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Comparative effects of soil and water salinity on oil quality parameters of *Brassica juncea*

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Abstract

Effect of soil and water salinity on oil quality of Indian mustard was evaluated. Based on germination test, five salt tolerant genotypes were selected out of 26 at seedling stage and used for field study. Pot experiment was carried out on two crop seasons under three salinity levels of irrigation water (EC_{iw} Control, 12 and 15 dS/m) while field experiment was conducted under three saline environments *viz*. Control, ECe 9 and 12 dS/m. Soil salinity was monitored over the entire crop cycle. Harvested seeds were subjected to seed analysis for oil, protein, erucic acid and crude fiber content using a pre-standardized Fourier Transform Near Infrared Reflectance Spectrometer. Under increasing salinity (water and soil); oil, protein and crude fibre content decreased, 7-10%; 18-19% and 36-42%, respectively, while erucic acid content increased by 30-42% compared to control. The reductions and increments varied for different genotypes. Comparative study of salinity in pot and field showed that soil salinity at ECe 9 dS/m have similar effect, as water salinity at EC_{iw} 12 dS/m over the oil and quality parameters in mustard. Consideration was given in this experiment to determine appropriate breeding strategies for screening and development of superior oil quality genotypes under salinity conditions.

Keywords : *Brassicas; erucic acid; oil quality; protein; salinity*

Introduction

Brassicas are the third most important edible oil source in the world, after soybean and palm. In India, rapeseed-mustard is one of the important oilseed crops grown mainly for edible oil in about 7.0 m ha of arid and semi-arid regions of the country. Some of these areas are endowed with poor quality underground waters, which are also used for irrigating the crop at different critical growth stages. The productivity of the crop is very poor (1257 kg/ha), compared to world's average 1856 kg/ha (FAO, 2013). In India, the salt affected soils have been estimated to be 6.73 million ha and out of these, oilseed brassicas are being cultivated in around 2 million ha which comes under semi-arid region, affected with varying degrees of soil salinity (Singh *et al.*, 2014). Salinity affects the growth and development of *Brassicas* in various ways. The most common adverse effects of salinity are the reduction in plant height, size and yield as well as deterioration of the product quality (Zamani *et al.*, 2011). Furthermore, nutritional imbalance as a result of depressed uptake of nutrients, shoot transport, chlorophyll breakdown and impaired distribution of mineral ions retarded the development of seeds and early maturity of plants under high salinity treatments might be responsible for the reduced oil content (Ali *et al.*, 2013; Mahmood *et al.*, 2007). Soil salinity significantly affects the lipid components of Indian mustard (*Brassica juncea* L.) seeds. With increasing salt levels, total and neu-tral 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.

Indian mustard varieties exhibit quite high contents of erucic acid in oil (more than 40%). This high amount of erucic acid in edible oils has been reported to impair myocardial conductance, causes lipidosis in children and increases blood cholesterol (Ackman *et al.,* 1977). Because of the adverse effects of high erucic acid in the oil of Indian mustard varieties, the varietal improvement programme in India aims at reducing the erucic acid level up to internationally accepted norms (less than 2%) which necessitates the screening of a large number of samples with limited seed availability especially in potential germplasm.

In addition, the high fiber content (12–13%) in the seed meal reflects lower values of metabolizable energy and may negatively influence protein digestibility and the bioavailability of minerals such as magnesium and zinc (Simbaya *et al.,* 1995).

Due to scanty rainfall and drought, soil and water salinity is increasing hence the genotypes with least effect of higher soil and water salinity on oil and quality parameters are need of the hour. In the present experiment the evaluation was done on higher soil salinity (ECe 12 dS/m) and irrigation water salinity (ECe 15 dS/m). The present study was conducted to evaluate the effect of salinity on oil quality component characters of popular varieties of mustard released for normal soil along with salt tolerant genotypes. The aim to determine the appropriate breeding strategies for the development of superior oil quality genotypes under saline conditions was also under taken**.**

Materials and Methods

The experimental materials comprised of twenty six popular Indian mustard genotypes developed by different research institutes of India (Table 1). Salt tolerance behaviour of these selected genotypes was studied at germination (in plastic tray) and whole plant stages in pots (20 kg capacity enamelled pots) and field conditions. Different behaviour of these 26 Indian mustard genotypes at germination stage was evaluated by placing the counted seed in plastic trays, filled with normal (ECe 2 dS/m) and saline soil (ECe 12 dS/m). Saline soil was prepared according to ICAR-CSSRIs standard protocol by saturating the normal soil with saline solution comprising of NaCl, $CaCl_2$, $MgSO_4$ and $MgCl_2$ (Cl: SO_4, of 4:1 and Ca: Mg, of 3:1 ratio, and sodium absorption ratio of 11.8), in normal tap water. Germination percentage was recorded at 3rd day after sowing and two weeks old seedlings were randomly taken for seedling length and dry weight. The percent decrease in germination was used to assess salinity tolerance at seedling stage. Further, top five genotypes tolerant to salinity at seedling stage were used for field study. Whole experiment was sub-divided in two experiments; Pot study and field study.

Pot study

Pot study was conducted in controlled condition in the nethouse of ICAR-Central Soil Salinity Research Institute (ICAR-CSSRI), Karnal, India during *rabi* 2012-13 and 2013-14. The plants were grown in 20 kg. capacity enamelled pots in sand culture and irrigated with three levels of saline irrigation water (EC_{iw} 2, 12 and 15 dSm^{-1} in ¼ strength Hoagland's solution) maintained in root zone throughout the experiment. Saline irrigation waters were prepared by adding NaCl, $CaCl_2$ and Na_2SO_4 and maintaining Na: Ca and Cl: SO_4 ratio as 4:1 respectively. The pots were arranged in a factorial experiment based on a factorial randomized block design (RBD) extern 3 replicates. The pots were irrigated with saline waters on daily basis by flushing the soil out of root zone.

Field study

The best performing five genotypes out of 26 (CS 8000-1-2-8; CS 15000-1-2-2-2-1; CS 2200-2-6; CS13000-3-1-1-4-2 and CS54 (national check for salinity evaluation trials) were evaluated in a factorial randomized block design with two replicates at the Experimental Research Farm, Karnal (Non-saline field/control) and out station experimental farm, Nain, Distt. Panipat (Saline field) of the ICAR-CSSRI, during *Rabi* 2012–13 and 2013–14 under four environments *viz*. ECe Control (2.8), 9, 12 and 15 dS/m and irrigated with water having pH=7.7 and EC_{iw} 2.8 dS/m at both Research farms. No germination was recorded in field at 15 dS/m hence data were recorded only for ECe Control (2.8), 9 and 12 dS/m. The salinity of

the saline soil is above the threshold limit for mustard under field condition ECe 8.2 dS/m (Singh *et al.*, 2014).

Oil quality parameters estimation

The whole seeds harvested from the two experiments mentioned above, were subjected to the seed analysis for oil, protein, erucic acid and crude fiber content using a pre-standardized Fourier Transform Near Infrared Reflectance Spectrometer (FT-NIR, Perkin Elmer, Massachusetts, USA). FT-NIR was standardized as by Singh *et al.* (2013) and calibration models were developed for oil [Coefficient of determination (r^2) = 0.907, relative prediction deviation (RPD) = 4.67]; protein [Coefficient of determination (r^2) = 922, relative prediction deviation (RPD) = 4.71]; erucic acid [Coefficient of determination (r^2) = 0.902, relative prediction deviation (RPD) = 4.65] and crude fibres [Coefficient of determination (r^2) = 0.903, relative prediction deviation (RPD) = 4.74]. The r^2 values shown by the equations for quality parameters determination in mustard seed indicated excellent quantitative information (Shenk and Westerhaus, 1996). On the other hand, on the basis of the RPD statistics, the equation was higher than 3, indicated a high prediction ability and thus being useful for estimation (Williams and Sobering, 1996).

Statistical analysis

All the statistical analyses for quality characters were worked out using the SAS 9.3 software (SAS Institute Inc., Cary, USA).

Results and Discussions

Effect of salinity on germination

Under control condition germination ranged from 80% in genotype LES-49 to 100% in genotype

Table 1: Effect of salinity on germination of different Indian mustard genotypes

Genotypes	Germination %		
	C*	S**	Reduction (%)
LES 48	96	76	20.8
PDZ-2	82	68	17.1
NPJ 179	92	72	21.7
PDZ-1	94	52	44.7
Hybrid –PJR	84	70	16.7
Hybrid-322R	95	72	24.2
NPJ183	92	68	26.1
NPJ184	90	66	26.7
NPJ177	98	72	26.5
LES 49	80	60	25.0
NPJ 178	98	80	18.4
LES 49	94	64	31.9
NPJ 180	90	70	22.2
NPJ 181	87	64	26.4
NPJ 182	94	78	17.0
RGN 298	96	78	18.8
Kranti	95	78	17.9
RH 1006	82	68	17.1
CS 8000-1-2-8	97	86	11.3
CS 13000-3-1-1-4-2	95	83	12.6
CS 2200-2-6	96	84	12.5
CS 15000-1-2-2-2-1	96	85	11.5
RH1003	96	64	33.3
CS 54	88	76	13.6
DRMR 81	92	62	32.6
DRMR 15	100	76	24.0
Mean	92.3	72.0	

*C=Control; **S=Salinity (12 dS/m)

DRMR-15, whereas, under saline condition, it varied from 52% in genotype PDZ-1 to 86% in genotype CS8000-1-2-8 (Table 1). Considering low percent reduction (<15%) in germination, genotypes CS 8000-1-2-8 (11.3%), CS 15000-1-2-2-2-1 (11.5%), CS 2200-2-6 (12.5%), CS13000-3-1-1-4-2 (12.6%) and CS54 (13.6%) were characterized as tolerant to salinity at germination and seedling stage.

Analysis of Variance

Mean squares of the salinity levels under pot and field study were significant for oil, protein, erucic acid and crude fiber content indicated significant differences of these traits for control and salinity. Significant mean square of genotypes indicating significant genetic variation was detected for all the traits. The significant interaction effect of salinity levels x genotypes for oil, protein, erucic acid and crude fiber content under pot and filed study (except for erucic acid and crude fiber content) revealed the variable response of genotypes by expression of traits over the salinity (Table 2).

In addition, the genotypes followed a similar pattern of performance in both cropping seasons (2012–13 and 2013–14) as the mean sum of squares due to year; year × salinity and year × genotype were non-significant for these traits. Therefore, the effect of salinity on quality characteristics was pooled over the two years.

Table 2: Summary of analysis of variance for oil and quality components for salinity levels in mustard genotypes under pot and field (pooled over 2 years)

Pot house experiment

Source of variation	D.F.	Mean sum of squares			
		Oil (%)	Protein (%)	Erucic acid (%)	Crude fibres (%)
Salinity (S)	2	134.0**	246.6**	1311.8**	326.2**
Genotypes (G)	25	0.3**	1.0**	22.5**	1.4**
S X G	50	0.2**	0.2**	1.0**	0.1**
Error	156	0.04	0.1	0.1	0.1

Field experiment

Source of variation	D.F.	Mean sum of squares			
		Oil (%)	Protein (%)	Erucic acid (%)	Crude fibres (%)
Salinity (S)	2	35.1**	33.2**	556.0**	63.3**
Genotypes (G)	4	0.3**	1.0**	24.4**	0.5**
S X G	8	0.1*	0.3*	1.2	0.1
Error	15	0.1	0.1	6.2	0.1

*, ** *Significant at the 5% and 1% levels of probability, respectively.*

Effect of salinity on oil and quality parameters

Mean comparison of oil and quality parameters for salinity levels showed that oil, protein and crude fiber content were decreased whereas erucic acid content was increased at high levels of salinity under both the conditions. The mean seed oil content showed a range of 35.82 (EC_{iw} 15 dS/m) to 38.43% (control) under pots conditions and 34.92 (ECe 12 dS/m) to 38.60% (non-saline condition) in the field experiment over the salinity levels (Table 3).

With the increase in salinity in field conditions, the seed oil content decreased by 10% at ECe 12 dS/m

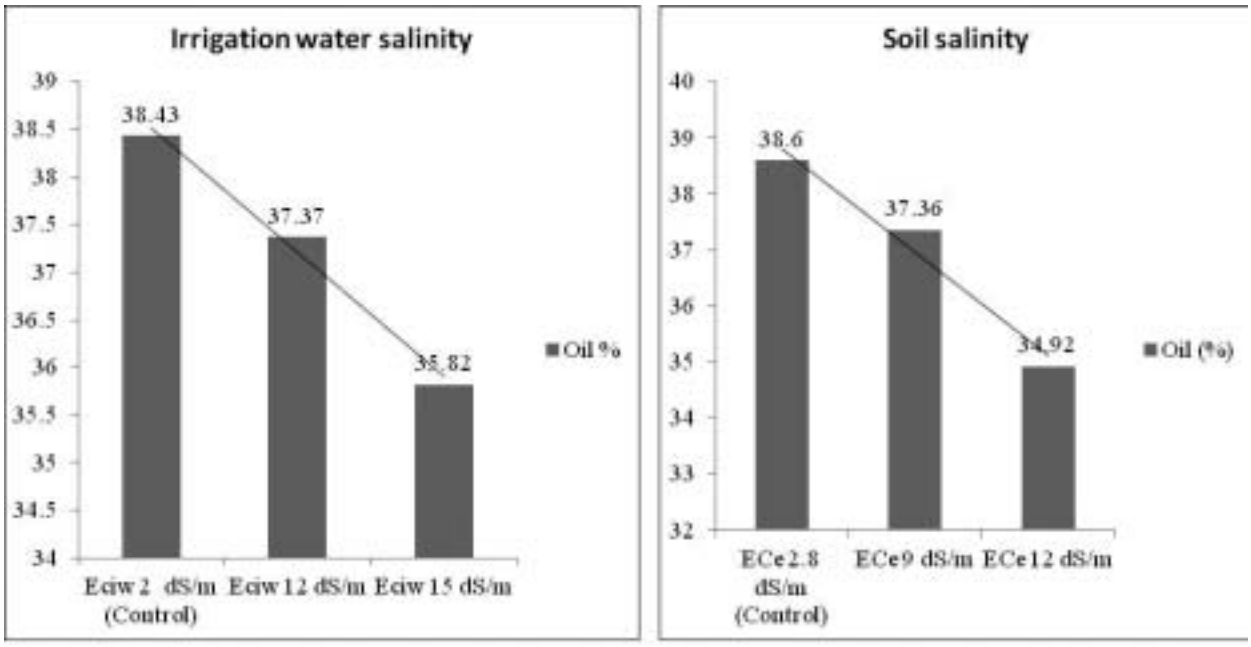

Fig 1: Effect of salinity (water and soil) on oil content of Indian mustard

as compared with non-saline condition, whereas, the oil content decreased by 7% at EC_{iw} 15 dS/m compared with the control under pots (Fig 1). The reduction in seed oil content might be due to an increase in the osmotic pressure of the soil solution and imbalances in nutrients and essential elements (Toorchi *et al.,* 2011) or the retarded development of seed and early maturity of plants in high salinity treatments (Flagella *et al.,* 2004; Cucci *et al.,* 2007).

Mean seed protein content exhibited a range of 15.84 (EC_{iw} 15 dS/m) to 19.39% (control) under pot conditions and 15.83 (ECe 12 dS/m) to 19.47% (non-saline condition) in the field experiment over the salinity levels. Whereas mean crude fiber content showed a range of 7.23 (EC_{iw} 15 dS/m) to 11.31% (control) under pot conditions and 6.96 (ECe 12 dS/m) to 11.99% (non-saline condition) in the field experi-ment over the different salinity levels (Table 3).

Table 3: Mean comparison of oil and quality components for salinity levels in mustard genotypes under pot and field (pooled over the two years)

Pot house experiment				
Salinity levels(EC_{iw} dSm^{-1})	Oil (%)	Protein (%)	Erucic acid (%)	Crude fibres (%)
S_1= 2 (Control)	38.4a	19.4a	27.1c	11.3a
S_2= 12	37.4b	17.8b	30.9b	9.1b
S_3= 15	35.8c	15.8c	35.2a	7.2c
LSD (P = 0.05)	0.6	0.8	0.1	0.7
Field experiment				
Salinity levels (ECe dSm^{-1})	Oil (%)	Protein (%)	Erucic acid (%)	Crude fibres (%)
S_1= 2.8 (Control)	38.6a	19.5a	28.7c	12.1a
S_2= 9	37.4b	17.7b	32.8b	9.5b
S_3= 12	34.9c	15.8c	43.2a	7.1c
LSD (P = 0.05)	0.2	0.3	2.4	0.3

Means with the same letter in each column have not statistically significant difference

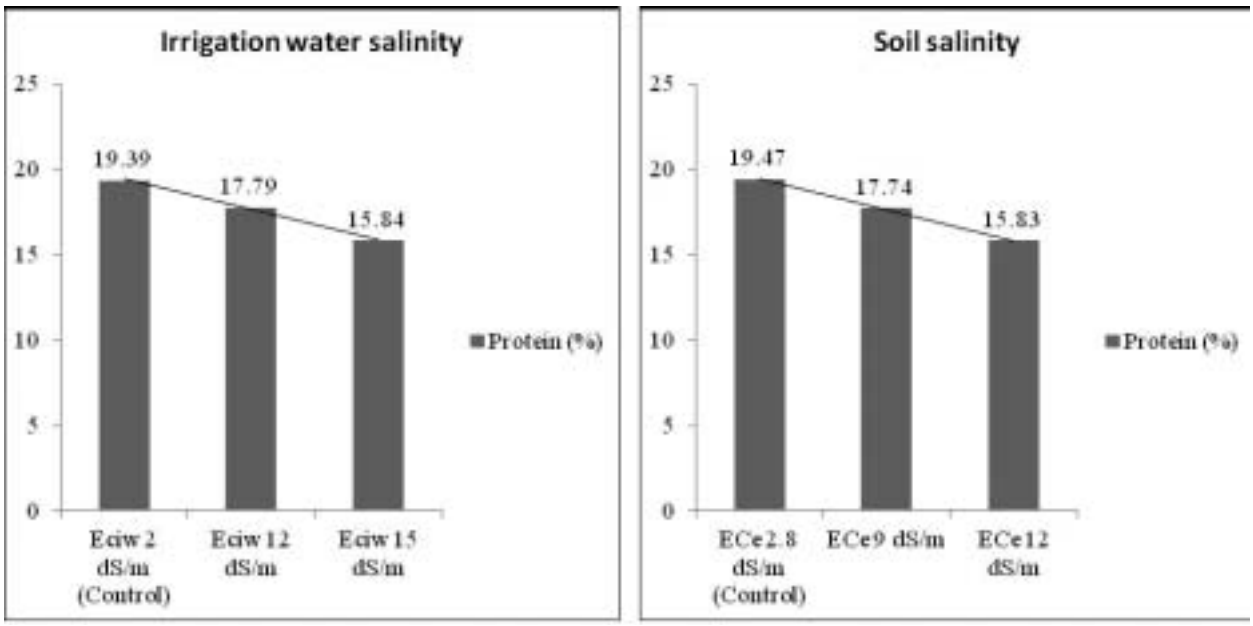

Fig 2: Effect of salinity (water and soil) on protein content of Indian mustard

Protein and crude fiber contents decreased by 18% and 36% (EC_{iw} 15 dS/m) in pots and 19% and 42% (ECe 12 dS/m) in field conditions, respectively, at high salinity levels compared with the control (Fig 2 and 3). The reduction in the protein and crude fiber content may be due to a failure of the plants to make full use of nitrogen compounds or reduction of nitrogen supply for the synthesis of amino acids and proteins. The accumulation of nitrogen compounds is more rapid than their utilization in building more cells and organs (Olfa *et al.*, 2009).

The erucic acid ranged from 26.96 (control) to 35.16% (EC_{iw} 15 dS/m) in pot and 28.74 (control) to 43.20% (ECe 12 dS/m) in the field experiments (Table 3). It increased by 30% in the pots at EC_{iw}

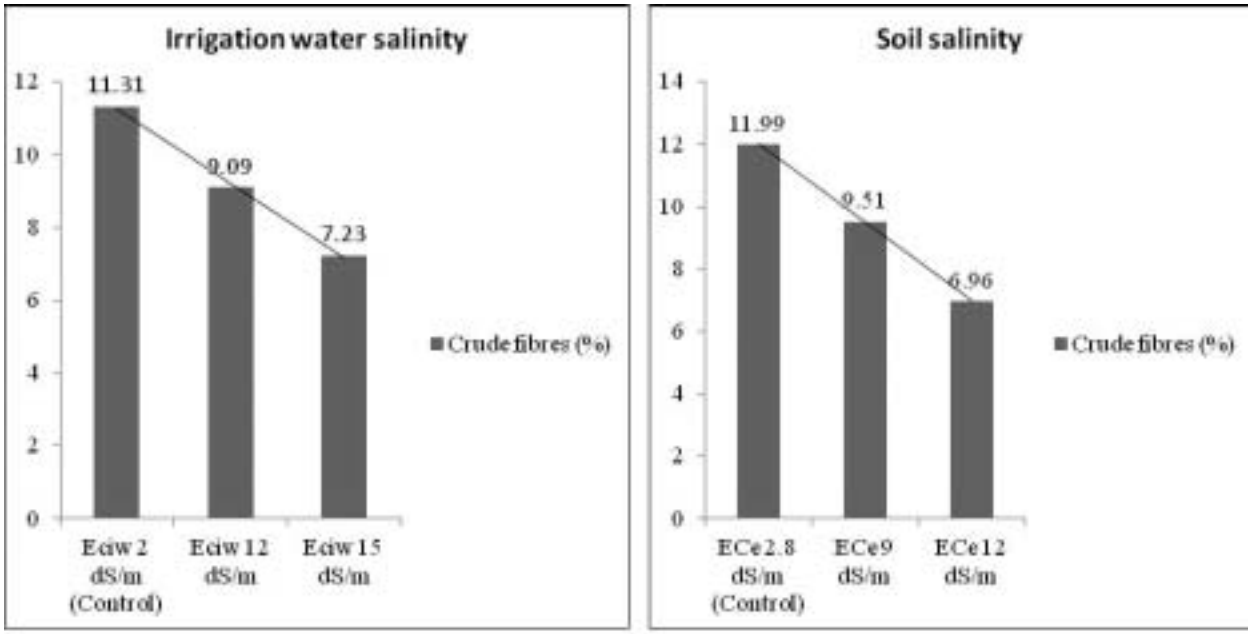

Fig 3: Effect of salinity (water and soil) on crude fiber content of Indian mustard

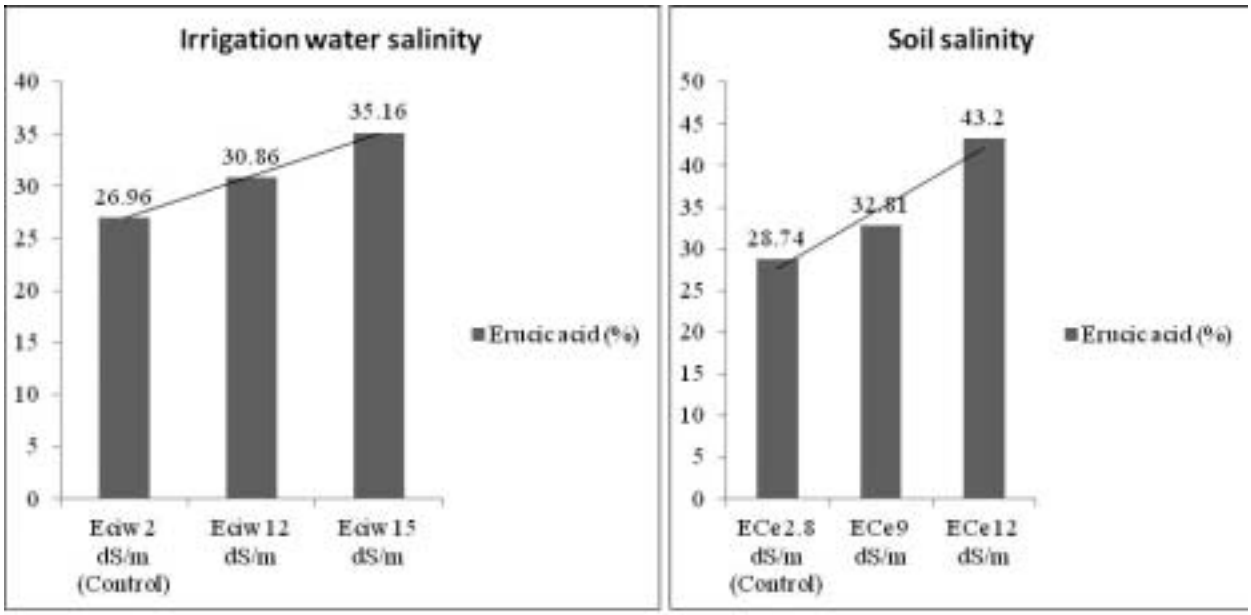

Fig 4: Effect of salinity (water and soil) on erucic acid content of Indian mustard

Table 4: Maximum and minimum values (given in parentheses) of oil and quality traits showed by genotypes at control and higher salinity (soil and water) levels

Location		Control			
		Oil%	Protein%	Erucic Acid%	Crude Fiber%
Field ECe 2.8 dS/m	Max	CS 15000-1-2-2-2-1 (38.9%)	CS 15000-1-2-2-2-1 (20.0%)	CS 54 (30.9)	CS 15000-1-2-2-2-1 (12.35)
	Min	CS 13000-3-1-1-4-2 (38.4%)	CS 54 (19.3%)	CS 13000-3-1-1-4-2 (25.9%)	CS 8000-1-2-8 (11.6%)
Pot EC_{iw} 2.0 dS/m	Max	LES 49 (38.9%)	LES 49 (20.5%)	NPJ184 and CS 2200-2-6 (29.9%)	NPJ177 (12.1%)
	Min	Kranti (38.1%)	Kranti and CS 13000-3-1-1-4-2 (18.8%)	PDZ-1 (24.8%)	LES 49 (10.7%)
		Higher Salinity			
Field ECe 12 dS/m	Max	CS 15000-1-2-2-2-1 (35.6%)	CS 2200-2-6 (16.6%)	CS 2200-2-6 (43.8%)	CS 15000-1-2-2-2-1 (7.5%)
	Min	CS 13000-3-1-1-4-2 (34.5%)	CS 54 (15.2%)	CS 8000-1-2-8 (41.1%)	CS 13000-3-1-1-4-2 (6.5%)
Pot EC_{iw} 15 dS/m	Max	DRMR 15 and CS 54 (36.3%)	PDZ-1 (16.7%)	NPJ184 (39.3%)	NPJ177 (7.9%)
	Min	Kranti (35.2%)	NPJ 182 (15.3%)	PDZ-1 (32.2%)	RGN 298 (6.4%)

15 dS/m compared to the non-saline; whereas, by 42% in the field at a high salinity level of ECe 12 dS/m compared with control (Fig 4).

Increasing erucic acid content with higher salinity may be due to changes in the fatty acids and the ratio of the unsaturated/ saturated in brassica (Wu *et al.*, 2005; Mansour and Salama, 2004). In addition, under conditions of salt stress, fatty acid contents increased compared with normal conditions

probably due to the involvement of some fatty acids in cell wall stability. These fatty acids increase the activity of some of the involved enzymes in salt stress resistance. Different compositions of fatty acids play an important role in the transport of protective compounds such as glycine- betaine (Xu *et al.*, 2001).

The genotypes which showed minimum reduction in oil, protein, crude fiber content and least increment in erucic acid content at higher salinity (both soil and water) may be used as potential donor in the Indian mustard breeding programme for oil and quality parameters (Table 4). The comparative study of salinity in pot and field showed that soil salinity at ECe 9 dS/m have similar effect as irrigation water salinity at EC_{iw} 12 dS/m over the oil and quality parameters in mustard.

Conclusions

The majority of plant breeders working on bio-saline problems have treated salinity stress as NaCl. While convenient, such artificial formulations do not reflect the major ion compositions of naturally occurring saline soils. Expectations that a line selected for tolerance to NaCl alone will exhibit equal performance under ionically more complex saline conditions are unreasonable. Selection for salt tolerance is difficult as the soil shows patches and many a times selection may be misleading. In any given environment, the concentration of soluble salts changes temporally and spatially. Sources of irrigation water are also likely to change in their quality during the course of the growing season. These represent important variables that must be monitored and assessed for the development of appropriate breeding strategies. Considerations must be given to breed for production in a specific saline environment.

Although with the result of salt increasing level oil, protein and crude fibre content were decreased, but increased erucic acid content. This reduction and increment varied for different genotypes. In this experiment oil, protein and crude fibre content decreased 7-10%; 18-19% and 36-42%, respectively, while erucic acid content increased by 30-42% compared to control salinity level in mustard varieties released for normal soil conditions.

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