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Breeding Mustard (*Brassica juncea*) for Salt Tolerance: Problems and Prospects

Jogendra Singh, Parbodh Chander Sharma and Vijayata Singh

Abstract

Salt stress is currently one of the most critical factors, reducing agricultural production. Indian mustard (*Brassica juncea*) is a major oilseed crop in these areas. However, salt affects as much as 50–90% worldwide yield reduction. Salt tolerance is a very complex factor controlled by a number of independent and/or interdependent mechanisms and genetic modification that lead to many changes in physiology and biochemistry at the cellular level. The classical methods of plant breeding for salt tolerance involves the widespread use of inter and intraspecific variations in the available germplasm which is essential for any crop development program. This large germplasm is then tested under various salt levels in microplots, which is a quick, reliable, reproducible and inexpensive method of salt tolerance. Genotypes that have shown better indications of stress tolerance without significant yield reduction are considered to be tolerant and are also used as potential donor in the breeding programs. In this way, ICAR-Central Soil Salinity Research Institute (ICAR-CSSRI), Karnal developed and produced five varieties of Indian mustard that tolerate high salt namely, CS 52, CS 54, CS 56, CS 58 and CS 60 in the country, and many other high-quality pipeline lines exploration and development. These salt-tolerant species work better under conditions of salt stress due to various manipulations (physiology, genes and molecular level) to fight salt stress has led to detrimental effects. Recent molecular tools to add classical breeding systems to improve saline-tolerant mustard varieties in a short span of time, including the Marker Assisted Selection (MAS) and backcrossing, that have helped using simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP) markers to identify quantitative trait loci (QTLs) that control the polygenic traits like tolerance of salt and seed yield.

Keywords: mustard, *Brassica juncea*, salinity, salt tolerance, antiporter, antioxidant genes, QTLs

1. Introduction

Globally the total area of saline soil is 397 million ha and 434 million ha of sodic soil. Of the 230 million irrigated fields, 45 million ha (19.5%) are salt-affected and almost 1500 million ha of arable agriculture, 32 million (2.1%) salt-affected [1]. Of the world's salt-affected land, an estimated 6.73 million ha are in India. In addition, arid and semi-arid areas are associated with salty groundwater, which must be used for irrigation, due to the unavailability or diversion of quality water outside for

agricultural purposes. The use of this salty groundwater makes the soil unsuitable for growing crops. Salt stress is currently one of the most critical factors, reducing agricultural production (**Figure 1**).

Reclamation of these salt affected areas is of paramount importance to bring more and more areas under cultivation. This is necessary to enhance the food availability for feeding the burgeoning population in the country. Generally, there are three approaches being followed for the reclamation of these salt affected soils. Of these, the engineering solution is beyond the reach of resource poor farmers due to its prohibitive cost and community based application. The chemical amendment approach is generally followed by the farmers, even for which the subsidies are required to be provided by the Governmental agencies. Further, the smaller land holdings with the resource poor farmers also act as a deterrent in the adoption of these technologies. Thirdly, the biological reclamation approach, by developing salinity and alkalinity tolerant crops, is cost effective and is also economically feasible.

Vast literature is available on the effects of salinity on crop plants. Higher amount of salt reduces the productivity of many agricultural crops [2]; salt stress has three way effects that reduce water potential and cause ion toxicity and imbalances [3]. Salt stress affects other major processes such as germination, germination speed, root/shoot dry weight and the Na^+/K^+ in the root and shoot [4]. Hence, salt tolerance is important during the life cycle of any plants. Excessive salinity reduces the productivity of many agricultural crops [2], Salt stress has three fold effects which reduces water potential and causes ion imbalance and toxicity [3]. These studies have revealed the complex and polygenic nature of plant salt tolerance. Potential of genetic approach towards solving the problems of soil salinity and alkalinity is now widely recognized and this approach may more relevant to areas that often facing hard constraints of availability of resources. The existence of sufficient heritable variability may help for Genetic adaptation of crops to salinity which permits the identification and selection of salt tolerant strains and traits confer salt tolerance. Modern varieties have a relatively narrow genetic base and are poorly adapted to adverse environments such as salinity. However, endemic genotypes from problem environments may provide the basic germplasm for breeding salt tolerant varieties with acceptable yield potentials. Notwithstanding, the, genetic variations for salt tolerance among agricultural crops are very less, because most of the cultivated genotypes have been selected from normal environment where salt tolerant traits must have been gradually discarded, however variability for salt

Figure 1.
View of natural salt affected soil.

tolerance are similar in many wild progenitors due to where natural selection in response to salty habitats.

Large amount of variability is present amongst different crops with respect to their behavior under salt stress and this has also been documented. Further, variability is also available within a particular crop for their performance under varying rootzone salinity. The availability of such kind of variability in a crop is an essential requirement for the improvement in its salt tolerance character besides retaining or incorporating the other desirable beneficial characters. Further, the pool of variability in any crop can also be enhanced by subjecting them to mutagenic agents, which can further be screened for the desired characters. Screening whole plants and the large amount of germplasm available for a particular crop for salinity tolerance in the field situations is really time consuming, labour intensive and herculean task. Keeping these factors in view, rapid screening methodologies have also been developed for screening large number of germplasm for salinity tolerance under solution culture in laboratory conditions. When plant breeders are faced with a task of breeding crop varieties which are to be used under specific problem conditions, the criteria of selection is essential to any advancement which may be possible. In case of salt resistance, it would seem that it is essential to work hand to hand with the plant physiologists and soil scientists, in conditions which would make reliable selection possible and to determine if parameters can be developed which can make selection possible and effective. Further, without a concerted and concentrated research effort, problem such as breeding for salt tolerance cannot be effectively pursued.

Brassicaceae are an important group of edible oil and vegetable plants of the Brassicaceae family. The group has six cultivated species, namely, *Brassica campestris*, *Brassica nigra* and *Brassica oleracea* are diploid; *Brassica juncea*, *Brassica napus* and *Brassica carinata* are oligo-tetraploids (**Figure 2**).

Brassicaceae is the third most important edible oil source in the world, after soybean and palm, grown in more than 50 countries around the world. China, Canada, India, Germany, France, UK, Australia, Poland and the USA are the major producers of various varieties of Brassica. Globally, India accounts for 21.7% and production area 10.7% [1]. In India, oil-seed Brassicaceae are cultivated at about 2.3 million ha salt affected fields out of 6.9 million total cultivated area, which fall under the arid region, affected by varying levels of saline soils [5]. *B. rapa*, *B. napus* and *B. juncea* are mainly grown for oil and seed meal. The most serious effects of salt stress in Brassicaceae are a decrease in crop height, size and yield and product quality [6]. Salt stress has significantly affected the lipid components of mustard seeds. With the increase in salt, the total amount of neutral lipids decreased significantly, while phosphor-lipids and glycol-lipids increased. The fatty acid profiles of whole, neutral and polar lipid fractions are severely affected. The dry weight of the plant decreased significantly in high salt levels (ECe 8 to 12 dS/m) and the maximum weight was observed in ECe 4 dS/m [7]. Brassica varieties showed a lower percentage of oil content in seeds under saline soil conditions (ECe = 13 dS/m and SAR = 12.70). It may be due to excessive absorption of toxins ions that interfere with metabolic processes.

In addition, unhealthy nutritional imbalances due to stress-induced nutrient uptake; depletion in the germination, chlorophyll and mineral ions slow down seed growth and early crop maturity under high salt intake can be attributed to reduced oil content [8, 9]. Further the salinity also significantly reduced net photosynthesis, stomatal conductance, water use efficiency and transpiration under during the formation of siliqua results in the greater yield loss [10].

Higher salt (EC > 12 dS/m); decreased the oil, protein and crude fiber content by 5–7%, 15–20% and 29–34% respectively, while the content of erucic acid increased by 12–17% [5]. However, its growth and productivity are greatly reduced by salt.

Figure 2.
The Triangle of U diagram shows the genetic relationship between the six species of the genus Brassica. Three of the Brassica species were derived from three ancestral genomes, denoted by the letters AA (campestris), BB (nigra) and CC (oleracea). Alone each of these diploid genomes produced a common Brassica species.

This situation can be mitigated in a way that includes water conservation and irrigation, crop management and crop production. There is a great deal of interest in the breeding stress-tolerant species, because significant genetic variations for salt tolerance exist between and within Brassica, which requires being exploited by selection and breeding. However, programs to develop salt tolerance species are hampered by traits complexity, inadequate genetic and physiological knowledge of tolerance-related factors, and a lack of an effective selection background. Improved mustard varieties with high salt tolerance and consumer accepted oil quality are required to achieve high yields and to increase the cultivated area under this stressful environment.

2. Development of salt tolerance in Brassicas

2.1 Germplasm characterization: right way to screen for salt tolerance

Salt tolerance is a complex characteristics that you can learn for the following reasons: (a) salt tolerance can only be tested under stressful conditions, which can affect many plant responses; (b) salt tolerance is a quantitative factor that requires effective and efficient methods of quantifying tolerance levels; (c) “salt” in “salt stress” is often misunderstood as it may contain different mineral salts, such as NaCl, MgCl₂, and CaCl₂; without excessive use of NaCl in salt, we cannot ignore the damage due to other ions; and (d) other abiotic stresses like drought, excess acidity and alkalinity, are often associated with salt exposed plants, making this difficult to study. Therefore, effective and efficient methods should be used, including plant culture under salt conditions, characterization and quantification of a salt tolerance level, in the first phase of the study.

Plants that grow under certain controlled conditions (e.g. hydroponics) are often used for salt tolerance studies because there is very little natural saline soil that can provide a representative and stable environment [11]. Large pots under the controlled conditions (Microplots/hydroponics) required for growth of Brassica plants and seedlings, while very less experiments for yield evaluation have been conducted in salt affected land. It is noteworthy that the salt tolerance of the Brassicas may be determined by a variety of genes, expressed by salt tolerance responses at various stages of development [12–15].

2.2 Control of salt stress environments

Diversification of locations, maximization of replications and monitoring of the environmental conditions during crop growth often provide a good control over the factors responsible for performance of a genotype or a set of genotypes. At ICAR-Central Soil Salinity Research Institute (ICAR-CSSRI), for large scale screening of varieties at germination and seedling stage, shallow-depth germination trays provided with a polythene sheet lining on the inner face are being used. They allow a simulation of germination response of the field nature, giving not only a quantitative indication of relative germination and survival rates but also the relative delays in germination, which is a characteristic of the different genotypes under salinity as well as sodicity stress. Apart from this, microplots of various sizes were constructed at the Institute filled with artificially prepared saline soil or original salty soil brought from salt affected fields, so that soil is uniform all through the profile. This way desired level of sodicity and salinity in these microplots can be maintained uniformly. Data obtained from microplots containing desired levels of saline or alkali soils, have been found to be well correlated with those collected from satisfactorily conducted field experiments. The field gradient of soil salinity is determined by soil tests at small intervals of space and a long strip running full length across the salinity/ sodicity gradient is allotted to each genotype. Further, irrigation with saline waters of predetermined composition is also practiced to establish desired soil salinity levels particularly when relative sensitivity of different growth stages are sought to be compared.

The genotypes with good germination rates has shown a reduction in fresh and dry weight in the vegetable phase under salt stress than in poorly developed ones. Therefore, salt tolerance trials throughout the life cycle or in areas where salt is most sensitive, will be required to compare salt tolerance in different lines [16]. Methods of artificial salt stress, such as slow compression and shock of salt, can lead to results different from those of field testing [17]. The enforcement of salt stress by the gradual exposure to NaCl instead of salt shock has been recommended in genetic and molecular studies because it reflects natural phenomena of salt stress. However, the ideal type of gradual salt impose is technically difficult [18, 19]. Researchers are looking for a simpler or more accurate approach to predicting salt tolerance so that they can better select tolerant plant species or tolerant genotypes. The ability to accumulate photosynthates, proline and glycine-betaine, as well as ion precipitation can be used as a means of biochemical or physiological selection for salt tolerance in canola [20, 21]. The accumulation pattern for various salt overly sensitive (SOS) transcripts after 24 hours of salt stress in various cultivars showed a strong positive association with salt tolerance among Brassica species [22]. Cell membrane stiffness associated with antioxidant enzyme activities (superoxide dismutase, catalase and peroxidase) can be particularly effective in identifying canola with high salt tolerance. To date, no uniform index has been used to test salt tolerance [23].

2.3 Development of salt tolerant cultivars: conventional methods

Breeding salt tolerance in crop plants is considered one of the ways to combat the global problem of increasing soil salinity in agricultural land. Stresses under adverse soil conditions are very complex and are often associated with climate hazards. The salt stress varies from place to place even during the season. Soil salinity is often associated with unhealthy nutrient inequalities (deficiencies/toxins) and other problems and plants adapted different types of strategies to overcome on it (**Figure 3**).

The interaction between soil salinity and other environmental factors influences the plant’s response to that salt stress. Such problems are due to the slow evolution of plant species that thrive in adverse edaphic areas [24]. Therefore, it is necessary that the genetic material of plants should be tested in targeted areas with sufficient salt stress to find reliable sources of tolerance. Developing crop varieties with increased salt tolerance are considered to be the most promising, energy-saving and economical method than major engineering processes and soil rehabilitation techniques that have exceeded the limits of smallholder farmers [25].

2.3.1 The genetic basis of salt tolerance in Brassicaceae

Exploration of the heritable potential of a certain trait within the existing germ-plasm for a given crop would provide information on factors such as salt tolerance for plant breeders. The both additive and non-additive gene actions involved of in the inheritance of characteristics. High narrow-sense heritability estimates were observed for Ca^{2+} , K^+ , Na^+ , K^+/Na^+ , $\text{Ca}^{2+}/\text{Na}^+$ and stress tolerance index, indicating the prime importance of additive effects in their genetic control [26]. Higher estimates of GCV, PCV, heritability and genetic advance (% of mean) under saline condition was observed for main shoot length, number of pods on main shoot and yield per plot, indicated that these characters might be controlled by additive genes [27, 28]. Salt tolerance was mainly controlled by dominant genes with an additive

Figure 3.
Problems due to Salt stress and combating strategies in plants.

effect. The dominant effect played a major role and over-dominance might have existed in salt tolerance [29, 30]. The traits like main shoot length, number of pods on main shoot and yield per plot could be improved effectively by selection as these might be controlled by additive genes. Indian mustard, which was thought to be the moderately salt-tolerant species, also showed a decrease in shoot length and root length, electrolyte leakage, protein content, K^+/Na^+ ratio due to differential regulation of Na^+ in root and main stem by inhibition of entry from roots to shoot and retain higher photosynthetic characteristics than other species [10]. The fencing of selection processes should therefore be based on such indicators as a priority in the development of the most productive varieties of Indian mustard for saline condition.

In an effective breeding program, the discovery of a large variety of potential variants in a plant's genetic pool is a prerequisite; such genepools are needed to provide the required genetic diversity. Genetic diversity provides parental material from well-adapted landraces to enhance local adaptation. It helps to overcome the tendency to find a problem in the soil and provides a basis for fulfilling the needs of the novels. The conventional methods of improving plant salt tolerance generally employ selection for seed yield and there are few examples of producing salt tolerant varieties following these approaches at ICAR-CSSRI. These varieties are extremely popular with the farmers and their certified seeds are in great demand. The areas under their cultivation is fast expanding and increasing every year. The adoption of these varieties by the farmers has helped in great deal to enhance their economic status.

2.3.2 Bulk method

Using this methods of breeding researchers at the ICAR-Central Soil Salinity Research Institute (ICAR-CSSRI), Karnal has developed five cultivars of salt-tolerant Indian Mustard (*Brassica juncea*); CS 52, CS 54, CS 56, CS 58 and CS 60 (**Table 1**).

In this method, space planting of F_1 was done and harvested in bulk, while the planting of F_2 to F_6 generations done at commercial seed rate and spacing and harvested in bulk (**Figure 4**). The size of population in each generation was about 30,000 plants. These were space planted in the F_7 generation, and, only 5000 plants with desired characters confers to salt tolerance under salinity (EC_e 12.0 dS/m) and sodicity (pH 9) conditions were selected. Seeds of these selected plants were separately harvested. Individual plant progenies were grown in multi-row plots. Weak and inferior progenies were rejected and only 300 individual homozygous plant progenies with desirable traits were selected and harvested in bulk. A preliminary yield trial was conducted for two years for agronomic traits and resistance/tolerance to disease and mustard aphid infestation, along with the national check varieties. Replicated yield trials were conducted for three years under saline and alkaline conditions in salt-affected soils [30].

2.4 Development of salt tolerant cultivars: non-conventional methods

If genetic diversity is fully utilized by continuous selection, then diversity may be sought through alternatives such as chemical and radiation, protoplast fusion, or recombinant DNA techniques. Different laboratories are undertaking studies on elucidating salt tolerance mechanisms following molecular and biotechnological approaches. Efforts for the sequencing of *Brassica juncea* genome is underway at different locations although a draft sequence has been published but a clear understanding of the agriculturally important traits is lacking. In the meantime, we are

Parameter/Variety	CS 52	CS 54	CS 56	CS 58	CS 60
Year of development	1997	2005	2008	2017	2018
Plant height (cm)	170–175	160–170	198–202	180–185	182–187
Maturity duration (days)	130–135	121–125	132–135	130–135	125–132
Seed type	Medium	Bold	Medium	Bold	Bold
1000-seed weight (g)	4.5–5.0	5.0–5.5	4.5–5.0	5.0–5.5	5.0–5.2
Salinity tolerance (ECe dS/m)	6–9	6–9	6–9	6–11	6–12
Sodicity tolerance (pH)	8.5–9.3	8.5–9.3	8.5–9.3	8.5–9.4	8.5–9.5
Yield in non stress(t/ha)	1.8–2.0	2.0–2.4	2.2–2.6	2.6–2.8	2.5–2.9
Yield in salt stress(t/ha)	1.5–1.6	1.6–1.9	1.6–1.9	2.0–2.2	2.0–2.2
Oil Content	37–38%	38–39%	38–39%	39–40%	40–41%
Time of sowing	Upto 15th October	Upto 15th October	Upto 15th November	Upto 25th October	Upto 25th October
Recommended ecology	Salt affected Areas				

Table 1.
Salinity tolerant cultivars of Brassica species developed through conventional breeding.

Figure 4.
Development of salt tolerant Indian mustard variety CS 60 (a) Bulk Method; (b) Genotype CS 60 under saline field (ECe 15 dS/m).

suggesting some studies that would help in further evaluating mustard germplasm for these traits through molecular techniques and will providing basis for development of salt tolerant brassica through non-conventional methods.

2.4.1 The molecular basis of salt tolerance in Brassicaceae

More recently, research into salt tolerance in plants has shifted from genetic mapping to molecular characterization of salt responsive genes. Increased understanding of biochemical pathways and mechanisms that involved in plant stress response has made it clear that many of these methods are common defense mechanisms that can be used by salt, drought and cold, although sometimes alternatives

signaling pathways may be used. The molecular mechanism of salt tolerance expressed in model plants will facilitate the identification of target genes and the development of transgenic salt-tolerant plants in Brassica plants (**Figure 5**). Overexpression of antiporters (SOS1, SOS2, SOS3, ENH and NHX) as well as antioxidant genes (MPK1, DHAR3, APX1, APX4 and MDHAR6) in mustard play an important role in reducing the effects of salt and enhance salt tolerance [10].

The SOS pathway consists of the plasma membrane Na^+/H^+ antiporter SOS1, the protein kinase SOS2, and the Ca^{2+} binding protein SOS3. An increase of Na^+ concentration elevate the intracellular Ca^{2+} , and SOS3 binds Ca^{2+} and activates SOS2 to form a compound that phosphorylates membrane-derived plasma SOS1. Finally, over-expression of SOS1 leads to Na^+ efflux overhead [31]. In addition, AtHKT1 is involved in the recirculation of Na^+ from shoots to roots, possibly by promoting Na^+ movement into phloems in shoots and translocation into roots. The role of AtNHX1 in salt tolerance through increased Na^+ compartmentation in the vacuoles [32–35]. SOS1 and SOS3 are constitutively expressed in Brassica plants, while the pattern of SOS2 expression amongst *Brassica* species was found to be very unique. The expression of SOS2 may be elevated by salinity stress in the roots of all the *Brassica* species except for *B. juncea*, which maintains high SOS2 transcripts even under non-stress conditions, indicating a very unique feature of *B. juncea* [22]. Strong correlation between transcript abundance for SOS pathway related genes and salinity stress tolerance was observed in *Brassica* crops [36]. Currently, transgenic plants have been used to test the effect of overexpression of certain plant genes, which are known to be controlled by salt stress. Efforts have been made to increase transgenic Brassica with genetic predisposition, using genes that have a proven role in ion homeostasis, accumulating osmolytes, etc., to make them more tolerant of salt stress.

Transgenic *B. rapa* spp. chinensis plants that express the gene for choline oxidase (codA) from *Arthrobacter globiformis* have shown significantly higher net photosynthesis under high salinity conditions than wild-type plants [37]. The deception of these genes can help reduce the effects of ionic toxicity and cellular homeostasis as well as the conditioning of photosynthetic traits that lead to a promising yield under salt stress. Therefore, with the genetic improvement of agro-morphological characteristics of salt tolerance in Indian mustard, researchers should pay close

Figure 5.
The existence of a more efficient salt scavenging system composed of ionic module (SOS1, SOS2, SOS3, ENH and NHX) and oxidative module (MPK1, DHAR3, APX1, APX4 and MDHAR6) in the salt tolerant mustard.

attention to the photosynthetic attributes and pyramiding of antiporters and antioxidant genes for high economic productivity under salt stress. The overexpressing LEA4-1 plays an important role in the salt tolerance at vegetative stage in *B. napus* while BnLEA4-1 increase tolerance to salt stress in *Arabidopsis* [38]. Similarly Glutathione (GSH) and γ -ECS (Glutamylcysteine synthetase) gene from *B. juncea* (BrECS) plays an important role in cell function and metabolism as an antioxidant and provides plants with improved salt tolerance by maintaining the cellular nature of GSH redox to avoid attacks from salinity-derived reactive oxygen species [39].

2.4.2 Quantitative trait loci (QTLs) for salt tolerance

The QTL mapping is the best way to identify the underlying genes, though it is difficult and time-consuming. Creating an association map, which uses the highest number of historical recombination events/relics that occur throughout the evolutionary process of mapping population, enables genetic engineering in small genomic regions [40]. Exciting results have been obtained from independent studies on salt tolerance in the Brassicaceae, particularly in *Arabidopsis*. Most of the identified QTLs that control salt tolerance were different from each other, because the difference in mapping populations and the features under investigation. Normal QTL for germination percentage was detected at 20 cM in chromosome 1 associated with the RAS1 gene, a poor salt-tolerant controller during seed germination and early growth [41]. Another QTL found at 50 cM in chromosome 4 of the candidate AT4G19030 gene [42], whose level of expression reduced by ABA and NaCl [43]. These results suggest a complex genetic network regulating salt tolerance with differential genetic determinants in different accessions. Other QTLs of various traits are embedded: for example, salt responses and root-length QTLs on chromosomes 1 and 3, indicating that these two loci may contain gene-regulating salt tolerance expressed by root growth. However, genome-wide association studies with larger samples are considered to be more reliable and highly productive.

However, studies on QTLs or genes that regulate salt tolerance in Brassica plants are still very limited. To date, the practice of breeding salt tolerance in Brassica has been unsuccessful due to the unavailability of the polymorphic and cross transferability markers and highly salt sensitive lines. Concerns have resulted in a comprehensive breeding program for the development of high-yielding salt-tolerant mustard at the ICAR-Central Soil Salinity Research Institute (ICAR-CSSRI), Karnal and also leading to the changing salt tolerance paths of Brassica juncea by mutation results in the development of highly salt sensitive mutant CS 614-1-1-100-13 and CS 245-2-80-7 that are being used in recombinant inbred lines for mapping of QTLs. Researchers and farmers are trying to understand the salt-tolerance mechanisms and the screen for stable salt-tolerant genotypes to be used in the breeding programs. Efforts have also been made to develop salt-tolerant Brassica transgenic plants with a gene-specific role in ion homeostasis and osmolyte accumulation [44].

3. Predicted model for deciphering salinity tolerance mechanism in Indian mustard

Based on our findings on we have developed a model for the salt tolerance mechanism in Indian mustard (**Figure 6**) and conditioning the differential functions of antiporter and antioxidant transcripts in the mitigation of detrimental effect of salt stress [45]. Model suggested the three-way effect of salt stress on mustard plants; (i) Decreasing stomatal conductance results in the decreased intercellular CO₂ which caused diminishing activities of photosynthetic enzymatic machinery and

Figure 6.
A predicted model for the salt tolerance mechanism in Indian mustard.

decline in net photosynthesis rate. (ii) Production of reactive oxygen species (ROS) which disrupt the membrane system and limited the carboxylation process results in the least photosynthesis. (iii) Imbalance in the cellular ionic concentrations due to increased uptake of Na^+ and decreased K^+ uptake which caused ion toxicity. This ion toxicity leads to decrease in leaf area and early leaf fall down and limited carboxylation results in declined photosynthesis rate. The salt tolerant mustard genotypes counteract on these toxic paths by activation of antioxidant gene network for ROS scavenging and antiporter gene complex that enhanced sequestration of Na^+ in roots and reduced toxic Na^+ transport to shoots, hence, makes mustard plant tolerant to salt stress.

4. The conclusion

Modern agriculture certainly requires commercial crops that tolerate salt for the purpose of crop trade. Genetic adaptation of crops to salinity requires that sufficient heritable variability exists within species to permit selection of salt tolerant strains and that those plant characteristics that confer salt tolerance be identified. Modern varieties have a relatively narrow genetic base and are poorly adapted to adverse environments such as salinity. However, endemic genotypes from problem environments may provide the basic germplasm for breeding salt tolerant varieties with acceptable yield potentials. Notwithstanding, the, genetic variations for salt tolerance among agricultural crops are very less, because most of the cultivated genotypes have been selected from normal environment where salt tolerant traits must have been gradually discarded, however variability for salt tolerance are similar in many wild progenitors due to where natural selection in response to salty

habitats. Recent in-depth studies have identified various pathways at physiological and cell levels in which wild plants respond to salt stress. Due to the close relationship and significant variability between and within the Brassica species show great potential for breeding salt tolerance in Brassica plants. However, it is clear that to connect the salt tolerance factor and the QTL site to the chromosome, a proper breeding system assisted by markers is a prerequisite.

Conflict of interest

All the authors declare that they have no conflict of interest.

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