. Due to salt stress cultivated lands and crop land are highly affected by 20 % and 50 % respectively [21]. Vital [22] reported that soil texture and composition adversely affected by one of the major environmental extreme that is salt stress. Shinwari [23] observed that when salt concentration is high, and then due to nutrients and ionic ~~unbalances~~inbalances, it ~~reduce~~ reduces the normal morpho-physiological and other biological processes. Masood [24] examined that negatively inhibiting reactive oxygen species (ROS) caused cell injury due to high to low salt stress. By decreasing essential nutrients normal plant growth changed and plant death occurs. ~~(~~KUMAR, 1995 reported that polyploidy species of ~~brassica~~ Brassica are less sensitive to salt stress as compared to diploid species of ~~brassica~~ Brassica (*Brassica rapa*). In many ~~brassica~~ Brassica species seed germination is ~~inhibits~~ inhibited due to high concentration of salt in soil and in irrigation water. But in some Brassica species, although germination occurs ~~but~~ plant growth and development show retardation [25]. In many plant species N (nitrogen) uptake and its absorption process is affected by salt stress. Various enzymes of *B. juncea* such as nitrite reductase (NiR), glutamine synthetase (GS), glutamate dehydrogenase (GDH) and asparagines synthetase (ASN) are also affected by high level of salt stress [26]. Siddiqui [27] reported that in many important *Brassica napus* genotypes salt stress also affects the growth and total fatty acid (TFA) contents. At high NaCl levels plant biomass was decreased by 25 to 35 % . In all canola genotypes at very high salt stress plant biomass and TFA value decreased by 55 and 25% respectively from 0 to 200 mmol. Membrane lipid degradation affects the overall decrease in biomass and TFA at high salt stress level. As salt stress level increased, then poly-unsaturated fatty acid decreased while the mono-unsaturated fatty acids increased.

In *B. juncea* plant biomass, root and shoot length and CO_2 assimilation is also changed by salt stress [28]. Due to salt stress in many Brassica species 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 [29]. Canola growth and productivity are extremely changed through different salt concentration, although it shows adequate level of resistance to salinity [30]. Different genotypes of ~~brassica~~ Brassica respond differently to salt stress [31]. All salt stress levels (50, 100, 150 and 150 mmol) cause reduction in shoot, root fresh and dry weights of canola plants [32]. As NaCl levels increased (0, 100, 150, 200, 250 and 300 mM), percent relative water contents (RWC) decreased. It means with increasing NaCl level, loss of water occurred from leaves [33]. Sergeeva [34] also reported that many imported *B. rapa* genotypes ~~response~~ respond differently at different concentration of salinity levels. *B. napus, B. juncea* and *B. rapa* genotypes showed reduction in the amounts of chlorophyll **a**, **b** and **a**+**b** up to several folds [35].

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

Cold stresses reduce plant productivity

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One of the most important environmental stresses that disturb the yield and other agronomic important characters of many crop plant species is cold stress [36, 38]. Frost stress affects the canola crop mainly at the stage of reproduction. During reproduction stage spring and winter temperature affects some of the important stages like gametogenesis, pollination, fertilization and embryogenesiss [39, 41]. Due to poor pollen formation, few mature seeds formation occurs at low temperature [42]. Shah [43] observed that whole plant death occurs if frost stress affects ~~at~~ the seedling stages. The rate of injury of frost stress extremely depends on many important factors such as duration and amount of cold stress, different stages of plant growth and moisture content. High frost stress (−16 °C) mainly changes the seedling growth. Wilting of leaves, bleaching and in high stress plant death also occurs due to frost stress. Among spring, hybrid and winter types a significant level of change in growth was observed due to cold stress.

5. Cold stress symptoms and its after effects

Symptoms of cold stress can develop only after a cold stress; however minor symptoms of herbicide damage may often be confused with symptoms caused by low temperature or nutrient deficient soil. As temperature ~~increased~~increases, cold stress ~~will be decreased~~decreases. Improbably symptoms of nutrient stress occur at the stage of cotyledon because at this stage nutrient demands are usually low [44]. Fig. 1 shows that purpling of leaves is not due to herbicide injury; rather it is a result of production of anthocyanin caused by low temperature. It may be toward the base, on the leaf margins or may cover whole leaves of young plant. As temperature ~~increased~~increases, symptoms ~~will be~~ reduce~~d~~. Fig. 2 reveals cupping caused by low temperatures and symptoms quickly disappear as temperature~~s~~ increases.

Fig.1. Purpling of leaves. **Fig 2 ~~cupping~~ Cupping of leaves**

The wilting symptoms can cause injury of maximum water from cells. In canola genotypes the blackened cotyledons and/or leaves act as indicator to cold stress. The canola is more sensitive at cotyledons stage than at three- to four-leaf stage. The slow growing seedling shows low susceptibility than that of fast growing ~~sedding~~ seedling canola genotypes [45].

Fig 3. An unaffected cotyledon

Fig 4. Severely affected cotyledon

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

One of the most important but least studied abiotic stresses which affect plant growth and productivity around the world is ~~high~~ heat stress. The direct effects of high temperature stress depend on the crop species and its adaptability. It disturbs normal plant growth and development, mainly at the primary stages of plant growth. At high temperature level of ascorbate peroxidase and gene expression in canola hypocotyl also increased. However it increased upon high temperature for short time duration. Metabolic processes and availability of nutrients at seedling stage also due to the up regulation of these proteins [46]. The ideal temperature for the germination of Brassica *napus* is 28 °C and plant growth and development start to be reduced when temperature rises above 28°C [47]. The influence of low to high temperature gradient on plants usually depends on some important aspects like anti-oxidant enzyme concentrations, plant species/ cultivar used, type of organs, time period of exposure, magnitude of stress and growth stages [48]. Proline defends the proper structure of protein from denaturing by stabilizing the cell membrane through interaction with phospholipid bilayer and also adjusts the osmotic pressure between cytoplasm and environment. So, as temperature level increased proline content also increased [49]. ~~While~~ On the otherhand at harsh temperature chlorophyll content was decreased [50]. Pollen viability, grain development, fertilization process and anthesis time are mostly affected at flower and grain filling stage because these stages are more sensitive for temperature stress. 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% decline in plant yield was recorded in many genotypes. The rapeseed mustard genotypes, BPR-549-9, BPR-540-6 and BPR-349-9 exhibited more heat tolerance at terminal growth stage and gave better yield and other morpho-physiological response than that of other accessions [51]. Zhang [52] reported that floral sterility and yield loss of many economically important *Brassica napus* cultivars occurred when temperature ~~rise~~ rose above 27 ° C. In all three important *Brassica* species (*B. rapa, B. juncea and B. napus*) low flower number are mainly due to high heat stress at vegetative growth stage. Yield of Brassica species expressively increased with maximum number of flowers. While reduced seed size per flower caused reduction in yield. Therefore, it is most important for heat tolerance genotypes to gain high number of flowers and also vigorous seed size.

7. Breeding for crop improvement

Creation of the Triangle of U is the important participation towards the improvement of Brassica species, which ultimately led to the development of canola [53]. The origin of the current tetraploid Brassica species is the result of three common diploid species (*B. campestris*, *B. nigra* and *B. oleracea*). These tetraploid species act like main grounds for existing development and improvements for both vegetables and oilseed crops within the genus [54]. In different Brassica species a wide assortments of loci for several physiological and morphological traits have been determined and recognized also through both classical and molecular methods. Several limitations such as male-sterility and self-incompatibility were overcome through the use of embryo rescue techniques, interspecific and intergeneric hybridization [55].

For the development of improved Brassica cultivars microspore culture and protoplast fusion have been employed successfully. Morrison [56] observed that at the stage of protoplasts fusion gained from different species hybrid formation has the potential to combine different genome which is almost impossible otherwise. These systems of hybridization along with recent biotechnology have strengthened the exploration of novel techniques to develop unique desirable cultivars.

8. Development of transgenic abiotic stress tolerant *Brassica* species

The transgenic plants expressing transgene show more abiotic stress tolerance as compared to non-transgenic plants [57].

9. ROLE OF GENES TO OVERCOME THE ABIOTIC STRESSES

Through different gene transfer approaches using *Agrobacterium*-mediated transfer; Transgenic plants against biotic and abiotic stress can be produced. This will increase tolerance against drought, salinity and low temperature in *Arabidopsis thaliana* [58]. Ofori [59] observed that with recent advances in molecular biology have shown that numerous genes ~~are~~ could be induced under abiotic stress conditions. Under the existence of specific induced or constitutive promoters these genes these genes have been isolated, characterized and transformed to plants. The ~~resulted~~ resulting transgenic plants showed tolerance to these life-threatening environmental disorders and played a vital role in the improvement of sustainable agriculture. Many effective, rapid, direct and indirect transformation protocols have been established ~~to~~ in a wide range of plant species [60].

Table 1

Transgenic Plant	Transgene	Against	References
Brassica napus *Brassica napus* *Brassica juncea* *Brassica juncea* cv. Varuna	*BnSIP1-1* Differentially expressed genes (DEGs) Glyoxalase I Lectin	Salt and Osmotic stress Drought Drought and salt stress Drought and salt stress	[61, 64]
Brassica napus *Brassica napus* Var. Wester	*AtDWF4* *DREB*	Drought and heat stress Salt stress	[65, 66]
Brassica oleracea var. botrytis	APX, SOD	Salt stress	[67]
Brassica napus	Vacuolar Na+/H+ antiporter*BnNHX1*	Salt stress	[68]
Brassica napus	Vacuolar Na+/H+ antiporter*AtNHX1*	Salt stress	[69]
Brassica napus	*AtCBF1*	Frost stress	[70]

10. ROLE OF GENES TO OVERCOME THE ABIOTIC STRESSES

Several transgenic *Brassica* species has been created against these environmental extremes that show~~s~~ tolerance as compared to non-transgenic plant. The detailed information of these transgenic *Brassica* species against abiotic stresses is shown in Table 1.

11. CONCLUSION

The drought, salt, frost and high temperature stresses significantly affect the morpho-physiological processes of some important *Brassica* species. In summary;

- Development and identification of abiotic stress (cold, heat salt) 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~~ provides 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 abiotic stresses.

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