PLANT STRESS PHYSIOLOGY

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Salt Tolerance in *Brassicas*: Present Status and Future Thrust Areas

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INTRODUCTION

Among the multitude of abiotic stresses, salinity stress brings about severe growth retardation and yield loss of different crops, which needs to be addressed and answered urgently. In arid and semi arid lands, the plants are subjected to different stresses throughout their life cycle; some of these plants can tolerate these stresses in different ways depending upon plant species and type of stress. Excessive salinity reduces the productivity of many agricultural crops. Around the globe 932.2 million hectare area is affected with salinity and sodicity stresses (Metternicht and Zinck, 2003), out of which, nearly 6.73 million hectare area is affected by these stresses in India. Further, the arid and semiarid areas in different states are also associated with saline underground water, which have to be used for irrigation, due to unavailability or diversion of good quality water to other than agricultural purpose. Use of such waters for irrigation further renders the soils unfit for crop cultivation. Salt stress has three fold effects which reduces water potential and causes ion imbalance and toxicity (Flowers *et al.,* 1977).

Salt stress affects some major processes such as germination, speed of germination, root/shoot dry weight and Na^+/K^+ ratio in root and shoot (Sharma, 2003; Javid *et al.,* 2012). Plants also respond differently to salinity stress at different growth stages, which makes this an even more challenging issue. The *Brassicas* commonly known as rapeseed-mustard are important group of edible oils and vegetables crops belonging to *Brassicaceae* or *Cruciferous* family. This group comprises of six cultivated species, namely, *Brassica campestris/rapa* ($2n$= 20, AA), *Brassica nigra* ($2n$= 16, BB) and *Brassica oleracea* ($2n$= 18, CC) are diploids; *Brassica juncea* ($2n$= 36, AABB), *Brassica napus* ($2n$= 38, AACC) and *Brassica carinata* ($2n$= 34, BBCC) are digenomic tetraploids, which were evolved in

nature following hybridization between the constituent diploid species (Fig. 1). *Brassicas* are the third most important edible oil source in the world, after soybean and palm and is grown in more than 50 countries across the globe. China, Canada, India, Germany, France, UK, Australia, Poland and USA are the major cultivators of its different species of *Brassica*. During 2013-14, the estimated area, production and yield of rapeseed mustard in the world was 34.19 mha, 63.09 mt and 1.85 tones/ha, respectively (ICAR-DRMR 2016).

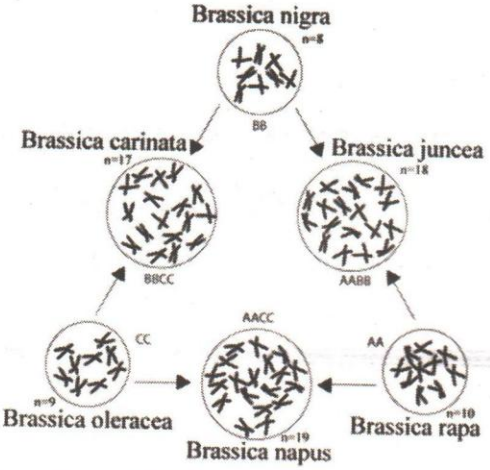

Fig. 1: The Triangle of U diagram showing genetic relationship between six species of the genus *Brassica*. Three of the *Brassica* species were derived from three ancestral genomes, denoted by the letters AA (*rapa*), BB (*nigra*) or CC (*oleracea*).

Globally, India account for 21.7% area and 10.7% production (FAO 2013) of rapeseed mustard. In India, oilseed *Brassicas* are being cultivated in around 2 million ha out of the 6.73 million ha total salt affected soils, which comes under semi-arid region and affected with varying degrees of soil salinity (Singh *et al.*, 2014). *Brassica* species *B. rapa, B. napus* and *B. juncea* are grown predominantly for oil and seed meal. India is the second largest country in rapeseed mustard production and more than 85% of its area under rapeseed mustard is occupied by Indian mustard *B. juncea* alone. The most common adverse effects of salinity on *Brassica* are the reduction in plant height, size and yield as well as deterioration of the product quality (Sharma, 2003, Zamani *et al.*, 2010; Javid *et al.*, 2012). Soil salinity also affects the lipid components of the seeds of Indian mustard (*Brassica juncea*). Under increasing salt levels, total and neutral lipids declined considerably, while phospholipids and glycolipids increased. The fatty acid profiles of total, neutral and polar lipid fractions were affected substantially. Erucic acid in total and neutral lipids decreased, while it was absent in the polar lipid fraction. In total and neutral lipids, oleic and linoleic acids increased. The amounts of linoleic and linolenic acids in the polar lipid fraction increased with rising salinity. Plant dry weight drastically declined at higher salinity levels (ECe 9 and 12) whereas the maximum weight was observed at ECe 6 (Sharma *et al.*, 2003). *Brassica* cultivars showed comparatively lower percentages of oil content in seeds

the final validation for salt tolerance potential is always carried out under natural conditions in salty land.

Researchers are seeking an easy method or an ideal trait value to forecast the salt tolerance to enable more efficient selection of tolerant crop types or tolerant genotypes. Photosynthetic capacity, proline and glycine-betaine accumulation ability, and ion discrimination can be used as potential biochemical or physiological selection criteria for salt tolerance in canola (Munir *et al.,* 2013; Ulfat *et al.,* 2007). The transcript accumulation pattern for various salt overly sensitive (SOS) members after 24 hrs. of salt stress in various cultivars showed a strong positive correlation with salt tolerance among *Brassica* species (Kumar *et al.,* 2009). Relative cell membrane permeability and activities of antioxidant enzymes (superoxide dismutase, catalase and peroxidase) could be very effective in identifying canola cultivars with high salt tolerance. So far, there are no uniform criteria applied to the assessment of salt tolerance (Ashrasf and Ali 2008).

Improving salt tolerance in crop plants following breeding and molecular approaches is envisaged as one way to combat the worldwide problem of increasing soil salinity in agricultural land. Stresses under adverse soil conditions are often compounded with climatic hazards. Seasonal as well as location specific variations are also observed in different stresses. Soil stresses are often associated with nutritional imbalance (deficiency/ toxicity). The various problems due to high soil salinity and strategies adapted by plants to overcome these situations are depicted below (Fig. 2).

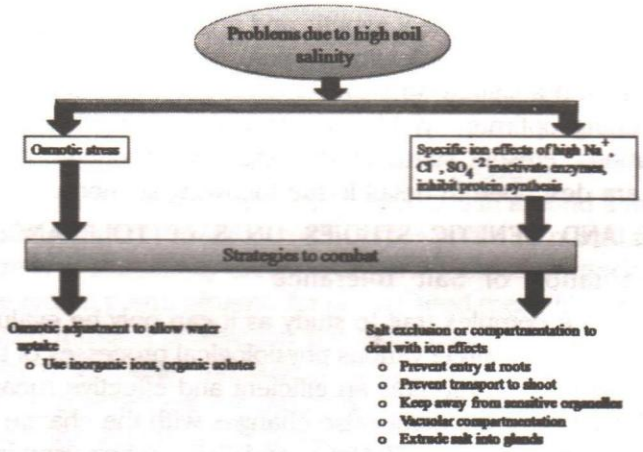

Fig. 2: Problems due to Salt stress and combating strategies in plants.

The interaction between soil stresses and other environmental factors influence the plant's response to that stress. Such complexities are responsible for the slow adaptability of high yielding crop varieties in adverse edaphic environments. It is, therefore, necessary that crop genotypes must be screened at target sites having adequate stresses in order to identify dependable sources of varietal tolerance. Breeding crop varieties for increased salt tolerance is considered as an economical approach compared to major engineering and soil amelioration approaches that are costlier to the marginal farmers. For a successful breeding program, presence of a great magnitude of heritable variation in the gene pool

of a crop is a prerequisite; such gene pools are necessary to provide the variability needed. Genetic diversity provides parental material from well-adapted landraces to enhance local adaptation. It helps to overcome susceptibilities to problem soil and also provides the foundation for breeding for novel requirements. If the genetic variability has been totally utilized through continued selection, then variability may be sought through other means such as chemical and radiation mutagens, protoplast fusion, or recombinant DNA techniques.

Further, the pool of variability in a 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. There is meager information available on salt tolerance of Indian mustard at germination and seedling emergence stages under different kinds of stresses. Different genotypes of Indian mustard were shown to differ in their tolerance to soil salinity and alkalinity at seed germination and seedling growth (Sharma and Gill 1995, Gill and Sharma 1999) and also at whole plant level (Sharma and Gill 1994, Sharma 2003).

Germplasm Evaluation in Salt Stress Environments

In general, selection for salt tolerance is made by evaluating large number of germplasm lines in germination trays, pots, microplots and field situations. 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. Further, earthern, glazed or plastic pots of different capacities, filled with coarse sand or representative soil are used to evaluate germplasm lines at different growth stages. Microplots pertains to a series of dug-out cavity structure made of brick-mortar-concrete materials measuring 2x2m, or 6x3m with a depth of about 0.8m, each filled with artificially prepared soil or original soil of different grades brought from salt affected fields, so that soil is uniform all through the profile. It is possible to create and maintain desired levels of sodicity and salinity in these microplots in a manner very much comparable to field conditions minus the soil heterogeneity. The plot size in these microplots is kept only one row. Data obtained from microplots containing desired grades 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. The plots generally measured 2 to 3 rows of each variety, 20-30m long. A set of check varieties representing resistant and susceptible types, replicated many times along with genotypes to be evaluated, to take note of general growth conditions. 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.

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 research effort, problem such as breeding for salt tolerance cannot be effectively pursued. The conventional methods of improving plant salt tolerance generally employ selection for seed yield following pedigree method. The advancement of generations were made following pedigree selection simultaneously in moderate stress and high stress sodicity and salinity environments, known as 'Parallel Pedigree Method' for the development of salt-tolerant varieties in problem soils. Backcross breeding has been used to induce salt tolerance in the prevailing genotypes. There are few examples of producing salt tolerant varieties following these approaches at CSSRI. With the sustained breeding efforts over the years, different salt tolerant high yielding varieties have been developed in the country with CSSRI as the major contributor. Besides seven salt tolerant varieties in rice, four in wheat, three salt tolerant varieties have also been developed in Indian mustard. 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 of Haryana and Uttar Pradesh has helped in great deal to enhance their economic status.

Rapid Screening Methodology

An appropriate and reliable methodology was developed for screening of large number of mustard genotypes for salt tolerance during germination and seedling emergence growth stages under laboratory conditions (Sinha *et al.,* 2003). Different mustard genotypes were evaluated under solution, sand and soil culture to arrive at a consensus salinity level in solution culture, which is a true representative of soil salinity conditions in field. Seedling emergence in solution, sand and soil cultures were observed to be significantly correlated. Significant positive correlation (r=0.92) was recorded between seedling emergence at 26 dS m^{-1} in solution culture and 12.8 dS m^{-1} in soil culture. In conclusion, screening of Indian mustard genotypes for salt tolerance at seed germination and seedling emergence stages can be done rapidly in solution culture in laboratory. This will help in accelerating the progress towards improvement of salt tolerance in Indian mustard.

Evaluation of *Brassica* Species and their Genotypes under Salinity and Alkalinity Stress

There are six major species of *Brassica* grown in different parts of the world. Amongst these, three are diploid, *B. compestris* (2n=20, AA), *B. nigra* (2n=16, BB) and *B. oleracea* (2n=18, CC), whereas the other three species are amphidiploids i.e. *B. juncea* (2n=36, AABB), *B. napus* (2n=38, AACC) and *B. carinata* (2n=34, BBCC). Amongst these six species of *Brassica* genus, evaluated for their performance and ionic accumulation under salinity, *B. juncea* genotypes recorded maximum mean seed yield and accumulated minimum mean Na. *B. nigra* genotypes were recorded to be sensitive to salinity stress. Significantly

higher shoot and root weight besides higher seed yield was demonstrated in amphidiplods compared to diploids under salinity stress (Ashraf *et al.,* 2001). The amphidiploids accumulated lower Na^+ and higher K^+ in shoots and roots than that of diploids. They have assessed that salt tolerance have likely come from A and C genome. Kumar *et al.* (2009) have also evaluated the performance of six *Brassica* species under salinity stress and shown *Brassica juncea* to be more tolerant compared to other *Brassica* species.

Evaluation of 158 genotypes, collected from different sources, under a range of salinity stress conditions upto 22 dS/m, in mixed salt solution containing NaCl, $CaCl_2$ and Na_2SO_4 in Hoagland solution, showed decline in seed germination by 51 and 82% at EC 18 and 22 dS m^{-1} respectively. Significant differences were observed amongst different genotypes for seed germination under different salinity stress levels. The regression equation for the pooled data of 158 genotypes was calculated to be $y = -3.8544\ x + 114.17$, ($R^2 = 0.87$) where y and x represent seed germination and salinity level respectively. Further, alkalinity stress showed higher yield reduction compared to salinity and also imposes additional stress when present in conjunction with mild salinity (Javid *et al.,* 2012). It was also demonstrated that alkaline salinity reduced uptake of essential nutrients and Na^+ exclusion that resulted on more deleterious effects on growth and development compared to salinity alone.

Stage Sensitivity

Maas (1986) has already documented salt tolerance of different crops but to a relatively constant salinity in the root zone. However, the exposure of plants to varying salinity levels at different growth stages would change the response of crop plants. Various experiments reveal that the salt tolerance observed during germination and emergence stages does not correlate with later growth stages. In Indian mustard, crop behavior to salinity stress changes as the crop matures. Indian mustard is sensitive at germination and seedling emergence stages whereas its tolerance to salinity stress increases at a later developmental stage. At EC 15.5 dS m^{-1}, seed yield declined by 84, 68 and 56% upon saline irrigation at germination, stem elongation and flower initiation growth stages (Gill and Sharma, 1999). Salinity level for 50% yield reduction (EC_{50}) will be 7.2, 10.5 and 7.1, when saline waters were used from germination stage, stem elongation and pod formation stages respectively, in Indian mustard.

Effects on Photosynthesis

Plant growth measured as biomass production is in reality the integration of net photosynthesis over time; therefore factors limiting plant growth are also the factors that limit net photosynthesis. Plant growth is affected by salinity stress, which is a consequence of several physiological processes including photosynthesis. Short term effects of salinity imposition on photosynthesis were studied at 2, 24 and 120 hrs of salinity imposition. Though the water stress symptoms were observed immediately after the salinity imposition, yet the effects on transpiration rate (E), stomatal conductance (g_s), assimilation rate (P_N) and internal CO_2 were not observed even after 2 hrs. of salinity treatment. The deteriorating effects of salinity were observed at 24 hrs. of saline irrigation with respect to above mentioned parameters. Further, the decline in assimilation rate was also observed at 24 hrs after salinity imposition with an average decline of about 40-50% compared to control.

Even under high salinity, the plant tried to maintain its photosynthetic activity as the effects varied greatly with respect to different leaves. The assimilation rate declined drastically in lower leaves approximately upto position 5 from base where about 60-80% of the effect was observed. The upper leaves were still maintaining higher assimilation rate under salinity compared to lower leaves (Sharma, 2003). Further, the long term effects of salinity on the above mentioned parameters were studied in genotypes Varuna, CS 330, CS 609, ST 63 and CS 33, differing with respect to 1000 seed weight and grain yield under salinity and irrigated with saline water of EC 12 and 15 dS/m for 45 days. The plants generally adjusts to long term salinity application as evidenced by their lower transpiration rates (36 and 41%), stomatal conductance (45 and 59%) and in turn their effects on lowering the assimilation rates by 25 and 35% in leaves under 12 and 15 dS m^{-1}salinity respectively, compared to control. This results in reduced photosynthesis leading to reduction in grain yield under salinity.

Salt Tolerance at Whole Plant Level

Large numbers of germplasm lines developed at CSSRI and the released varieties of mustard were evaluated over a range of salinity stresses under sand culture, microplots and in field situations, over a period of time. The better performing lines under field situations were again tested for their salt tolerance potential under microplots and in pots under sand culture conditions. Different salinity levels were applied at germination stage and maintained throughout the experiment. It was shown that the better performance of a genotype was associated with higher shoot and root fresh and dry weight at seedling stage, minimum percentage reduction in grain yield under salinity, maximum mean susceptibility index values, more number of pods/plant at higher salinity levels, lower accumulation of Na in shoot and higher in root, higher K levels both in shoot and root and lower shoot Na/K ratio. Recently, Chakraborty *et al.* (2012) have also shown lower Na and higher K accumulation in leaves, stem and roots of salt tolerant varieties CS 52 and CS 54 compared to salt sensitive varieties Varuna and T 9 both at flowering and post flowering stages.

Using Conventional Breeding approaches researchers at Central Soil Salinity Research Institute (CSSRI), Karnal have generated three high yielding salt tolerant varieties of Indian mustard (*Brassica juncea*); CS 52, CS 54 and CS 56 (Table 1).

Assessment of salt tolerance has also been performed in *Brassica* and its closely related species. There is significant interspecific and intraspecific variation for salt tolerance. Comparing salt tolerance of amphidiploids and diploid *Brassica* species under salt stress revealed that shoot and root weights and seed yield of the three amphidiploid species were significantly greater than those of their ancestral diploids. It could be suggested that the salt tolerance of these species originated from A and C genomes (Ashraf *et al.,* 2001; Nazir *et al.,* 2001). Variation of salt tolerance within six common *Brassica* species plus *Eruca sativa* and *Brassica tournefortii* was explored by assessing the morphological, physiological and biochemical parameters. *B. juncea*, which had been thought to be the most salt-tolerant species, showed the least decrease of shoot length and root length, and lesser electrolyte leakage, higher proline content and higher K^+/Na^+ ratio than the other species (Kumar *et al.,* 2009).

TABLE 1

Salinity tolerant cultivars of *Brassica* species developed through conventional breeding

Parameter / Variety	CS 52	CS54	CS56
Year of release	1997	2005	2008
Parentage	Sel. from DIRA 343	B 380 X NDR 8603	RH 851 X Pusa Bold
Plant Height (cm)	170	160	202
Maturity duration (days)	135	121	132
Grain type	Medium	Bold	Medium
1000 seed weight (g)	4.0	5.3	4.4
Salinity tolerance (ECe dS/m)	6 - 9	6 - 9	6 - 9
Sodicity tolerance (pH)	9.3	9.3	9.3
Yield in Non stress(q/ha)	18-20	20-24	22-26
Yield in Salt stress(q/ha)	15-16	16-19	16-19
Time of sowing	Up to 15th October	Up to 15th October	Up to 15th November
Recommended States / Areas	Uttar Pradesh, Punjab, Haryana and Rajasthan		

Classical Genetics of Salt Tolerance in *Brassica*ceae

Exploration of the heritable potential of a certain trait within the existing germplasm for a given crop would supply information on factors such as salt tolerance for 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^+, Ca^{2+}/Na^+ and stress tolerance index, indicating the prime importance of additive effects in their genetic control (Rezai and Saeidi, 2005). 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 (Sinha *et al.,* 2002; Kumar and Mishra, 2006). Salt tolerance was mainly controlled by dominant genes with an additive effect. The dominant effect played a major role and over-dominance might have existed in salt tolerance (Qiu and Li, 2009; Long *et al.,* 2013). 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. Hence framing of selection criteria should be based on such traits on priority in the development of high yielding variety in Indian mustard for saline condition.

MOLECULAR BASIS OF SALT TOLERANCE IN *BRASSICA*CEAE

Molecular approaches comprise of marker assisted breeding and transgenic approaches. Marker assisted breeding include identification of salt tolerant and sensitive genotypes, their crossing and development of large number of recombinant inbred lines (RILs), which should be more than 250. Stringent phenotyping of these RILs under target environments for different characters followed by genotyping of these RILs with a large

The SOS pathway comprises the plasma membrane Na^+/H^+ antiporter SOS1, the protein kinase SOS2, and the Ca^{2+} binding protein SOS3. With the increase of Na^+ concentration at the cellular level, elevation of intracellular Ca^{2+} was recorded. Further, SOS3 binds Ca^{2+} and activates SOS2 to form a compound which phosphorylates the SOS1 localized on plasma membrane. Martinez-Atienza *et al.*, (2007) have demonstrated that over-expression of *SOS1* results in an efflux of more Na^+. *SOS1* and *SOS3* are constitutively expressed in *Brassica* crops, while the expression pattern for *SOS2* amongst *Brassica* species was found to be very unique (Kumar *et al.*, 2009). *SOS2* may be upregulated 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* (Kumar *et al.*, 2009). Strong correlation between transcript abundance for *SOS* pathway related genes and salinity stress tolerance was observed in *Brassica* crops (Chakraborty *et al.*, 2012; Kumar *et al.*, 2009).

TABLE 2

Salinity tolerant transgenic *Brassica* developed through genetic engineering

Species	Genes	Encoding protein	Phenotype	Reference
B. napus	*codA*	Choline oxidase	Moderate salinity tolerance and enhanced shoot growth	Huang *et al.*, 2000
	AtNHX1	Vacuolar Na^+/H^+ antiporter	Salt tolerance, growth, seed yield and seed oil quality	Zhang *et al.*, 2001
	PR10	Pathogenesis related (PR)	Enhances germination and growth in the presence of NaCl	Srivastava *et al.*, 2004
B. juncea	*codA*	Choline oxidase	Tolerance to salinity stress, enhanced growth	Prasad *et al.*, 2000
	PgNHX1	Vacuolar Na^+/H^+ antiporter	Tolerance to salinity stress, exhibited normal growth	Rajagopal *et al.*, 2007
B. oleracea	*betA*	Betaine aldehyde	Salinity tolerance	Bhattacharya *et al.*, 2004
B. campestris	*Lea*	Group3 Late embryo-genesis abundant	Salinity and drought tolerance	Park *et al.*, 2005

Currently, transgenic plants have been used to test the effect of over-expression of specific prokaryotic or plant genes, known to be up-regulated by salinity stress. Attempts have been made to raise transgenic *Brassica* with candidate gene approach only, making

use of the genes having proven role in ion homeostasis, osmolytes accumulation etc., to make them more tolerant to salinity stress (Table 2). Transgenic *B. rapa* spp. *chinensis* plants expressing a choline oxidase (*codA*) gene from *Arthrobacter globiformis* showed a significantly higher net photosynthetic rate and a higher photosynthetic rate under high salinity conditions than wild-type plants (Wang *et al.,* 2011). In addition, *AtHKT1* is involved in the recirculation of Na^+ from shoots to roots, presumably by promoting Na^+ movement into phloems in shoots and translocation into roots. The function of *AtNHX1* in salt tolerance through increased Na^+ compartmentation in the vacuoles (Berthomieu *et al.,* 2003; Apse *et al.,* 1999; Zhang and Blumwald 2001; Zhang *et al.,* 2001).

Dalal *et al.,* (2009) proved that *LEA4-1* plays a crucial role in salt stress tolerance during the vegetative stage of *B. napus* and that transgenic *Arabidopsis* plants over-expressing *BnLEA4-1* have enhanced tolerance to salt stress. Glutathione (GSH) plays an important role in cell function and metabolism as an antioxidant. Bae *et al.,* (2013) developed transgenic plants by introducing the ã-*ECS* (*Glutamylcysteine synthetase*) gene from *B. juncea* (*BrECS*) into rice. Over-expression of *BrECS* confers plants with significantly enhanced tolerance to salinity by sustaining a cellular GSH *redox* state to avoid attacks from reactive oxygen species produced by salt.

QTL Mapping of Salt Tolerance

Although QTL mapping remains the best method for identifying causal genes, it is quite laborious and time consuming. Association mapping, which utilizes a higher number of historical recombination events that have occurred throughout the entire evolutionary process of the population, enables mapping of the genes in smaller genomic regions (Nordborg and Tavaré, 2002).

Interesting results have been obtained by independent studies on salt tolerance in *Brassicaceae*, especially in *Arabidopsis*. Most of the mapped QTLs controlling the salt tolerance were different from each other, since the mapping populations were different and the investigated traits were not all the same. A common QTL for percent germination was detected at 20 cM on chromosome 1 which co-localized with the gene *RAS1*, a negative regulator of salt tolerance during seed germination and early growth (Ren *et al.,* 2010). Another QTL located at 50 cM on chromosome 4 for candidate gene AT4G19030 (Lee *et al.,* 2006), the expression level of which is reduced by ABA and NaCl, was predicted (DeRose-Wilson and Gaut, 2011). These results suggest a complicated genetic work controlling salt tolerance and that the genetic determinants are different in different accessions. Some QTLs for different traits were overlapped: for example, QTLs for root length and response on chromosome 1 and 3, indicating that these two loci may contain genes controlling root length and those for salt tolerance exhibited by root growth. However, genome-wide association studies with larger samples are considered to be more reliable and fruitful.

However, studies on QTLs or genes controlling salt tolerance in *Brassica* oil crops are still very limited. To date, the breeding practice of salt tolerance in *Brassica* crops has been largely unsuccessful due to non availability of salt sensitive line of *Brassica*. The concerns prompted an intensive breeding program to develop high yielding cultivars with

salinity tolerance at Central Soil Salinity Research Institute (CSSRI), Karnal which results in developed salt sensitive mutant lines of *Brassica juncea* CS 614-1-1-100-13 and CS 245-2-80-7 which are utilizing in development of recombinant inbred lines. Researchers and breeders endeavor to understand the mechanisms of salt tolerance and screen for stable salt-tolerant genotypes to use in breeding programs. Attempts have also been made to develop salt-tolerant transgenic *Brassica* crops with candidate genes with proven roles in ion homeostasis and osmolytes accumulation (Zhang *et al.,* 2004).

Characterisation of Transcriptome under Salinity Stress in *Brassica* Species

Brassica exhibits considerable intra and interspecific genetic variability for salt tolerance (Ashraf and McNeilly 2004; Purty *et al.,* 2008). Tolerant species of *Brassica* always displayed maximum accumulation of compatible solutes with minimum levels of H_2O_2, membrane injury and lipid peroxidation levels as compared to other species (Joshi *et al.,* 2011). SOS pathway has been proposed to be the major candidate responsible for salinity tolerance in this species, considering only few gene expression studies (Kumar *et al.,* 2009; Chakraborty *et al.,* 2012). However, salinity tolerance is a very complex trait regulated by several independent and/or interdependent pathways. Therefore, transcriptome study is highly imperative to investigate the changes in gene expression in response to salinity stress and understand the functional genetic basis of salinity tolerance in *Brassica* species.

Considering the large size of genome, partial sequence information and limited genetic resources available, exploiting genetic potential for genome-assisted breeding and trait improvement in *Brassica* species is greatly hampered.One of the very important species of *Brassica* ie., *B. juncea* is a natural amphidiploid (AABB, 2n = 36) of *B. rapa* (AA, 2n = 20) and *B. nigra* (BB, 2n = 16) with haploid (1X) genome size estimated to be 534 Mbp (Johnston *et al.,* 2005). Till now, the whole genome sequence of only *B. rapa* has been attempted (Wang *et al.,* 2011) with very limited sequence information available for *B. juncea* and *B. nigra* genomes in public databases. Recently RNA sequencing followed by de novo assembly has been adopted in *B. juncea* with the aim of deciphering global view of transcriptome and comparative expression analysis. Sun and coworkers used Illumina short-read technology with a tag-based digital gene expression system to study the molecular mechanism of stem swelling in the tumorous stem mustard *B. juncea* var. tumida Tsen et Lee (Sun *et al.,* 2012). Also, Liu and coworkers performed RNA sequencing to explore the molecular mechanism governing seed pigmentation in *B. juncea* (Liu *et al.,* 2013). Recently, SNP markers were developed for Varuna and Heera lines of *B. juncea* and synteny was analysed between two constituent genomes using RNA-sequencing data (Paritosh *et al.,* 2014). However, to the best of our knowledge, genome-wide analysis of gene expression in response to abiotic stresses has not been undertaken in any of the *Brassica* species.

Sharma *et al.,* (2015) reported transcriptome sequencing of *B. juncea* var. CS52 under normal growth conditions and in response to salinity stress. A thorough analysis of high expressing genes under control conditions and differentially expressed genes in response to salinity stress revealed key signaling components and pathways contributing to salinity tolerance. Real-time qPCR-based expression analysis of key abiotic stress-

responsive genes in two contrasting cultivars of *Brassica* (salt-tolerant, *B. juncea* and salt-sensitive, *B. nigra*) highlighted constitutive expression of most of them in the tolerant variety under normal growth conditions. Data generated by them is indeed a good platform for more focused investigations of salinity tolerance mechanisms in *Brassica* genotypes and initiating functional genomic studies for trait improvement.

RNA sequencing is a highly useful application of next generation sequencing technologies for annotating the whole genome and quantitative analysis of gene expression (Wang *et al.,* 2009). Sharma *et al.,* (2015) obtained over 200 million reads paving way for 53,669 predicted unigenes from RNA-seq libraries of CS52 variety of *B. juncea* (Indian mustard) seedlings under control conditions and in response to salinity stress. Only 47.4% of these were represented by *Brassica* Expressed Sequence Tags (ESTs) in public databases suggesting that 52.6% of these unigenes, were novel compared to EST sequence available for *B. juncea* and constituent genomes (*B. rapa* and *B. nigra*). *Brassica* species share very close genetic relationship with arabidopsis and whole genome of *B. rapa* has already been sequenced. So the annotation information available for *B. rapa* and Arabidopsis can be successfully used for annotation of the predicted genes. Using these resources and protein database at NCBI, Sharma *et al.,* (2015) annotated 67% of the predicted unigenes. The annotation of rest 33% unigenes remains unknown (Sharma *et al.,* 2015). Exploring this unknown region still remains a daunting task for researchers across the globe as valuable information might be residing in this part of the transcriptome.

Previous researchers have always observed a significant up regulation of several pathways including proline biosynthesis, calcium signaling, sulfur assimilation and ROS detoxification associated with salinity stress response (Sharma *et al.,* 2015). Sharma *et al.,* (2015) observed elevated expression of ABA biosynthetic enzyme coding gene, 9-cis-Epoxycarotenoid Dioxygenase 4 *(NCED4)* in response to salinity stress, whereas, the genes involved in carbon assimilation, carbohydrate metabolism and jasmonic acid biosynthesis were mainly down regulated most likelt as an acclimation response to divert resources to tolerate the salt stress and prevent photodamage (Sharma *et al.,* 2015).

Glutathione is another very important antioxidant molecule which acts as a sensor of redox and plays an important role in maintaining lower levels of ROS via the glutathione-ascorbate cycle (Gill and Tuteja, 2010). The glutathione-ascorbate cycle involves several antioxidant metabolites and enzymes. Superoxide dismutase (SOD) catalyze dismutation of toxic superoxide radical into oxygen or hydrogen peroxide (H_2O_2). Oxidation of ascorbate to monoaldehydroascorbate by ascorbate peroxidase scavenges H_2O_2. MDHA is either directly converted back to ASC by monodehydroascorbate reductase or converted to dehydroascorbate which is again converted to ASC by dehydroascorbate reductase. DHAR uses GSH which is regenerated from its oxidized form GSSG by action ofglutathione reductase (GR), glutathione peroxidase (GPX) or glutathione S-transferase (GST), leading to removal of ROS. Sharma *et al.,* (2015) observed significant induction of several ROS detoxification genes including SOD, GPX, DHAR, APX, MDHAR in response to salinity stress though their initial transcript levels in *B. juncea* were also much higher compared to those in salt-sensitive cultivar. They also observed up regulation of delta1-pyrroline-5-carboxylate synthetase (P5CS) gene that catalyzes the rate-limiting step in proline biosynthesis in

response to salinity stress. Many researchers have previously suggested constitutive expression of abiotic stress responsive genes in tolerant varieties as a critical factor contributing to stress tolerance (Taji *et al.,* 2004; Ruiz and Blumwald, 2002).

Sulfur assimilation pathway includes activation of sulfate to adenosine 5′-phosphosulfate (APS) by ATP sulfurylase (ATPS). Subsequently, APS is reduced to sulfite by APS reductase (APR). Sulfite is reduced to sulfide and incorporated into cysteine which is direct precursor for the synthesis of glutathione (GSH). Sharma *et al.,* 2015 observed significant increase in the transcript levels of both ATPS and APR genes in response to salinity stress. In fact, APR is among the highly expressed genes in *B. juncea* seedlings even in the absence of stress with expression of both genes much higher in tolerant cultivar compared to sensitive one (Sharma *et al.,* 2015). Earlier studies proposed that efficient Salt Overly Sensitive (*SOS*) pathway that comprises *SOS3, SOS2* and *SOS1* could be a major factor in determining salt tolerance in *Brassica* genotypes (Chakraborty *et al.,* 2012). Sharma *et al.,* (2015) did not observe significant change in their transcript levels in response to salinity stress. This might be due to the fact that *SOS* genes are mostly regulated at posttranslational level (Ma *et al.,* 2006). Enhancer of *SOS3* pathway, *ENH1*, displayed very high transcript levels in *B. juncea* in the absence of stress and was further up regulated in response to salinity stress.

Recently, a nuclear calcium sensing pathway comprising of a calcium binding protein, RSA1 (SHORT ROOT IN SALT MEDIUM 1) and a bHLH transcription factor, RITF1 has been shown to play critical role in stress tolerance in Arabidopsis (Guan *et al.,* 2013). The RSA1-RITF1 complex regulates expression of key genes involved in detoxification of ROS and maintaining Na^+ homeostasis in response to salt stress (Guan *et al.,* 2013). RITF1 was among the top 1% genes exhibiting 128 folds up regulation in response to stress in the study conducted by Sharma *et al.,* (2015) suggesting that it plays very important role in regulating gene expression in response to salinity stress and may serve as a molecular marker of stress tolerance. *RITF1* also regulates the expression of *SOS1* indicating a link in both the calcium signaling pathways. Ultimately, activation of antioxygenic enzymes seems to be the major reason for improved growth under salinity stress condition by preventing oxidative damage.

Altogether, it can be summarized that multiple pathways may be involved in salinity tolerance in *Brassica* species. The transcriptomic data generated will serve as a highly valuable resource to support genome analysis, develop expression arrays, molecular marker identification and, initiating functional and comparative genomic studies in *Brassica* species.

Proteome Dynamics to Salinity Stress in *Brassica* Species

NaCl is the major component of salinity (Golldack *et al.,* 2014) and exposure to high concentrations of NaCl can lead to various physiological alterations, including water deficit, ionic toxicity, nutritional disorders, stunted growth, photosynthesis and protein synthesis depression. excess reactive oxygen species (ROS) generation and oxidative stress (Yadav *et al.,* 2011; Barkla *et al.,* 2013; Shavrukov 2013; Tang *et al.,* 2014). Salinity stress induces extensive proteome alteration in crop plants and proteomic analysis has

proved to be a very efficient approach to study plant salt-stress tolerance (Tahir *et al.,* 2013; Sobhanian *et al.,* 2011). A database consisting of 2171 salt-responsive proteins was recently constructed based on proteomics studies from 34 plant species, which has immensely helped our understanding of the mechanisms underlying plant salt response and tolerance (Zhang *et al.,* 2012). Two-dimensional polyacrylamide gel electrophoresis (2-DE) still remains the fundamental approach in proteomic research and has been applied intensively in studies on abiotic stress response in crops (Razavizadeh *et al.,* 2009; Gao *et al.,* 2011; Ma *et al.,* 2012).

Interestingly, only limited proteomic studies on salinity stress response have been reported for *Brassica* species. A previous study on canola under salt stress detected 44 and 31 differentially accumulated proteins in leaf proteome of the salt-tolerant genotype Hyola 308 and salt-susceptible genotype Sarigol, respectively; 46 proteins were identified using mass spectrometry analysis (Bandehagh *et al.,* 2011). Recently, a proteomic analysis of seedling roots from Hyola 308 and Sarigol detected 20 and 21 proteins that responded to salt-stress treatments, respectively; However, only 19 proteins were identified (Ali Bandehagh *et al.,* 2013). The salinity stress induced proteome alterations and the roles of the salt-responsive proteins in salt stress adaptation remain unclear and require further focused investigation.

Recently Jia *et al.,* (2015) analyzed proteomic and physiological responses to salt stress in *Brassica napus.* Salt-responsive proteins were separated by 2-DE and identified by MS analysis. They identified approximately 800 protein spots on silver-stained 2-DE gels in the pH range of 4–7 under salt stress in *Brassica* leaves by the PDQuest software. Forty-four protein spots were observed to be reproducibly and significantly ($p < 0.05$) altered in abundance by more than two-fold in at least one time point after salt treatment (Table 3).

ATP synthase (chloroplast) was identified in seven spots and 60-kDa chaperonin was identified in three spots, whereas chloroplast ribulose-1, 5-bisphosphate carboxylase/ oxygenase activase, heat shock protein 70 and chitinase were identified in two spots. The identified salt-stress-responsive proteins were further divided into seven categories on the basis of their functions including Chlorophyll biosynthesis (2.38%), photosynthesis (28.57%), energy synthesis (19.05%), respiration (4.76%), protein metabolism (23.81%), and damage repair and defense response (21.43%).

Stress can impair photosynthesis indirectly by reducing chlorophyll content (Tang *et al.,* 2014). However, the impact of salinity on chlorophyll biosynthesis remains unclear. A recent study on rice seedlings suggested that down regulation of chlorophyll biosynthesis by salt stress could be attributed to decreased activities of chlorophyll biosynthetic pathway enzyme (Turan and Tripathi, 2015). Glutamate 1-semialdehyde aminotransferase (GSA-AT) is a key enzyme in plant chlorophyll synthesis (Grimm 1990), and GSA-AT antisense transformants showed varying degrees of chlorophyll deficiency (Hartel *et al.,* 1997; Tsang *et al.,* 2003). Jia *et al.,* (2015) observed that the abundance of GSA-AT decreased after 24 h of salt-stress treatment. However, the GSA-AT protein level recovered to control levels at 48 h and 72 h of NaCl treatment which might be attributed to salt tolerance mechanisms in *Brassica napus* seedlings. Consistent with this finding, the reduction in Chlorophyll content in salt-stressed seedlings was not significant after 72 h of treatment.

TABLE 3

Identification of differentially accumulated proteins under salt stress in leaves of *Brassica napus*. (Adapted from Jia *et al.*, 2015)

Accession no	Protein name	Species	Cellular location
chlorophyll biosynthesis			
28972461	glutamate 1-semialdehyde aminotransferase enzyme	*Brassica napus*	Chloroplast
photosynthesis			
262400776	photosystem I subunit VII	*Brassica napus*	Chloroplast
15228194	Sedoheptulose-1,7-bisphosphatase	*Arabidopsis thaliana*	Chloroplast
383470439	Chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase activase	*Brassica oleracea*	Chloroplast
15222551	phosphoribulokinase	*Arabidopsis thaliana*	Chloroplast
50313237	Lhcb6 protein	*Brassica rapa*	Chloroplast
22571	33 kDa oxygen-evolving protein	*Arabidopsis thaliana*	Chloroplast
31323256	chlorophyll a/b binding protein	*Brassica oleracea*	Chloroplast
1620920	23kD protein of oxygen evolving system of photosystem II	*Brassica juncea*	Chloroplast
383470439	chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase activase	*Brassica oleracea*	Chloroplast
15983513	transketolase-1		Chloroplast
406311	clpA	*Brassica napus*	Chloroplast
energy synthesis			
8745523	ATP synthase beta subunit	*Brassica napus*	Chloroplast
5708095	ATP synthase gamma chain, chloroplast precursor	*Arabidopsis thaliana*	Chloroplast
19554	F1-ATPase alpha subunit	*Brassica napus*	Mitochondrion
262400757	ATP synthase CF1 beta subunit	*Brassica napus*	Chloroplast
262400756	ATP synthase CF1 alpha subunit	*Brassica napus*	Chloroplast

Contd...

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Accession no	Protein name	Species	Cellular location
8745523	ATP synthase beta subunit	*Brassica napus*	Chloroplast
8745523	ATP synthase beta subunit	*Brassica napus*	Chloroplast
8745523	ATP synthase beta subunit	*Brassica napus*	Chloroplast
Respiration			
15241286	pyruvate dehydrogenase E1 beta	*Arabidopsis thaliana*	mitochondrion
433335660	malate dehydrogenase	*Brassica oleracea*	mitochondrion
Protein metabolism			
167138	cyclophilin, partial	*Brassica napus*	plasma membrane
289365	60-kDa chaperonin, partial	*Brassica napus*	mitochondrion
115447473	20S proteasome alpha subunit B	*Oryza sativa*	cytoplasm
134104	60 kDa chaperonin subunit beta	*Brassica napus*	chloroplast
134104	60 kDa chaperonin subunit beta	*Brassica napus*	chloroplast
397482	heat shock protein 70 cognate	*Arabidopsis thaliana*	nucleus
18400195	heat shock protein 60-3A	*Arabidopsis thaliana*	mitochondrion
2655420	heat shock cognate protein HSC70	*Brassica napus*	nucleus
532212746	elongation factor Tu	*Brassica rapa*	chloroplast
16221	chaperonin hsp60	*Arabidopsis thaliana*	cytoplasm
Damage repair and defense response			
312837924	Fe superoxide dismutase 1, partial	*Brassica rapa*	peroxisome
20067415	glutathione transferase	*Triticum aestivum*	peroxisome
22653413	dehydroascorbate reductase	*Brassica juncea*	mitochondrion
15239735	thiazole biosynthetic enzyme	*Arabidopsis thaliana*	chloroplast
15010596	FtSH2	*Arabidopsis thaliana*	chloroplast
19849246	cinnamyl alcohol dehydrogenase	*Lolium perenne*	cytoplasm
6048743	chitinase	*Brassica juncea*	cytoplasm
6048743	chitinase	*Brassica juncea*	cytoplasm
8885622	N-glyceraldehyde-2-phosphotransferase-like	*Arabidopsis thaliana*	cytoplasm

Photosynthesis is one among the key physiological process affected by salinity (Munns *et al.,* 2006; Chaves *et al.,* 2009). Proteomic analysis by Jia *et al.,* (2015) showed that 12 proteins related to photosynthesis were salinity responsive, which includes six photosynthetic proteins and six enzymes crucial for carbon assimilation (Table 3). Interestingly, most of the proteins involved in the light reaction, including photosystem I subunit VII, Lhcb6 protein, 33 kDa oxygen-evolving protein, Chl a/b binding protein and 23kD protein of oxygen evolving system of photosystem II, were upregulated in response to salt stress. However, the protein abundance of enzymes in CO_2 assimilation, including Ribulose bisphosphate carboxylase (RuBisCO) large chain, sedoheptulose-1, 7-bisphosphatase and phosphoribulokinase, was significantly decreased after salt-stress treatment (Jia *et al.,* 2015). Altogether it can be hypothesized that the salt-induced reduction in photosynthesis capacity could be mainly attributed to impaired CO_2 fixation in *Brassica* species. RuBisCO activase (RCA) protein was significantly upaccumulated in *Brassica* napus seedlings after salt stress treatment, especially at the 48 h time point (Jia *et al.,* 2015). RCA is a key regulatory enzyme that catalyzes the activation of RuBisCO (Ashraf and Harris 2013). RCA has been implicated in the maintenance of photosynthesis at low CO_2 levels because of the reduction in stomatal conductance caused by salinity (de Abreu *et al.,* 2014). Therefore, the up-accumulation of RCA might contribute to salt tolerance in *Brassica* species (Jia *et al.,* 2015).

Salinity stress negatively affect protein synthesis and disrupt protein folding in the endoplasmic reticulum, leading to prolific unfolding of proteins (Wang *et al.,* 2011). Proteomic analysis by Jia *et al.,* (2015) indicated that elongation factor Tu, which is involved in protein biosynthesis and chaperones (cyclophilin, 60 kDa chaperonin/ HSP60 and HSP70) related to protein folding, was significantly induced in salt-stressed leaves of *Brassica* species. By contrast, a protein associated with protein degradation (20S proteasome) was decreased by salinity stress treatment. Chaperonins play a major role in protein folding and assembly and may function to protect and repair vulnerable protein targets under stress conditions (Quireshi *et al.,* 2007; Fernandez-Garcia *et al.,* 2011). On the contrary, previous proteomic analysis of *Brassica napus* seedling roots identified chaperonin hsp60 as a down-regulated protein in response to salinity (Ali Bandehagh *et al.,* 2013). This may be due to the use of varying plant genotypes or different tissues. This clearly depicts the role of proteins related to protein metabolism in salt-stress tolerance in *Brassica* species.

Salt stress often also leads to oxidative stress caused by the generation of excess reactive oxygen species ROS (Lyon *et al.,* 2011). Jia *et al.,* (2015) reported the up regulation of antioxidant enzymes, such as FeSOD 1, glutathione transferase and dehydroascorbate reductase, which might promote ROS scavenging and mitigate the oxidative damage under salt stress in *Brassica napus* leaves. They also reported up-accumulation of thiazole biosynthetic enzyme, THI which might help to restore DNA stability and alleviate oxidative stress caused by NaCl treatment in *Brassica napus* leaves. FtSH, a cell division assisting protein, has been reported to play a role in attenuating the detrimental effects of salinity on the photosynthetic machinery (Barkla *et al.,* 2013). Jia *et al.,* (2015) reported enhanced abundance of FtSH2 in salt-stressed *Brassica napus* leaves which might have contributed

to the restoring of the photosynthetic proteins at 48 h and 72 h after salt-stress treatment in these species. Three up-accumulated proteins related to the defense response have been reported in *Brassica napus* leaves, including cinnamyl alcohol dehydrogenase, chitinase and N-glyceraldehyde-2-phosphotransferase-like protein. Cinnamyl alcohol dehydrogenase (CAD) is a key enzyme in lignin biosynthesis and enhanced CAD might increase the extent of lignification, which might represent a salt-adaptation response in plant roots (Sanchez-Aguayo *et al.,* 2004; Chen *et al.,* 2007; Zhao *et al.,* 2013). Chitinase belongs to the pathogenesis-related (PR) protein family, which plays important roles in biotic and abiotic stress resistance (Fernandez-Garcia *et al.,* 2011). These results suggested that chitinase might help to enhance resistance against salt stress in *Brassica napus.* A study in *Brassica carinata* showed that N-glyceraldehyde-2-phosphotransferase was up-regulated in the resistant genotype upon pathogen attack (Subramanian *et al.,* 2005). The involvement of N-glyceraldehyde- 2-phosphotransferase in salt stress resistance/tolerance is unclear, may represent a novel salt-stress-responsive protein in *Brassica napus* leaves (Jia *et al.,* 2015).

ATP synthase is another very important salt-responsive enzyme and plays crucial roles in plant salt tolerance (Liu *et al.,* 2014). Jia *et al.,* (2015), in their proteomic study, identified eight spots as ATP synthase subunits, whose abundances changed under salt-stress conditions. Most of these proteins were down-accumulated, especially at the 24 h time point, and the enzyme activity analysis result was consistent with this finding. So this suggests that energy synthesis might be repressed in salt-stressed *Brassica* napus seedlings. However, the response of ATP synthase to salinity depends on the plant species and genotype. For example, the studies in rice (Zou *et al.,* 2011) and black locust (Wang *et al.,* 2013) showed that ATP synthase was induced upon salinity. Plant respiration response to salt stress has also been studied (Rai *et al.,* 2014). Jia *et al.,* (2015) observed in *Brassica* species that the abundance of the respiration enzyme pyruvate dehydrogenase was 2.2-fold higher after 72 h of salt stress treatment, while malate dehydrogenase was decreased by 0.66-fold at the 24 h time point.

CONCLUSIONS AND FUTURE PROSPECTS

The current scenario's of edible oil's production in India requires importing oilseeds as well as edible oil to meet the requirements of the population. The import budget can be curtailed by expanding cultivation of *Brassica* to problematic soils, especially salt affected soils. Salt tolerance potential of the cultivated Brassicas needs to be enhanced for the very commercial purpose of the crop. Recent in-depth investigations at physiological and molecular levels have identified many ways by which wild type plants cope with salinity stress. Thanks to close relationship and the significant inter and intra specific variation within *Brassica* species which shows huge potential for breeding for salt stress tolerance in *Brassica* crops. With the advent of publication of *Brassica* A genome sequences from *B. rapa* (Wang *et al.,*. 2011) and sequencing of the *Brassica* C genome from *B. oleracea,* (http:// www.ocri-genomics.org/bolbase/), along with microarray studies data together with the known genes involved in salt tolerance in model plants like *Arabidopsis* will serve as a fine platform for identification of 'candidate genes'. Recombinant inbred lines from salt tolerant and sensitive *Brassica juncea* genotypes have also been

developed at CSSRI. The identified salt tolerant germplasms as well as DNA markers delineating QTLs for these traits can be used in marker-assisted selection (MAS) breeding programs of *Brassica* crops for areas affected by salt stress.

The transgenic approach has promised a substantial improvement in desired traits at isolated levels. Engineered genes encoding organic osmolytes, plant growth regulators, antioxidants, late embryogenesis abundant proteins, and transcription factors have been introduced into transgenic lines which performed well under controlled stress conditions. Translating this success under field situations will take us further subject to the country's policies on such issues. It is also imperative to note that most salt tolerant transgenic lines have been developed using a single gene transformation, which may not be as productive as using transformation of many genes. Enhancing crop stress tolerance by transferring a number of target genes would be a more logical approach. Advances in transcriptomic studies would certainly support genome analysis, develop expression arrays, molecular marker identification and, initiating functional and comparative genomic studies in *Brassica* species. Finally, marker assisted selection and breeding along with genes identification through proteomics and their transfer will promote molecular breeding of salt tolerant *Brassica* species in the future.

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ISBN 978-81-7132-832-1