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## Molecular breeding for development of salt tolerance in brassica

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**Abstract**

Mustard is one of the major oil yielding crops of India and their improvement for salt tolerance is one of the major goals for increasing the area in salt affected soil. There are several approaches like traditional breeding, molecular breeding and transgenics has been implemented for improvement of salt tolerance in brassica. Of these approaches molecular breeding is of the marvelous approach to incorporate the salinity tolerant trait in brassica cultivars. Several salt tolerance genes like BrERF4, DREB1A, SOS1, AtHKT1, BrECS, BnLEA4-1 has been identified from the brassica species and Arabidopsis for various level of salinity tolerance and their introgression through marker assisted back cross breeding may increase the tolerance level. The salt tolerance brassica cultivars has been developed at several level of tolerance like germination, vegetative as well as seed yield stage.

**Keywords:** Mustard, salt tolerance, molecular breeding and yield

**Introduction**

India's social security and overall economic welfare is mainly played by agriculture sector. With 5.8% world edible oil production, 6.1% world oil meal production, 3.9% world oil meal export, 11.2% world oil import and 9.3% world edible oil consumption, India has the 5<sup>th</sup> biggest edible oil economy in the world after USA, China, Brazil and Argentina (Vision 2050, 2015) [58]. In India there are nine main oilseed crops grown for their unique oil rich characteristics. Among the nine oilseed crops grown in the country, seven of them are edible (viz. soybean, rapeseed-mustard, groundnut, sunflower, safflower, sesame, niger) whereas two of them (castor and linseed) are non-edible oilseed crops. India is the largest producer of the oilseeds in the world and the oilseed sector 12-15 per cent of global oilseeds area, 6-7 per cent of vegetable oil production and 9-10 per cent of the total oil consumptions (Jha, 2012). However, the growth in the domestic production of oilseeds has not been able to keep pace with the growth in the demand in India. Due to low and unstable yield of most oilseed crops, the country now imports nearly half of the annual consumptions of 168 million tones. The major biotic and abiotic stresses are the main cause for the low production of oilseed crops and are predicted to be worsened with anticipated climate change. Among the various abiotic stresses saline and sodic (alkaline) soil is one of the major constraints since most of the oilseed crops are grown in marginal lands which are highly affected with salts. A tentative estimate showed that the salt affected soils will constitute about 15.5 million ha area in the country by 2030 (CSSRI 2030 Vision) (Table1). Therefore for achieving self sufficiency in oilseed production and nutritional security of the country, development of salinity tolerant oilseed crop species is an urgent necessity.

In case of saline soil, due to high salt concentration in soil, plants face nutrients imbalance and accumulation of toxic elements whereas in case of sodic tolerance, major damage is due to iron deficiency caused by a high soil pH. Plant growth was affected by salinity stress due to ionic and osmotic effects (Agrawal *et al.*, 2015). The higher salt concentration in salinity affected soil causes plant wilt and salt ions like Na<sup>+</sup> and Cl<sup>-</sup> affect healthy growth and development of the plant (Farhoudi *et al.* 2007). In many areas of the country the area of salt affected soil is increasing due to use of poor quality of water for irrigation and soil salinization. Plants adapted to the salinity tolerance by fine tuning its complex physiological, metabolic and molecular gene networks (Gupta and Huang, 2014) [19]. Recent studies revealed that the stress signaling and sensing components can play pivotal roles in developing salinity tolerance to the crop plants (Dienlein *et al.*, 2014).

**Table 1:** Extent and distribution of salt affected soil in India (CSSRI, Karnal)

S. No	State	Saline Soils (ha)	Alkali Soils (ha)	Coastline Saline Soil (ha)	Total (ha)
1	Andhra Pradesh	0	196609	77598	274207
2	A & N Islands	0	0	77000	77000
3	Bihar	47301	105852	0	153153
4	Gujarat	1218255	541430	462315	2222000
5	Haryana	49157	183399	0	232556
6	J & K	0	17500	0	17500
7	Karnataka	1307	148136	586	150029
8	Kerala	0	0	20000	20000
9	Maharashtra	177093	422670	6996	606759
10	Madhya Pradesh	0	139720	0	139720
11	Orissa	0	0	1471138	147138
12	Punjab	0	151717	0	151717
13	Rajasthan	195571	179371	0	374952
14	Tamil Nadu	0	354784	13231	368015
15	Uttar Pradesh	21989	1346971	0	1368960
16	West Bengal	0	0	441272	441272
	Total	1710673	3788159	1246136	6744968

### Salinity stress in rapeseed-mustard

Out of seven edible oilseed crops grown in the country, rapeseed-mustard is the second most important edible oilseed crop after soybean contributing nearly 27% in edible oil pool of the country (Singh *et al.* 2013) [51]. In India, rapeseed-mustard crops include Indian mustard (*Brassica juncea*), yellow sarson (*B. rapa* L. var. *yellow sarson*), brown sarson (*B. rapa* L. var. *brown sarson*), toria (*B. rapa* L. var. *toria*), black mustard (*B. nigra*) and taramira (*Eruca sativa*), among which Indian mustard (*B. juncea*) is the most important edible oilseed crop contributing 7.8 Mt production during 2013-14 (FAO 2015). *Brassica napus* (oilseed rape) is the major rapeseed-mustard crop grown internationally *viz* Australia, Canada and European Union whereas *B. juncea* (Indian mustard) is the major rapeseed mustard crop in the Indian subcontinent.

Rapeseed-mustard crops are grown under a spectrum of agro-climatic conditions ranging from rainfed to irrigated, normal to saline and alkaline soil, north-eastern to western hills and in different cropping system. Among major mustard growing states (Rajasthan, Haryana, Uttar Pradesh, Madhya Pradesh, Punjab, Bihar and West Bengal), Rajasthan is the largest producer with half of the total national production (Vision-2050, 2015). The seed meal of mustard is very nutritious and mainly used as livestock feed. Indian mustard is a natural amphidiploid (AABB, 2n = 36) of *B. rapa* (AA, 2n = 20) and *B. nigra* (BB, 2n = 16) with haploid (1X) genome size of 534 Mbp (Johnston *et al.*, 2005) [24]. Due to large genome size and complex genome very limited sequence information is available for *B. juncea* and *B. nigra* genomes. So far, *B. rapa* and *B. oleracea* whole genome sequencing have been done (Wang *et al.*, 2011; Liu *et al.*, 2014) [59, 25].

Salinity and alkalinity stress is considered as one of the major environmental factors, decreases rapeseed-mustard crop productivity world-wide. It is one of the major abiotic factors for this crop in irrigated as well as in rainfed areas, which leads to poor harvests (Purty *et al.*, 2008) [41]. As an effect of poor seed germination and hampered seedling development, the growth, yield, and oil production of mustard is considerably reduced due to salinity stress (Ashraf and McNeilly 2004) [42]. In a study conducted on Indian mustard, significant reductions in oil, protein and fiber contents with increased erucic acid percentage in response to salt stress was reported (Singh *et al.*, 2014) [51]. Therefore, evaluation, identification,

characterization and generation of improved Brassica cultivars with increased salt tolerance are required to maintain the optimum yield and quality in Brassica species.

### Development of salt tolerant Brassica

For developing salinity tolerance currently there are three basic approaches being used *viz* screening of preexisting genotypes, conventional breeding and molecular breeding for developing salt tolerant lines, and development of transgenic plants to introduce novel genes or to alter expression levels of the native genes to increase salinity tolerance (Purty *et al.*, 2008) [41].

### Screening of preexisting salt-tolerant genotypes of Brassica

The mechanism of salinity tolerance is a very complex trait since it is controlled by various mechanisms at cellular, tissue, organ and whole plant levels. Some of these mechanism/traits may only be functional at specific stage of plant growth and the effect of one mechanism may prohibit the effect of the others at certain plant developmental stages (Dubey, 1997; Yeo, 1998; Makela *et al.*, 1999) [15]. Both diploid and polyploid Brassica species have been cultivated and most of them have been classified as moderately salt tolerant. Several studies have shown that the amphidiploid species (*B. carinata*, *B. juncea*, and *B. napus*) poses superiority of the over the diploid species (*B. campestris*, *B. nigra*, and *B. oleracea*) in response to salinity stress (Malik, 1990; He and Cramer, 1992; Kumar, 1995) [34, 21]. Stebbins (1966) [54] has also studied that the polyploid species can generally withstand abiotic stresses better than their respective diploid ancestors. Ashraf and coworkers have suggested that the salt tolerance of amphidiploids has been acquired from the A genome of *B. rapa* and C genome of *B. oleracea* (Ashraf *et al.*, 2001) [42]. A significant inter- and intra-specific variation within the Brassica genera has been observed by several workers (Purty *et al.*, 2008; Hayat *et al.*, 2011; Su *et al.*, 2013; Sharma *et al.*, 2013) [41, 20, 14]. These variations are mainly due to differences in electrolyte leakage, proline accumulation and the K<sup>+</sup>/Na<sup>+</sup> ratio among the genotypes. These variations need to be exploited through mapping and cloning of salinity tolerance genes/QTLs and their incorporation to superior genetic backgrounds through MAS to develop salt tolerant mustard varieties.

**Table 1:** List of salt-tolerant-associated genes in Mustard

Species	Genes	Encoding protein	Reference
<i>B. rapa</i>	BrERF4	Ethylene-responsive factors	Seo <i>et al.</i> 2010
Arabidopsis	DREB1A	Dehydration response element binding protein	Kasuga <i>et al.</i> 1999
Arabidopsis	SOS1	Plasma membrane-bound Na <sup>+</sup> /H <sup>+</sup> antiports	Martinez-Atienza <i>et al.</i> 2007,
Arabidopsis	AtHKT1	Na <sup>+</sup> transporter	Berthomieu <i>et al.</i> 2003
Arabidopsis	AtNHX1	Vacuolar Na <sup>+</sup> /H <sup>+</sup> antiporter	Zhang and Blumwald 2001,
Arthrobacter globiformis	Coda	Choline oxidase	Wang <i>et al.</i> 2010 [60]
<i>B. napus</i>	BnLEA4-1	Late-embryogenesis abundant proteins in group 4	Dalal <i>et al.</i> 2009
<i>B. juncea</i>	BrECS	Glutamylcysteine synthetase	Bae <i>et al.</i> 2013

**Table 2:** Salinity tolerant cultivars and lines of Brassica species developed through breeding

Brassica species	Variety	Parameter for testing tolerance	Reference
<i>B. napus</i>	Dunkeld	Metabolites	Qasim (2003)
	ST9194	Germination	Puppala <i>et al.</i> (1999)
	Rapora, Mytnitskii, Chisayanatane	Seed yield	Pokrovskii (1990)
<i>B. juncea</i>	Common Green	Vegetative stage	Kwon <i>et al.</i> (1997)
	Varuna	Germination, Seed yield	Rai (1977), Kumar (1984)
	TH 68	Germination	Singh <i>et al.</i> (1984) [51]
	RH 30, Pusa Bold, Kranti, CS4, CS15, Pant Rai 2030, PR 1002, RH 7818 and DIRA 337	Seed yield	Dhawan <i>et al.</i> (1987), Kumar (1995), Uma <i>et al.</i> (1992), Sinha (1991), Kumar (1984),
	BM-1, LL-84, P-15, KS-5,1 CSTR 330-1, CS12,	Biomass and seed yield	Ashraf (2004), Ashraf <i>et al.</i> (1994) [41], www.plantstress.com/files/ saltkarnal.htm
	CSTR 600-B-10,	Germination, Vegetative stage	www.plantstress.com/files/ saltkarnal.htm
<i>B. carinata</i>	C90-1191, Yellow Dodella	Germination and seedling growth	Ashraf and Sharif (1997) [43]
	C90-1115, 77-321	Seed yield	Ashraf and Sharif (1997) [42]
<i>B. rapa</i>	BSH1	Germination	Paliwal (1972)
	BSH1	Seed yield	Kumar (1984)

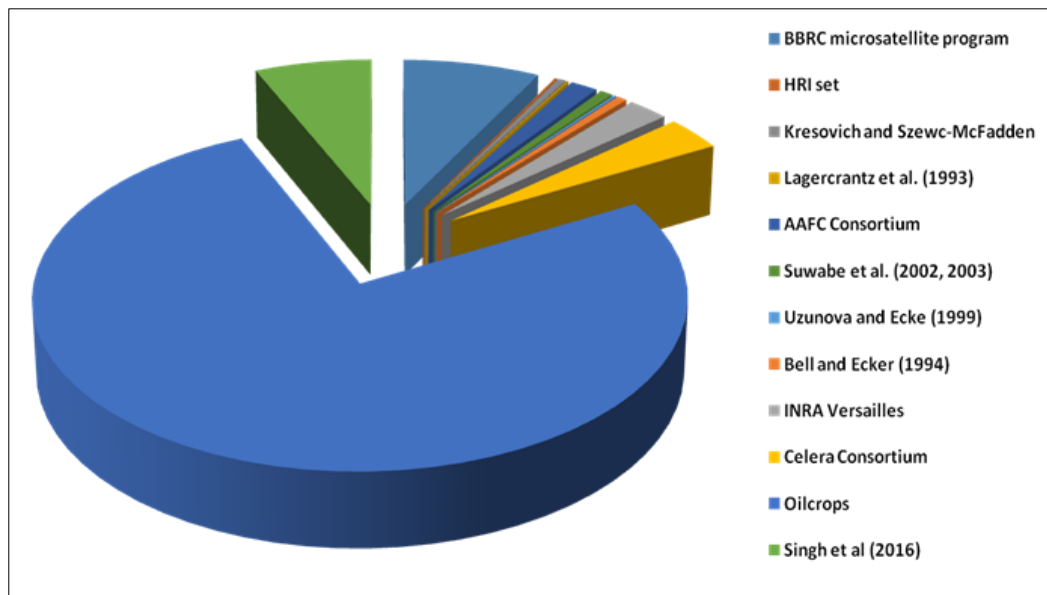
To cope with the worldwide problem of increasing soil salinity in agricultural land, conventional breeding approaches is envisaged as one way for enhancing salt tolerance in Brassica crop plants. Abiotic factors under adverse soil conditions are complex and often associated with climatic hazards. These stresses occur in a range from location to location and even from season to season and often associated with nutritional imbalance (deficiency/toxicity) due to salinity and alkalinity problems. The interaction between salt stresses with other environmental factors affects the plant's response to that stress. These factors are responsible for high yielding crop varieties to adapt such adverse edaphic environments. Therefore it is necessary that crop genotypes must be screened at target sites having ample stresses in order to select stable sources of varietal tolerance. Breeding for salinity tolerant crop varieties is the best approach because it is promising, energy-efficient and economical approach than major soil amelioration techniques and engineering processes which have become highly labor demanding and costly and thus gone beyond the limits of marginal farmers (Ashraf and McNeilly, 2004) [42]. Presence of a great magnitude of heritable variation in the gene pool of a crop is a prerequisite for a successful breeding program. Genetic diversity available in these gene pools gives parental material from well-adapted landraces to improved cultivars. When the genetic diversity has been utilized through continued selection, then variability may be hunted through other means like mutation breeding, protoplast fusion and recombinant DNA technologies. Several salt tolerant varieties of rapeseed-mustard have been developed through conventional breeding approaches (Table 2). Central Soil Salinity Research Institute, Karnal, India has been developing various promising salt tolerant varieties of *Brassica juncea* (CS54, CS52, CS416, CSTR 330-1, CSTR 600-B-10, CSTR 610-10-1-1, and CS12). A *B. juncea* var.

CS54 with an average yield of 1.1 t/ha has been recently developed by CSSRI Karnal, which is a high yielding and salinity tolerant variety and recommended for cultivation in salt stressed soils having EC upto 7 to 8 dsm-1 and pH 9.2 – 9.3. Several varieties have been developed for other Brassica crops (*Brassica rapa* var. *toria*, *B. napus* and *Eruca sativa*) with higher salinity tolerance. Among these Brassica varieties which include Peelaraya, SPS-23-1, SPS-23-2, ORI-56-6, P-8-2, RL-18, and brown raya, P-8-2 has been found most tolerant varieties under salinity condition (Sadiq *et al.*, 2002) [47]. With the advancement of new technologies *viz* development of molecular markers for physiological traits, genomics and double haploid production has made significant headway in recent years.

The application of molecular markers in breeding programs is increasing rapidly its preciseness, easiness and reliability which increase the efficiency of the breeding programs. There are several sets of microsatellite markers for Brassicaceae are available in public domain which is listed in table 4. Rapid progress in DNA based molecular marker technology has led to the development of detailed molecular linkage maps for many plant species (Jain and Selvaraj, 1997) [22]. Marker Assisted Selection (MAS) is a nondestructive process which can select the genotype of a single plant without exposing it to the actual stress. With flanking DNA markers to a desired gene/QTL, it can be easily transferred to elite rapeseed-mustard cultivars through MAS. Salinity tolerance is found to be governed by polygenes as well such genes which condition the expression of quantitative traits are referred to as quantitative trait loci (QTL) (Tanksley *et al.*, 1996) [56]. Mapping and tagging of QTL for salinity tolerance with DNA markers has become crucial to the improvement of crops species (Ramchiary *et al.*, 2007) [45]. Presently molecular maps for different rapeseed-mustard crops have been made by

various types of DNA markers *viz* RFLP, RAPD, AFLP, SSR and SNPs (Kim *et al.*, 2006; Ramchiary *et al.*, 2010; Yadava *et al.*, 2012 and Paritosh *et al.*, 2014)<sup>[7, 64, 38]</sup>. The molecular maps generated with the help of molecular markers have been helping scientists to develop stress tolerance in plants. Furthermore, with these DNA based molecular markers, abiotic stress like salinity tolerance genes/QTLs can be

efficiently manipulated in combination with other genes controlling important attributes necessary to produce an elite cultivar (Foolad and Chen, 1999)<sup>[17]</sup>. Tagging and mapping of genes/QTLs for stress tolerance can also be used for cloning of stress tolerance genes which would be very helpful for studying underlying physiological and biochemical mechanisms.



**Fig 1:** Brassica SSR markers available in public domain.

#### QTL mapping of salt tolerance

There are several independent studies on salinity tolerance in Brassicaceae, especially in *Arabidopsis*. Many mapped QTLs responsible for salinity tolerance were different from one other, since the mapping populations used and screening and analysis methods were different. The QTLs for salinity tolerance in Brassicaceae family are summarized in table 3. In most of these studies, the screening of salt tolerance was conducted with an agar medium (Quesada *et al.* 2002; Galpaz and Reymond 2010; DeRose-Wilson and Gaut 2011; Yong *et al.*, 2015)<sup>[43, 18]</sup>. A common QTL was detected at 20 cM on chromosome 1 for percent germination (PC) across four RIL populations. This QTL was co-localized with the gene RAS1 which is a negative regulator of salinity tolerance at seed germination and early growth stage. This gene was cloned from a population derived from the cross Sha × Ler (Ren2010). Another QTL for per cent germination was detected at 50 cM on chromosome 4. A candidate gene AT4G19030 (Lee *et al.* 2006) was predicted (DeRose- Wilson and Gaut 2011), with reduced expression by ABA and NaCl. Other QTLs identified in these studies were distributed in different positions on the genome. These findings indicate that salinity tolerance is controlled by a complex genetic network and there are different genetic determinants in different genotypes. Some QTLs controlling other traits were overlapped, eg: QTLs for root length and response to salinity tolerance were located on chromosome 1 and 3, indicating that salinity tolerance is exhibited by root growth. QTL mapping is the best method for identifying causal genes and QTLs responsible for salinity tolerance, it is high labor demanding and time consuming. QTL mapping through association mapping, which utilizes larger number of historical recombination events that have accumulated during evolutionary process of the genotypes, enables high resolution mapping (Nordborg and Tavaré 2002). Using association

mapping approach with a population of 96 accessions, DeRose-Wilson and Gaut (2011) discovered ten genomic regions associated with salinity tolerance among them six QTLs were previously identified. However, the small size of the population taken for this study, the results of the association mapping might be underpowered (Zhao *et al.* 2007)<sup>[68]</sup>.

#### Conclusion

Salinity stress is one of the most important factors affecting edible oil production in the country. To overcome this problem, there is several mechanisms available (agronomic practices, irrigation scheduling, soil remediation *etc*) among which development of salinity tolerant oilseed varieties is the most efficient and economic strategy to produce more under salt affected soils. Great success has been achieved in development of salinity tolerant varieties in various crops. But for Brassica crops, so far there have been lesser achievements in developing salt tolerant cultivars in comparison to other crops. Only few studies of salinity tolerance have been done in case of rapeseed-mustard and there are very few salinity tolerant varieties have been developed either by conventional breeding or through molecular breeding. For rapeseed-mustard, groundnut and sesame there is very few reports available on identification of salinity tolerance genes/QTLs. However, screening for salt tolerant genotypes in these crops has been done and a wide spectrum of variability has been found in respect to the salinity response. In *Arabidopsis*, several salt tolerant genes have been identified and these findings may help in identifying salt tolerant QTLs and genes in rapeseed-mustard crops. Few salt tolerant varieties of some Brassica crops (CS 52, CS 54, NarendraRai) have been developed but they are region specific salinity. Mutation breeding can be used to produce salt tolerant genotypes followed by isolating, tagging and mapping the gene will help

to develop tolerant genotypes (Yadav *et al.*, 2016). Phenotyping genotypes to identify salt tolerant one in a large mutated populations is currently a big challenge but with advance phenomics techniques this issue can be overcome (Berger *et al.*, 2012). Lack of high density linkage map and other genomic tools can be generated by using NGS techniques in a faster way (Yadav *et al.* 2016). Transgenic approaches have big potential for developing salinity tolerance in oilseed crops, especially with upcoming genome editing techniques like TALEN and CRISPR/Cas9 (Rani *et al.*, 2016).

## References

- Agarwal N, Kumar A, Agarwal S, Singh A. Evaluation of Soybean (*Glycine max* L.) Cultivars Under Salinity Stress During Early Vegetative Growth. *Int. J Curr. Microbiol. App. Sci.* 2015; 4(2):123-134.
- Agarwal N, Kumar A, Agarwal S, Singh A. Evaluation of Soybean (*Glycine max* L.) Cultivars Under Salinity Stress During Early Vegetative Growth. *Int. J Curr. Microbiol. App. Sci.* 2015; 4(2):123-134.
- Ashraf M, McNeilly T. Salinity tolerance in Brassica oilseeds. *Cr Rev Plant Sci.* 2004; 23:157-174.
- Ashraf M, Sharif R. Inter-cultivar variation for salt (NaCl) tolerance in a potential oil-seed crop Ethiopian mustard (*Brassica carinata* A.Br.). *Arch Acker Pfl Boden.* 1997; 42:129-136.
- Ashraf M, Zafar ZU, Tufail M. Intra-specific variation for salt tolerance in a potential oilseed crop, brown mustard (*Brassica juncea* (L.) Czern. and Coss.). *Arch Acker Pfl Boden.* 1994; 38:449-458.
- Ashraf M, McNeilly T, Nazir M. Comparative salt tolerance of amphidiploid and diploid Brassica species. *Plant Sci.* 2001; 160:683-689.
- Bae MJ, Kim YS, Kim IS, Choe YH, Lee EJ, Kim YH, *et al.* Transgenic rice overexpressing the Brassica juncea gamma-glutamylcysteine synthetase gene enhances tolerance to abiotic stress and improves grain yield under paddy field conditions. *Mol. Breed.* 2013; 31:931-945.
- Berger B, de Regt B, Tester M. Trait dissection of salinity tolerance with plant phenomics. *Plant Salt Tolerance: Methods and Protocols*, 2012, 399-413.
- Berthomieu P, Conéjéro G, Nublát A, Brackenbury WJ, Lambert C, Savio C *et al.* Functional analysis of AtHKT1 in Arabidopsis shows that Na<sup>+</sup> recirculation by the phloem is crucial for salt tolerance. *EMBO J.* 2003; 22:2004-2014.
- CSSRI. Vision, 2011, 2030.
- Dalal M, Tayal D, Chinnusamy V, Bansal KC. Abiotic stress and ABA-inducible Group 4 LEA from Brassica napus play a key role in salt and drought tolerance. *J Biotechnol.* 2009; 139:137-145.
- Deinlein U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI. Plant salt-tolerance mechanisms. *Trends in Plant Sciences.* 2014; 19(6):371-379.
- DeRose-Wilson L, Gaut BS. Mapping salinity tolerance during Arabidopsis thaliana germination and seedling growth. *PLoS ONE.* 2011; 6:e22832.
- Dhawan RS, Sharma DR, Choudhary JB. Effect of salinity on germination and yield components in three species of Brassica. *Indian J Agr Sci.* 1987; 57:107-111.
- Dubey RS. Photosynthesis in plants under stressful conditions. In: *Handbook of Photosynthesis*, Pessaraki, M., Ed., Marcel Dekker, New York, 1997, 859-875.
- FAOSTAT, 2014. <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor>
- Foolad MR, Chen FQ. RFLP mapping of QTLs conferring salt tolerance during vegetative stage in tomato. *Theor Appl. Genet.* 1999; 99:235-243.
- Galpaz N, Reymond M. Natural variation in Arabidopsis thaliana revealed a genetic network controlling germination under salt stress. *PLoS One.* 2010; 5:e15198.
- Gupta B, Huang B. Mechanism of Salinity Tolerance in Plants: Physiological, Biochemical, and Molecular Characterization. *International Journal of Genomics.* Article ID 701596, 2014, 18. <http://dx.doi.org/10.1155/2014/701596>
- Hayat S, Mir BA, Wani AS, Hasan SA, Irfan M, Ahmad A. Screening of salt-tolerant genotypes of Brassica juncea based on photosynthetic attributes. *Journal of Plant Interactions.* 2011; 6(1):53-60.
- He T, Cramer GR. Growth and mineral nutrition of six rapid-cycling Brassica species in response to sea water salinity. *Plant Soil.* 1992; 139:285-294.
- Jain RK, Selvaraj G. Molecular genetic improvement of salt tolerance in plants. *Biotech. Annu. Rev.* 1997; 3:245-267.
- Jha GK. Edible Oilseeds Supply and Demand Scenario in India, report IARI, 2012.
- Johnston JS, Pepper AE, Hall AE, Chen ZJ, Hodnett G, Drabek J, Price HJ. Evolution of genome size in Brassicaceae. *Annals of botany.* 2005; 95(1):229-235.
- Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat. Biotechnol.* 1999; 17:287-291.
- Kim JS, Chung TY, King GJ, Jin M, Yang TJ, Jin YM, *et al.* A sequence-tagged linkage map of Brassica rapa. *Genetics.* 2006; 174(1):29-39.
- Kumar D. The value of certain plant parameters as an index for salt tolerance in Indian mustard (*Brassica juncea* L.). *Plant Soil.* 1984; 79:261-272.
- Kumar D. Salt tolerance in oilseed Brassicas - present status and future prospects. *Plant Breeding Abstracts.* 1995; 65:1439-1447.
- Kumar D. Salt tolerance in oilseed Brassicas - present status and future prospects. *Plant Breeding Abstracts.* 1995; 65:1439-1447.
- Kwon TR, Bourne WF, Harris PJC. Growth and ion accumulation of Brassica juncea L. cultivars differing in salinity tolerance. Poster paper presented at the 1997 Annual Meeting of the Society of Experimental Biology, University of Kent at Canterbury, 7-10 April 1997; and published as Abstract in *J Exp Bot.* 1997; 48:66.
- Lee BH, Kapoor A, Zhu J, Zhu JK. STABILIZED1, a stress-upregulated nuclear protein, is required for pre-mRNA splicing, mRNA turnover, and stress tolerance in Arabidopsis. *Plant Cell.* 2006; 18:1736-1749.
- Liu S, Liu Y, Yang X, Tong C, Edwards D, Parkin IA *et al.* The Brassica oleracea genome reveals the asymmetrical evolution of polyploid genomes. *Nature communications,* 2014, 5.
- Mäkela P, Kontturi M, Pehu E, Somersalo S. Photosynthetic response of drought-and salt-stressed tomato and turnip rape plants to foliar applied glycine betaine. *Physiol. Plant.* 1999; 105:45-50.
- Malik RS. Prospects for Brassica carinata as an oilseed crop in India. *Exp. Agric.* 1990; 26:125-129.

35. Martinez-Atienza J, Jiang X, Garcíadeblas B, Mendoza I, Zhu JK, Pardo JM *et al.* Conservation of the salt overly sensitive pathway in rice. *Plant Physiol.* 2007; 143:1001-1012.
36. Nordborg M, Tavaré S. Linkage disequilibrium: what history has to tell us. *Trends Genet.* 2002; 18:83-90.
37. Paliwal KV. Irrigation with saline water. Indian Agricultural Research Institute Monograph 2, New Delhi, 1972.
38. Paritosh K, Gupta V, Yadava SK, Singh P, Pradhan AK, Pental D. RNA-seq based SNPs for mapping in *Brassica juncea* (AABB): synteny analysis between the two constituent genomes A (from *B. rapa*) and B (from *B. nigra*) shows highly divergent gene block arrangement and unique block fragmentation patterns. *BMC genomics*, 2014; 15(1):1.
39. Pokrovskii VB. Promising forms of winter swede rape. *Selektsiya-i-Semenovodstvo Moskva.* 1990; 4:24-25.
40. Puppala N, Fowler JL, Poindexter L, Bhardwaj HL. Evaluation of salinity tolerance of canola germination. Perspectives on new crops and new uses. ASHS Press, Alexandria, VA, 1999, 251-253.
41. Purty RS, Kumar G, Singla-Pareek SL, Pareek A. Towards salinity tolerance in Brassica: an overview. *Physiology and Molecular Biology of Plants.* 2008; 14(1-2):39-49.
42. Qasim M, Ashraf M, Ashraf MY, Rehman SU, Rha ES. Salt-induced changes in two canola cultivars differing in salt tolerance. *Biologia Plantarum.* 2003; 46(4):629-632.
43. Quesada V, García-Martínez S, Piqueras P, Ponce MR, Micol JL. Genetic architecture of NaCl tolerance in *Arabidopsis*. *Plant Physiol.* 2002; 130:951-963.
44. Rai M. Salinity tolerance in Indian mustard and safflower. *Indian J Agr Sci.* 1977; 47:70-73
45. Ramchiary N, Padmaja KL, Sharma S, Gupta V, Sodhi YS, Mukhopadhyay A, *et al.* Mapping of yield influencing QTL in *Brassica juncea*: implications for breeding of a major oilseed crop of dryland areas. *Theor Appl Genet.* 2007. PMID:17646960
46. Rani R, Yadav P, Barbadikar KM, Baliyan N, Malhotra EV, Singh BK, *et al.* CRISPR/Cas9: a promising way to exploit genetic variation in plants. *Biotechnology letters.* 2016; 38(12):1991-2006.
47. Sadiq M, Jamil M, Mehdi SM, Sarfraz M, Hassan G. Comparative performance of *Brassica* varieties/lines under saline sodic condition. *Asian J Plant Sci.* 2002; 2:77-78.
48. Seo YJ, Park JB, Cho YJ, Jung C, Seo HS, Park SK, *et al.* Overexpression of the ethylene-responsive factor gene BrERF4 from *Brassica rapa* increases tolerance to salt and drought in *Arabidopsis* plants. *Mol. Cells.* 2010; 30:271-277.
49. Sharma P, Sardana V, Banga SS. Salt tolerance of Indian mustard (*Brassica juncea*) at germination and early seedling growth. *Environ Exp Biol.* 2013; 11:39-46.
50. Singh A, Avtar R, Singh D, Sangwan O, Thakral N, Malik V, *et al.* Combining ability analysis for seed yield and component traits in Indian mustard [*Brassica juncea* (L.) Czern & Coss.]. *Research in Plant Biology.* 2013; 3(2).
51. Singh BK, Mishra DC, Yadav S, Ambawat S, Vaidya E, Tribhuvan KU, *et al.* Identification, characterization, validation and cross-species amplification of genic-SSRs in Indian Mustard (*Brassica juncea*). *Journal of Plant Biochemistry and Biotechnology.* 2016, 1-11.
52. Singh H, Prakash J, Singh H. Effect of salinity on germination and early growth of toria (*Brassica campestris* var. *Toria*) genotypes. *Seed Res.* 1984; 12:22-24.
53. Sinha TS. Genetic adaptation of Indian mustard (*Brassica juncea*) to semi-arid saline soil condition. *Indian J Agric. Sci.* 1991; 61:251-254.
54. Stebbins GL. Chromosomal variations and evolution. *Science.* 1966; 152:1463-1469.
55. Su J, Wu S, Xu Z, Qiu S, Luo T, Yang Y, Huang B. Comparison of Salt Tolerance in Brassicas and some related species. *American Journal of Plant Sciences.* 2013; 4(10):1911.
56. Tanksley S, Grandillo S, Fulton T, Zamir D, Eshed Y, Petiard V *et al.* Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. *Theor Appl Genet.* 1996; 92:213-224.
57. Uma MS, Srinivas S, Hebbara M, Patil SG. Screening of Indian mustard (*B. juncea*) genotypes for saline vertisols Tungbhadra areas of Karnataka. *Indian J Agric. Sci.* 1992; 62:486-488.
58. Vision 2050. Vision Document. ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan, Indian Council of Agricultural Research, 2015, 1.
59. Wang X, Wang H, Wang J, Sun R, Wu J, Liu S *et al.* The genome of the mesopolyploid crop species *Brassica rapa*. *Nature genetics.* 2011; 43(10):1035-1039.
60. Wang X, Wang H, Wang J, Sun R, Wu J, Liu S *et al.* The genome of the mesopolyploid crop species *Brassica rapa*. *Nature genetics.* 2011; 43(10):1035-1039.
61. Wang QB, Xu W, Xue QZ, Su WA. Transgenic *Brassica chinensis* plants expressing a bacterial code. A gene exhibit enhanced tolerance to extreme temperature and high salinity. *J Zhejiang Univ. Sci. B.* 2010; 11:851-861.
62. Yadav P, Meena HS, Meena PD, Kumar A, Gupta R, Jambhulkar S, *et al.* Determination of LD50 of ethyl methanesulfonate (EMS) for induction of mutations in rapeseed-mustard. *Journal of Oilseed Brassica.* 2016; 1(1):77-82.
63. Yadav P, Vaidya E, Rani R, Yadav NK, Singh BK, Rai PK, *et al.* Recent Perspective of Next Generation Sequencing: Applications in Molecular Plant Biology and Crop Improvement. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences,* 1-15.
64. Yadava SK, Arumugam N, Mukhopadhyay A, Sodhi YS, Gupta V, Pental D, Pradhan AK. QTL mapping of yield-associated traits in *Brassica juncea*: meta-analysis and epistatic interactions using two different crosses between east European and Indian gene pool lines. *Theoretical and applied genetics.* 2012; 125(7):1553-1564.
65. Yeo AR. Molecular biology of salt tolerance in the context of whole-plant physiology. *J Exp. Bot.* 1998; 49:915-929.
66. Yong HY, Wang C, Bancroft I, Li F, Wu X, Kitashiba H, *et al.* Identification of a gene controlling variation in the salt tolerance of rapeseed (*Brassica napus* L.). *Planta.* 2015; 242(1):313-326.
67. Zhang HX, Blumwald E. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. *Nat. Biotechnol.* 2001; 19:765-768.
68. Zhao K, Aranzana MJ, Kim S, Lister C, Shindo C, Tang C, *et al.* An *Arabidopsis* example of Association Mapping in structured samples. *PLoS Genet.* 2007; 3:e4.