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SALINITY RESPONSE OF SOME AMPHIDIPLOIDS SPECIES OF BRASSICA (*BRASSICACEAE*) AT SEEDLING AND MATURITY STAGES

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Abstract

Studies were conducted to evaluate salt tolerance in some amphidiploids species of *Brassica (*Family: *Brassicaceae)* at seedling stage (under gravel culture) and at maturity (under saline field conditions). Ten locally adopted genotypes of *Brassica* (five genotypes of *Brassica napus* and five of *Brassica juncea)* were collected from NIA, Tandojam, NIFA Peshawer and ARI Tandojam. Three treatments were imposed (i.e. control, 6.0 and 9.0 dS/m NaCl). It was observed that at early seedling stage the genotypes Early raya & Toria selection of *Brassica juncea* species and Waster & Dunckled of *Brassica napus* species had better response to NaCl stress. The better performance of these genotypes under salinity stress might be due to their better osmotic adjustment. The genotype NIFA raya and Durr-e-NIFA of *Brassica juncea* and *Brassica napus* species were found more sensitive to NaCl stress, respectively. The physiological studies with respect to osmotic adjustment showed that almost all the genotypes had enhanced proline accumulation however in tolerant genotypes the relative increase (%) was higher. It was also observed that the tolerant genotypes had high K/Na ratio as compared to sensitive ones. The genotypes were also evaluated under natural saline field conditions, where salinity ranged from medium to very high (i.e. 8-16 dS/m). In the field no growth was observed above15 dSm^{-1}. Observation recorded in terms of plant height, number of branches $plant^{-1}$, number of slique $plant^{-1}$, slique length, grain wt $plant^{-1}$, grain yield $plot^{-1}$ and 100 grain wt., showed that among *Brassica juncea* genotypes, Early raya had < 50% decrease under saline condition in all the growth parameters followed by Sultan raya having < 50% reduction in 7 growth parameters. Whereas, among the *Brassica napus* genotypes Waster and Abaseen-95 performed better, showing < 50% decrease in all the growth parameters under saline field conditions. Based on these studies it is concluded that Early raya and Waster are the suitable genotypes to perform better under saline field conditions.

Key words: *Brassica* species, Salinity, Solute accumulation, Seedling and maturity stage.

Introduction

Rape-seed (*Brassica napus* L.) and mustard (*Brassica juncea* L. Czern.) contribute about 16% of the domestic production of edible oil in Pakistan, (Tanveer *et al.*, 2002). It is widely adopted in irrigated and non-irrigated areas of Pakistan under different soil conditions. In Sindh province it is mostly cultivated in September after the harvest of rice crop (Bhatti & Soomro, 1996). Though the brassica species are included in salt tolerant category (Mass & Hoffman, 1977), its yield decline is very high above the threshold values (i.e., 9.7 dS/m). *Brassica juncea* and *Brassica napus*, which are the natural amphidiploids of genus Brassica, are assumed to have more tolerance than diploid species (Ashraf and McNeilly, 2004). Higher salt tolerance in amphidiploids of genus Brassica has been acquired from the A genome of (*Brassica compestris*) and C genome of (*Brassica oleracea* L.) or A genome of (*Brassica compestris*) and B genome of (*Brassica nigra*). Variations in salt tolerance among the genotypes of Brassica at seed germination and other growth stages are well reported. The differences also occur in their physiological response eg. electrolyte leakage, proline accumulation and the K/Na (Puppala *et al.*, 1999); Mer *et al.*, 2000; Bybordi, 2010; Tunuturk *et al.*, 2011; Zamani *et al.*, 2010). According to Munns (2002), sensitive cultivars accumulate toxic ions more quickly than tolerant cultivars and this leads to leaf death and then absolute plant death. It is therefore, necessary to evaluate suitable Brassica genotypes on the basis of growth and yield performance under saline conditions. The present study is designed to evaluate salt tolerance in some locally adopted genotypes of amphidiploid species of genus Brassica (*Brassica napus* and *Brassica juncea*) and their physiological response at early seedling stage and under natural saline field conditions at crop maturity.

Material and Methods

Salt tolerance in *Brassica* at early seedling stage: The study was conducted at net house in cemented tanks (size 3.75 x 9.75 m), filled with coarse gravel (up to 30 cm depth) and a thin layer of river sand (2.5 cm depth). The experiment was laid out in randomized complete block design (RCBD) with three replicates. Three salinity treatments (i.e. control (1.56), 6.0 and 9.0 dSm^{-1}), were imposed gradually after two weeks of germination, through commercial sodium chloride (NaCl) salt. The beds were irrigated with modified Hoagland solution using commercial nutrients salts (Hoagland and Arnon, 1950). The experiment was terminated after three weeks of salinity treatment. Growth observations were recorded in terms of Shoot & Root length, Shoot & Root Fresh wt and Shoot & Root Dry wt. Plant samples (leaves) were collected, washed thoroughly and were subjected for the analysis of organic (proline) and inorganic (Na, K and K/Na ratio) solutes. Proline was estimated according to the method of Bates *et al.,* (1973). Sodium (Na^+) and Potassium (K^+) contents were estimated by flame photometer according to the standard methods as reported by Jackson (1962).

Salt tolerance in *Brassica* sp. under normal and saline soil (field studies): Brassica genotypes were evaluated under normal and saline field conditions at NIA, experimental farm. Two plots of size (6 m x 15 m) were selected on the basis of visual observation. Soil samples were collected at 0-30 cm depth. The values for electrical conductivity of non-saline site were less than 4.0 dSm^{-1}, ranged b/w 1.06–3.34 dSm^{-1}. On the other hand the selected saline site was patchy saline, where the salinity was gradually increasing from slightly saline (ECe > 4.0 dSm^{-1}) to very highly saline (ECe ≥ 20.7 dSm^{-1}). However, the genotypes were planted on selected high saline patches, where ECe ranged from 12 - 16 dSm^{-1}. The physio-chemical properties of the soil at both sides are presented in Table 1. To maintain the uniformity of soil salinity, sowing was done on small plots. The size of small sub plots was 1.35 m^2 (i.e. 0.9 m x 1.5 m). Three rows of 1.5 meter length were planted in each sub plot. The row spacing was 30 cm. The experiment was laid out according to randomized block design (RBD), with three replicates. Growth parameters (i. Plant height, ii. Number of branches, iii. Number of slique / plant, iv Slique length, v.Number of grain / slique, vi. Grain weight/ plant, vii. 100 grain weight, viii. Grain yield / plot) were recorded at crop maturity. The growth data regarding all the traits measured at crop maturity stage were statistically analyzed using analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) by MSTAT-C computer package (Anonymous, 1991). Oil contents in brassica seeds were determined in tolerant and sensitive genotypes using the Soxhlet extraction method (Anon., 2000).

Results

Salt tolerance in *Brassica* at early seedling stage: There was gradual decrease in shoot length with increasing salinity treatments (Table 2). Comparatively less decrease was observed at 6 dS/m (low salinity level) than at 9 dS/m (i.e. high salinity level). Least reduction in shoot length among the *Brassica juncea* genotypes was observed in Early raya (25.8%), whereas, maximum reduction was recorded in P-78(63.01%) at 9dS/m salinity. In case of *Brassica napus* species all the genotypes performed well with <50% relative decrease. The least affected genotype at 9.0 dS/m salinity was Dunckled showing 36.21%, while maximum reduction in shoot length was observed in Durre-e- NIFA. 47.42%.

Decrease in root length was also significant with increasing salinity level, reduction was high at 9 dS/m as compared to 6 dS/cm salinity level.The data with respect to individual genotypes also showed variation among the genotypes. The genotype Toria selection of *Brassica juncea* genotypes had maximum root length at high salinity levels. However, the least reduction at 9.0 dS/m was recorded as 9.62% in Early raya. Whereas, in case of *Brassica napus* species, maximum root length at 9 dS/m salinity levels was observed in Dunckled (5.67cm) with relative decrease of only 14.1% .The most affected genotypes were P-78 of *Brassica juncea* and Abaseen- 95 of *Brassica napus*, showing 31.3 and 28.5 % reduction in root length at highest salinity treatment, respectively.

The effect of salinity treatments on shoot fresh weight was also significant among the species and genotypes as well. Reduction in shoot fresh weight (SFW) was more prominent under 9.0 dS/m salinity levels as compared to 6.0 dS/m salinity levels. The reduction was > 50% in all the tested genotypes except Waster, where < 50% reduction was in shoot fresh weight was recorded. Among *Brassica juncea* genotypes, Early raya and Toria selection performed better (i.e. 9.66 and 9.54g), respectively. Whereas, minimum shoot fresh weight was recorded in P-78 (i.e. 4.65g). Variation among the individual genotypes of *Brassica napus* was also evident. Maximum values for shoot fresh weight at high salinity treatment were observed in Waster. While minimum values were recorded in Durr-e-NIFA (i.e., 5.27g).

There was also reduction in root fresh weight (RFW) due to salinity. Reduction at high salinity (9.0 dS/m) treatments was comparatively high, showed an overall 64.41 and 50.0% reduction in *Brassica juncea* and *Brassica napus* species, respectively.The genotype Sultan raya performed better among *Brassica juncea* species, showing maximum RFW (i.e. 0.245g) at 9 dS/m salinity treatment, whereas, the minimum RFW was recorded in NIFA raya (i.e. 0.167g). The data with respect to the genotypes of *Brassica napus* species showed that except Dunckled all the genotypes had less than 50% reduction in RFW. Maximum root fresh weight at high salinity treatment were observed in Durr-e- NIFA (0.27g) and minimum RFW were recorded in Shiralee (i.e. 0.16 g).

Presence of salinity was also found to reduce shoot dry weight (SDW) in genotypes of both *Brassica* species. Maximum SDW at 9.0 dS/m, in *Brassica juncea* genotypes was 0.63g in Toria selection and minimum in NIFA-raya (i.e. 0.30 g). In *Brassica napus* genotypes except Waster almost all the genotypes had more than 50% reduction at high salinity treatment (i.e. 9.0 dS/m). The SDW was 0.88g in Waster at 9.0 dS/m salinity and the least values for SDW were recorded in Durr-e- NIFA (i.e. 0.23 g).

Root dry weight (RDW), also decreased significantly, due to the presence of salinity of the medium. Reduction in RDW was > 50% in both *Brassica* species. The mean reduction in RDW was 72% for *Brassica juncea* genotypes and 62.5% for *Brassica napus* genotypes at 9.0 dS/m salinity treatments. Among the genotypes of *Brassica juncea*, maximum RDW at 9.0 dS/m, was recorded in Early raya (0.035g), whereas, as minimum in NIFA-raya (i.e. 0.019g). Root dry weight in case of *Brassica napus* genotypes was maximum in Waster (0.039g) at 9.0 dS/m salinity. On the other hand, minimum values for RDW were recorded in Durr-e-NIFA (i.e. 0.026g).

Table 1. Physiochemical properties of experimental sites (Non saline and Saline).

Sites	Soil properties							
	Soil texture	Soil ECe (dSm^{-1})	Soil pH	Na (meq L^{-1})	K (meq L^{-1})	Ca + Mg (meq^{-1})	SAR	Salinity class
Non saline	Silty clay loam	1.2 ---3.42	7.90 ---8.10	11.0 --- 35.0	2.5 --- 2.8	10.0 --- 22.0	4.9 --- 15.5	Non Saline
Saline	Silty clay loam	12.0 ---21.0	7.70 ---8.00	217 --- 670	1.0 –1.8	41.0 --- 41.5	48 --- 148	Saline - Sodic

Table 2. Performance of Brassica genotypes at early seedling stage under controlled conditions (Gravel culture).

Genotypes	Shoot length			Root length			Shoot fresh weight			Root fresh weight			Shoot dry weight			Root dry weight		
	Cont	6.0 dS/m	9.0dS/m	Cont	6.0 dS/m	9.0 dS/m	Cont	6.0 dS/m	9.0 dS/m	Cont	6.0 dS/m	9.0 dS/m	Cont	6.0 dS/m	9.0 dS/m	Cont	6.0 dS/m	9.0 dS/m
	Brassica napus																	
Durr-e–NIFA	15.95 cd	11.67 ef	9.73 ghi	6.20 abcd	5.70 cdef	5.60 cdefg	24.83 bc	10.57 fg	5.27 gh	0.50 a	0.30 de	0.27 def	1.46 abc	0.98 cde	0.23 g	0.06	0.05	0.03
Abaseen-95	18.77 ab	14.93 d	9.87 fghi	6.67 a	5.80 bcde	4.77 ghi	36.23 a	21.37 cd	8.41 fgh	0.50 a	0.44 abc	0.26 def	1.85 a	1.74 a	0.60 defg	0.09	0.08	0.03
Shiralee	16.41 cd	12.27 e	10.17 fghi	6.37 abc	4.13 i	5.10 efgh	14.50 ef	10.85 fg	6.74 gh	0.32 cde	0.29 de	0.16 fg	0.83 def	0.78 defg	0.50 efg	0.052	0.05	0.03
Waster	19.57 a	14.63 d	10.40 fghi	6.57 ab	5.70 cdef	4.95 fghi	21.40 cd	18.60 de	10.89 fg	0.47 ab	0.33 cde	0.23 efg	1.72 a	1.64 ab	0.88 def	0.08	0.08	0.04
Dunkled	17.24 bc	17.17 e	11.00 efg	6.60 ab	5.50 defg	5.67 cdef	29.50 b	9.93 fgh	6.90 gh	0.50 a	0.37 bcd	0.22 efg	1.98 a	1.10 bcd	0.51 efg	0.10	0.04	0.03
Mean	**17.59 A**	**13.13 B**	**10.17 C**	**6.48 A**	**5.37 B**	**5.22 B**	**25.29 A**	**14.26 B**	**7.64 C**	**0.46 A**	**0.35 B**	**0.23 C**	**1.57 A**	**1.25 A**	**0.54 B**	**0.08**	**0.06**	**0.03**
LSD (0.05)	1.85			0.84			6.23			0.128			0.55			NS		
	Brassica juncea																	
NIFA raya	18.50 bc	13.6 f	8.80 j	6.57 abc	5.63 cdefg	4.70 gh	26.03 c	11.87 f	4.85 hi	0.57 b	0.23 de	0.16 def	1.47 cd	0.95 e	0.30 g	0.085 abc	0.042 bcde	0.019 de
Sultan raya	18.63 bc	14.33 ef	10.33 i	6.27 bcd	4.93 gh	5.43 defg	16.50 e	15.93 e	5.12 h	0.37 c	0.37 c	0.25 d	1.57 bcd	1.38 d	0.41 fg	0.123 a	0.062 bcde	0.022 de
Toria Selection	21.47 a	17.83 cd	11.77 h	7.23 a	6.03 cdef	5.57 defg	34.30 a	28.30 b	9.54 g	0.53 b	0.53 b	0.20 def	1.75 ab	1.75 ab	0.63 f	0.092 ab	0.089 ab	0.032 de
Early raya	16.67 d	15.17 e	12.37 gh	5.64 cdefg	5.13 efg	5.10 fg	22.13 d	17.40 e	9.67 g	0.77 a	0.40 c	0.21 def	1.81 ab	1.71abc	0.62 f	0.085 abc	0.070 bcd	0.035 cde
P-78	19.47 b	13.17 fg	7.20 k	7.13 a	6.07 cde	4.90 gh	28.43 b	16.10 e	4.65 hi	0.70 a	0.35 c	0.20 def	1.87 a	1.11 e	0.41 fg	0.091 ab	0.059 bcde	0.024 de
Mean	**18.95 A**	**14.83 B**	**10.09 C**	**6.57 A**	**5.56 B**	**5.14 BC**	**25.48 A**	**17.92 B**	**6.76 C**	**0.59 A**	**0.38 B**	**0.21 C**	**1.69 A**	**1.38 B**	**0.47 C**	**0.095 A**	**0.064 AB**	**0.026 BC**
LSD (0.05)	1.27			0.95			1.47			0.10			0.26			0.05		

Table 3. Performance of Brassica genotypes under non-saline (Control) and saline field conditions.

Genotypes	Plant height		No. of slique plant^{-1}		Slique length (cm)		No. of grains slique^{-1}		Grain wt plant^{-}		100 grain wt.		[1]Grain yield plot^{-1}	
	Cont.	Saline	Cont.	Saline	Cont.	Saline	Cont.	Saline	Cont.	Saline	Cont.	Saline	Cont.	Saline
	Brassica napus													
Durr-e–NIFA	142 a	103 b	238b	224ab	7.38 a	7.10a	22.5 a	19.6abcd	17.0 ab	7.4d	0.32 ab	0.32 ab	259 b	89 d
Abaseen-95	154 a	132 ab	275 ab	225ab	6.88a	6.26 ab	20.6 ab	19.4abcd	19.0 a	12.9abcd	0.32 ab	0.32 ab	309 bc	268 bcd
Shiralee	164 a	130 ab	212 b	206b	7.48 a	6.20 ab	22.5 a	16.8 cd	8.1 cd	7.6 cd	0.42 a	0.42 ab	548 a	198 bcd
Waster	178 a	167 a	370 a	330 a	7.25 a	5.27 b	20.1 abc	17.3 bcd	15.0 ab	14.2 abc	0.36 ab	0.36 ab	350 ab	241 bcd
Dunkled	176 a	132 ab	216 b	212 b	7.74 a	6.77 a	22.0 a	16.3 d	10.5 bcd	7.4 cd	0.33 ab	0.33 ab	282 bcd	123 cd
Mean	**163 A**	**132 B**	**262 A**	**239 A**	**7.35 A**	**6.32B**	**21.54 A**	**17.88B**	**13.93 A**	**9.9 B**	**0.35 A**	**0.35 A**	**350 A**	**183 B**
LSD (0.05)	38.36		114		1.127		3.76		6.83		0.110		206	
	Brassica juncea													
NIFA- Raya	191 a	110 a	605 a	542 a	4.03 c	4.00 b	13.0 b	12.0 b	13.1 bc	3.2 b	0.31 b	0.26 b	685 a	203 b
Sultan Raya	157 ab	118a	449 ab	330 ab	5.54 ab	4.93 a	14.0 b	13 .0 b	18.3 ab	14.4 a	0.71 a	0.45 a	463 ab	189 b
Toria selection	132 b	128 a	268b	118 b	6.11 a	5.08 a	20.0 a	18.0 a	11.6 c	3.4 b	0.34 b	0.22 b	426 b	187 b
Early raya	184 a	115 a	410 ab	399 ab	4.76 bc	4.35 ab	14.0 b	12 .0 a	13.0 bc	11.0 a	0.37 b	0.33 ab	460 ab	233 ab
P-78	183 a	141 a	425 ab	386 ab	5.39 ab	4.95 a	14.0 b	13 .0 b	19.5 a	13.9 a	0.44 b	0.34 ab	555 ab	466 a
Mean	**169 A**	**122B**	**431A**	**355 A**	**5.16 A**	**4.66B**	**15.0**	**13.6 B**	**15.1**	**9.18**	**0.43 A**	**0.32 B**	**256**	**256**
LSD (0.05)	47.60		296		0.795		NS		6.27		0.133		248	

Salt tolerance in *Brassica* sp. under normal and saline soil (field studies): Brassica genotypes were also evaluated for salt tolerance under natural saline field. Growth and yield parameters were recorded at the crop maturity (Table 3).

It was observed that there was a significant decrease in plant height under saline condition. Comparatively higher values of plant height were recorded in *Brassica juncea* genotypes then *Brassica napus* genotypes under both saline and non saline conditions. The average values for the genotypic means of two species of Brassica under control and saline environments were 163 &132 cm for *Brassica napus* species and 169 & 123cm for *Brassica juncea* species. Maximum plant height of *Brassica napus* species in saline soil was observed in Waster i.e., 167cm, with only 6.18% rel. dec. While maximum reduction was observed in Durr-e-NIFA (27.5%). There was also a significant decrease in plant height in all the *Brassica juncea* genotypes under saline field. Plant height ranged from 157 to 191 cm under normal soil condition and 142 to 110 cm under saline conditions. The genotype Toria selection had minimum decrease (2.8%) and maximum decrease was observed in NIFA raya i.e., 42.2%.

The effect of salinity on slique formation was significant. All the genotypes of both Brassica species showed decrease in number of slique plant^{-1}. Relative decrease in *Brassica napus* genotypes was 2 to 20%, whereas, in *Brassica juncea* genotypes, the relative decrease was between 3 to 55%. Among the individual genotypes of Brassica species, maximum number of slique under saline conditions observed in NIFA- raya of *Brassica juncea* and Waster of *Brassica napus* species, showing 542 and 330 slique plant^{-1}, respectively. However, least decrease under saline condition was observed in Dunckled of *Brassica napus* (1.85%) and Early raya of *Brassica juncea* species (2.68%).

There was a significant decrease in slique length of all the genotypes of both Brassica species. All the genotypes were showing < 50% decrease under saline condition. Reduction in slique length was high in *Brassica napus* genotypes than *Brassica juncea* genotypes. Mean values under two environments (non saline and saline), were recorded as 7.35 and 6.32 in *Brassica napus* genotypes and 5.16 and 4.66 cm in *Brassica juncea* genotypes, respectively. Among the individual genotypes of *Brassica napus* species, maximum slique length under saline condition was observed in Durr-e- NIFA (7.10 cm) with least reduction of 3.75%. On the other side in genotypesof *Brassica juncea* species values for slique length were maximum in Toria selection (5.08 cm). It was also observed that though the values of slique length under salinity were maximum in Toria selection but the relative decrease was comparatively high (i.e.16.86%), whereas least decrease was observed in genotype Durr-e- NIFA (i.e. 0.74%).

Grain formation in Brassica genotypes was also reduced under salinity. Number of grains slique^{-1} in *Brassica napus under* non saline and saline conditions were 21.54 and 17.88, respectively. Grain numbers in genotypes of *Brassica juncea* under two environments were 15.0 and 13.6 under non saline and saline conditions, respectively. Among the genotypes of *Brassica napus* species maximum number of grains were observed in Durr – e -NIFA under saline conditions (i.e. 19.6). While decrease due to salinity was maximum in Dunckled (i.e. 26%). Similarly in case of *Brassica juncea* genotypes, maximum grains slique^{-1} were observed in Toria selection (i.e. 8.0) under saline conditions. The relative decrease was minimum in Sultan raya and P-78 (7.14%), However, all the genotypes of both *Brassica* species have <50% reduction under salinity.

Grain weight of brassica genotypes also reduced due to soil salinity. Mean values for the genotypes of *Brassica napus* species were 13.93 and 9.90 g plant^{-1}, under non saline and saline condition, respectively. Whereas, in case of *Brassica juncea* the grain weight was 15.1 g under non-saline and 9.18 g under saline field condition. Among the genotypes of *Brassica napus*, maximum grain wt. was observed in Waster (i.e. 14.20 g plant^{-1}), with least reduction of only 5.0%. Almost all the genotypes of *Brassica napus* had <50% reduction except Durr-e-NIFA. The reduction among the genotypes of *Brassica juncea*, species was comparatively more. Maximum grain wt. plant^{-1} under salinity was observed in Sultan raya (i.e. 14.40 g plant^{-1}), with relative reduction of 21.31%. The genotype Early raya comparatively had less grain wt plant^{-1} than Sultan raya, but the relative reduction was minimum (15.38%).

Grain yield of *Brassica juncea* genotypes was comparatively higher than *Brassica napus* genotypes (Table 3).The mean grain yield in *Brassica juncea* genotypes were recorded as 518 & 256 g plot^{-1} and 350 & 185 g plot^{-1} in *Brassica napus* genotypes, under non saline and saline condition, respectively. Among the individual genotypes of *Brassica napus* Abaseen -95 showed maximum grain yield (268 g plot^{-1}). The relative reduction was 13.27%. Grain yield in *Brassica juncea* genotypes also decreased significantly due to salinity. Among the individual genotypes the only genotypes showing < 50% reduction was P-78 having grain yield 466 g plot^{-1} with 16.04% relative decrease. The genotype Early raya was on the margin with grain yield 233 g plot^{-1} showing 49.35% decrease under salinity.

Almost all the genotypes had < 50% reduction in 100 grain weight. Mean decrease was 25.58% in *Brassica juncea* and 5.71% in *Brassica napus*. The data with respect to relative decrease under salinity showed that Waster and Abaseen-95 were the best, where the relative decrease was almost nil. All the genotypes showed < 50% reduction under salinity. In genotypes of *Brassica juncea* species, maximum values for hundered grain were recorded in Sultan raya (0.45 g). However, the relative reduction under saline conditions was minimum in Agati Sarheen i.e. 10.31% dec.

The growth performance of Brassica genotypes are summarized on the basis of < 50% reduction in different agronomical parameters (Table 4). It was observed that among the *Brassica napus* genotypes Abaseen-95 and Waster performed best, where relative decrease in all the recorded variables was less than 50%. Similarly among genotypes of *Brassica juncea* species, Early raya showed <50% decrease in all the growth parameters. Therefore Abaseen -95, Waster and Early raya were classed as tolerant genotypes (T). On the other hand, the genotypes Durr- e-NIFA & Shiralee of *Brassica napus* and NIFA raya & Toria selection of *Brassica juncea* species had > 50% relative decrease in two to three variables, showing sensitivity to salinity especially in case of grain yield and hence categorized as sensitive (S) to medium sensitive (MS).

Table 5. Summarized results of two studies (Gravel culture and field) for the selection of salt tolerance genotypes of *Brassica* species.

Categories	Species	Gravel culture studies (Early seedling stage)	Field studies (Maturity stage)	Genotypes common in two studies
Better performing	*Brassica juncea*	Early raya Toria selection	Early raya Sultan raya, P-78	Early raya
Brassica genotypes	*Brassica napus*	Wester Dunckled	Wester Abaseen-95	Wester
Poor performing	*Brassica juncea*	NIFA- Raya	NIFA- Raya	NIFA- Raya
Brassica genotypes	*Brassica napus*	Durr -e - NIFA	Durr -e - NIFA	Durr -e - NIFA

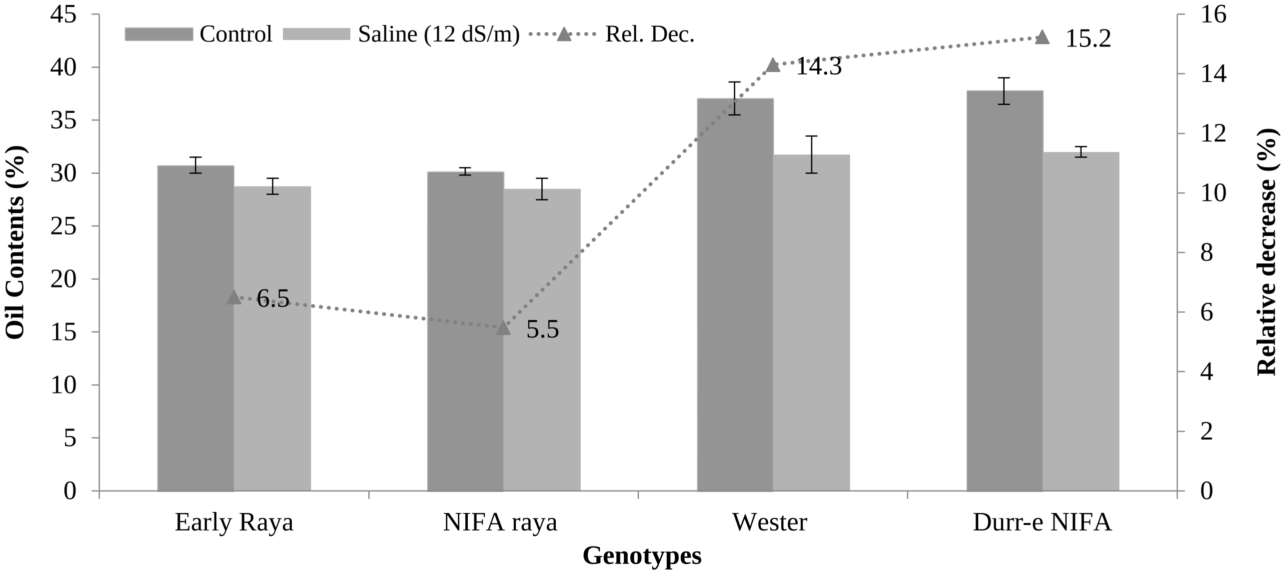

Fig. 1. Oil content (%) in tolerant and sensitive genotypes of two *Brassica* species.

The results of the two studies were also summarized to categorize Brassica genotypes into different categories (Table 5). Based on these studies (gravel culture and saline field), it is concluded that Early raya (*Brassica juncea*) and Waster (*Brassica napus*) are the suitable genotypes to perform better under saline field conditions.

The grain samples of tolerant and sensitive genotypes were analyzed for oil content in grain (Fig. 1). The data showed that the genotypes of *Brassica juncea* had comparatively less oil contents as compared to *Brassica napus* genotypes. The oil content under normal soil condition in *Brassica juncea* genotypes ranged between 30-31% and in *Brassica napus* genotypes it ranged as 37-38%. There was a decrease in oil content under saline environments in all the genotypes of both *Brassica species i*rrespective of tolerant and sensitive. Singh *et al.*, (2014) also reported significant reductions in oil, protein and fiber contents with increased erucic acid content in Brassica cultivars in response to salt stress. The decrease in oil contents was more in *Brassica napus genotypes* than the genotypesof *Brassica juncea*. The oil content under saline condition was 29% in *Brassica juncea* genotypes and in *Brassica napus* genotypes it was 32%. The data for the relative decrease under salinity showed that both the *Brassica juncea* genotypes had only 5-6% decrease in oil content under salinity. In *Brassica napus* genotypesthevalues for the relative decrease were bit higher (i.e.14 to 15 %) under salinity.

Solute accumulation: Accumulation of organic and inorganic solutes plays a vital role in osmotic adjustment of plant. Therefore organic (Proline) and inorganic (Na and K) solutes were also estimated in leave samples (Table 6). Proline accumulation in plant increased with the increasing salinity treatments, showing two to three folds higher accumulation under both salinity levels (i.e. 6 and 9 dS/m) than plants under non-saline treatment. Proline accumulation in two *Brassica* species at control, 6.0 and 9.0 dS/m salinity was ranged as (9.91, 29.64 and 29.47ug/g F wt) in *Brassica juncea* and (11.15, 29.42 and 29.18 ug/g F wt) in *Brassica napus* species, respectively. Proline accumulation at 9 dS/m salinity level among *Brassica juncea* genotypes was maximum in Sultan raya (30.59 ug/g F.wt), showing non-significant differences with NIFA raya, Early raya and P-78 when compared statistically. Similarly in case of *Brassica napus* genotypes the values for proline accumulation were also at par within Durr-e-NIFA, Shiralee and Waster genotypes (30.3 to 32.53 ug/g F.wt). Minimum proline accumulation was observed in Dunckled (24.08 ug/ g F.wt.) at 9.0 dS/m salinity treatment.

Table 4. Results showing < 50% reduction in different growth variables are classed in to different tolerance categories.

Genotypes	Plant height	No. of branches plant^{-1}	No. of slique plant^{-1}	Pod length (cm)	No. of grains slique^{-1}	Grain wt plant^{-1}	Grain yield Plot^{-1}	100 grain wt.	Variables < 50% red.
					A. *Brassica napus*				
Durr -e - NIFA	+	+	+	+	+	-	-	+	**6 (MS)**
Abaseen-95	+	+	+	+	+	+	+	+	**8 (T)**
Shiralee	+	+	-	+	+	+	-	+	**6 (MS)**
Waster	+	+	+	+	+	+	+	+	**8 (T)**
Dunkled	+	+	+	+	+	+	-	+	**7 (MT)**
					B. *Brassica juncea*				
NIFA- Raya	+	+	+	+	+	-	-	+	6 (MS)
Sultan Raya	+	+	+	+	+	+	-	+	7 (T)
Toria selection	+	+	-	+	+	-	-	+	5 (S)
Early raya	+	+	+	+	+	+	+	+	8 (T)
P-78	+	+	+	+	+	+	+	+	7 (MT)

Sensitive = (S). MS = Medium sensitive, MT = Medium tolerant, and T = Tolerant

Table 6. Solute accumulation (In-organic and organic) in leaves samples grown under control, 6.0 and 9.0 dS/m salinity treatments.

Genotypes	Na (%)			K (%)			K/Na ratio			Proline (µ mole g^{-1} F. wt)		
	Cont.	6.0 dSm^{-1}	9.0 dSm^{-1}	Cont.	6.0 dSm^{-1}	9.0 dSm^{-1}	Cont.	6.0dSm^{-1}	9.0 dSm^{-1}	Cont.	6.0 dSm^{-1}	9.0 dSm^{-1}
						Brassica napus						
Durr -e - NIFA	0.9 Klm	1.2 cdefgh	1.1 cdefghi	0.7 bcde	0.6 cdefg	0.5 defghi	0.7cd	0.5 fg	0.4 fgh	11.5 e	32.1 Ab	32.5ab
Abaseen-95	0.9 klm	1.0Ijkl	1.1 defghijk	1.3a	0.6 cdef	0.6 cdefgh	1.1a	0.6 def	0.5 efg	11.2 e	29.3 abc	29.2abc
Shiralee	1.0 jklm	1.0 fghijkl	1.3 ab	0.7bc	0.5 cdefghi	0.5 defghi	0.8 bc	0.5 efg	0.4 ghi	12.8 e	28.3bcd	30.0 ab
Waster	0.9 M	1.0 hijkl	1.0 ghijkl	0.8 b	0.5 cdefgh	0.7 bcd	0.9 ab	0.5 defg	0.7 cde	8.8 e	25.1 cd	30.1 ab
Dunkled	1.1 efghijkl	1.2 cdef	1.2bcd	0.8 b	0.6 bcde	0.5 efghi	0.8 bc	0.5 defg	0.4 ghi	11.2 e	32.3 Ab	24.1 d
Mean	**0.97 C**	**1.1 B**	**1.2 AB**	**0.9 A**	**0.6 B**	**0.5 B**	**0.8 A**	**0.5 B**	**0.5 B**	**11.1 C**	**29.4 B**	**29.2 B**
LSD (0.05)	0.14			0.22			0.17			4.6		
						Brassica juncea						
NIFA- raya	0.9 gh	1.0 efgh	1.7 a	1.0 ab	0.6 cd	0.5 d	1.2 ab	0.6 efg	0.3 hi	9.0 d	29.8 abc	30.4 abc
Sultan raya	0.8 gh	1.0 fgh	1.2 cdef	1.1 a	0.6 cd	0.5 d	1.4 a	0.7def	0.4 ghi	11.6 d	31.2 ab	30.6 abc
Toria selection	1.2 def	1.4 abc	1.5 ab	0.8bc	0.6 cd	0.5 d	0.7 def	0.5 fgh	0.4 hi	8.3 d	28.1 bc	27.0 c
Early raya	0.9gh	0.8 h	1.3 bcd	1.1a	0.6 cd	0.7 cd	1.2 ab	0.9 cd	0.5 efgh	9.6 d	29.9 abc	30.0 abc
P-78	1.0fgh	1.0fgh	1.2 cdef	1.0ab	0.7 cd	0.5 de	1.0 bc	0.7 de	0.4 gh	11.1 d	29.1 bc	30.0 abc
Mean	**0.96 B**	**1.0 B**	**1.4 A**	**1.0 A**	**0.65 B**	**0.54 B**	**1.1 A**	**0.68 B**	**0.4 C**	**9.9 B**	**29.6 A**	**30.0 A**
LSD (0.05)	0.31			0.23			0.25			3.85		

There was gradual increase of sodium (Na^+) uptake in Brassica genotypes due to increasing salinity levels. Na accumulation was comparatively more in *Brassica juncea* than *Brassica napus*. Mean Na^+ contents were ranged as (0.96, 1.02 and 1.40 %) in *Brassica juncea* and (0.87, 1.02 and 1.04 %) in *Brassica napus* at control, 6.0 and 9.0 dS/m salinity levels. Minimum Na^+ accumulation at 9.0 dS/m salinity levels was observed in P-78 (1.21%) and Waster (1.04%), among *Brassica juncea* and *Brassica napus* genotypes, respectively. On the other hand maximum Na^+ contents at 9.0 dS/m salinity level were recorded as 1.54% and 1.32% in Toria selection (*Brassica juncea*) and Shiralee (*Brassica napus)*, respectively.There was an overall decrease in potassium (K^+) uptake with increasing salinity level. Decrease in K^+ uptake in *Brassica napus* genotypes was comparatively less than *Brassica juncea* genotypes. The average K^+ contents were ranged as (1.05, 0.65 and 0.54 %) in *Brassica juncea* and (0.86, 0.56 and 0.53 %) in *Brassica napus* at control, 6.0 and 9.0 dS/m salinity. At high salinity patches, Potassium contents in Early raya and Waster were found maximum i.e., 0.65 and 0.69%, respectively. The genotypes P-78 (0.49%) and Dunckled (0.46%) showed maximum decrease in K^+ among both *Brassica* species. K^+/Na^+ ratio estimated under non saline and saline treatments showed a decreasing trend in K^+/Na^+ ratio with increasing salinity in all Brassica genotypes. The genotypes Early raya of *Brassica juncea* and wester of *Brassica napus,* had higher values of K^+/Na^+ ratio, i.e. 0.49 and 0.67, respectively. On the other hand, minimum K^+/Na^+ ratio values were observed in NIFA-raya and Dunckled i.e. 0.31 and 0.38, respectively.

Discussion

Early vigor at seedling stage ensures healthy plants growth that can face the adverse environmental conditions (Khan & Asim, 1998; Wilson *et al.,* 1999). The growth performance of brassica genotypes at early seedling stage showed significant variations due to salinity stress among all Brassica genotypes (Table 2). Jamil & Rha (2004) also reported significant reduction in sugar beet and cabbage (*Brassica oleracea & Brassica capitata*), due to salinity in the root and shoot length, which might be due to the inhibitory effect of ions (Jamil *et al.*, 2007; Brini *et al.*, 2009; Souhail & Chaabane, 2009; Oueslati *et al.*, 2010). Reduced plant growth in Brassica species as a result of salt stress has also been reported in our earlier studies (Shirazi *et al.,* 2011, Shirazi *et al*., 2015). It is assumed that for a better response of plant under salinity stress roots play a vital role by supplying plant nutrients and water to plant therefore rooting behavior may provide useful information regarding the salt tolerance potential of plants (Khan, 2008). Neumann (1995) also reported that salinity inhibit root growth rapidly and hence reduce water uptake and essential mineral nutrition from soil. All the genotypes of both Brassica species had less than 50% reduction in root length at 9 dSm^{-1} salinity level. Moud, (2008) have the openion that salt stress inhibits coleoptile growth more than root growth. On the contrary, Bybordi (2010) observed more reduction in root length as compared to shoot length in canola cultivars. Presence of salts in the growing medium, were also found to reduce the fresh weight in Brassica genotypes (Table 2). Maximum shoot fresh weight (SFW) at 9 dSm^{-1} was observed in Early raya (*Brassica juncea*) and Waster and (*Brassica napus*). Reduction in fresh weight may be attributed to osmotic effects (Jameel *et al.,* 2005), resulting in low water absorption (Wener & Finkelstein, 1995; Prado *et al.,* 2000). Among *Brassica juncea* species, Sultan raya had maximum root fresh weight (RFW) at 9 dSm^{-1} salinity, whereas, among *Brassica napus*, maximum RFW at high salinity treatment was observed in Durr-e-NIFA. Ashraf *et al.,* (2005) reported that depletion of O_2 deprives the plants of its primary energy source and accumulation of internal ethylene causes the inhibition of root elongation which consequently reduces root fresh and dry biomass. Reduction in shoot and root dry weight (SDW and RDW) due to salinity was also evident. Maximum SDW and RDW, among the genotypes of *Brassica juncea*, were recorded in Toria selection and Early raya (Table 2). The better response for SDW and RDW in *Brassica napus* genotypes was only observed in Waster. The results of the present study at vegetative stage suggest that among the tested genotypes Early raya and Toria selection of *Brassica juncea* and wester of *Brassica napus* have the potential to perform better under saline conditions.

Brassica genotypes were also evaluated under field conditions. It was observed that only those genotypes showed better response that have the genetic potential for salt tolerance at early seedling stage. Among the genotypes tested Early raya and Waster also showed higher values for plant height under saline field conditions (Table 3). The differences among Brassica genotypes in plant height might be due to the differences in genetic background (Sana *et al.,* 2003). Munns *et al.,* (1995) suggest that any varietal diversity in plant growth responses to salinity appears slowly and is caused by genotypic differences in rates of salt accumulation. According to Kingsbury *et al.,* (1984) the accumulations of excessive salts limit the cell wall elasticity and also modify the metabolic activities of the cell. In addition secondary cells appear sooner and cell wall becomes rigid, as a consequence the turger pressure efficiency in cell enlargement decreases. Reduction in plant height with increasing salinity, have also been observed by Ashraf *et al.,* (1999) and Akhtar *et al.,* (2002) in Brassica species. The effect of salinity on slique formation was significant. Sana *et al.,* (2003) reported that, time of flowering and number of silique in plant are critical components to determine grain yield. Salinity stress decreases growth period and consequently, plants decrease the silique number to survive. The numbers of slique were comparatively more in genotypes of *Brassica juncea* than the genotypes of *Brassica napus* (Table 3). Similar trend in slique formation more in genotypes of *Brassica juncea* was observed by Akhtar *et al.,* (2002). According to Lin (2004), reduction in slique number might be associated with the increase of ABA and pollen death. Following the flower decrease there was a fall in the number of siliqe at fruiting phase (Zadeh & Naeini, 2007). Sinaki *et al.,* (2007) also concluded flowering stage as one of the important reason for the reduction of slique in Brassica due to the induction of salinity stress. There was a significant decrease in slique length in all the genotypes of both *Brassica* species (Table 3). Comparatively more reduction in slique length in Waster was observed, however, in all the genotypes the relative reduction was within the economic limits (i.e. < 50%). Reduction in slique length under salinity stress was also reported by Zadeh & Naeini, (2007). Reduction in different yield parameters due to salinity also resulted in reduced grain formation in Brassica genotypes (Table 3).

One of the major reasons for decrease in seed number is the reduction in silique size (Baybordi, 2010). Numbers of grain were also high in *Brassica napus* genotypes as compared to *Brassica juncea* under both the growing environment. Increasing salinity of the soil also reflected on grain weight plant^{-1}. Grain weight plant^{-1} in Brassica genotypes decreased significantly due to salinity of the soil (Table 3). Decrease in seed weight might be due to prevention of assimilate transported to the seeds during seed filling stage. Munns *et al.,* (2006) also observed many disorders in reproductive stages when barley plants were exposed to salinity stress. Among the genotypes of *Brassica napus*, maximum grain weight was observed in Waster with only 5.0% relative decrease. The genotype Early raya of *Brassica juncea* comparatively had less grain wt plant^{-1} then Sultan raya and P-78, but the relative reduction was found minimum (15.38 %). Engqvist Beker (1993) reported seed weight and numbers of silique as most important component for the selection of high yielding genotypes. Mir *et al.,* (2010) had the opinion that maximum slique plant^{-1}, seed pod^{-1} and grain weight, are positively correlated with crop yield. Whereas, Sakr *et al.,* (2007) reported, numbers of seeds as a major growth parameter among yield components. According to Ashraf *et al.,* (1999) reduction in seed yield may be due to decreasing assimilates production associated with decreased plant size and yield. The decrease in yield components due to salinity stress lead to loss of final yield. It seems that ions accumulation in plant tissues at different growth stages is the main reason of yield decrease. The data with respect to relative decrease showed that under salinity in *Brassica napus* Waster and Abaseen-95 were the best. While in *Brassica juncea* Early raya had the minimum reduction (10.31 % dec.) followed by NIFA raya (16.13 % dec.). The results of the two studies were also summarized to categorize Brassica genotypes into different categories (Table 5).The better performance of these genotypes under salinity stress might be due to their better osmotic adjustment because the adaptability of plant under saline conditions depends largely upon better osmotic adjustment under stress. Presence of salts in higher concentration lowers the osmotic potential of the growing media, resulting in less availability of water and essential nutrients. According to Ashraf (2004) among many physiological indicators, osmotic adjustment and ion transport have recently gained ground because there are numerous reports in literature which show that plant with high capacity of osmotic adjustment by excluding ions from the cell or tissue and accumulating low molecular weight organic osmotica, show enhanced tolerance to salt stress (Mehboob *et al*., 2017). This was found true in the present studies; almost all the good performing genotypes of *Brassica napus* showed enhanced accumulation of proline in leaves, whereas, the poor performing genotype Dunckled of *Brassica napus* species showed slightly lower accumulation of proline (Table 6).

In *Brassica juncea* species, the genotypes NIFA-raya and P-78 in spite of its poor performance, showed enhanced accumulation of proline in leaves under NaCl stress. Kitri *et al.,* (1991), observed higher proline accumulation in salt-tolerant *Brassica juncea* plants with better growth than the control. In contrast to this Ashraf (1989) reported a negative relationship between proline accumulation and salt tolerance in *Vigna mungo*. High leaf proline levels in salt-sensitive barley have been well explained by Fidalgo *et al.,* (2004) who were of the opinion that NaCl induced oxidative stress caused an increased H_2O_2 accumulation due to inefficiencies in H_2O_2 scavenging in salt-sensitive potato cultivars, so they produced larger amounts of the antioxidant proline to compensate for the H_2O_2 scavenging. Restriction of Na^+ inside the plant or its restricted accumulation in root ensures better growth under salinity. The genotypes, Early raya and Waster were showing lower uptake of Na^+ ions in leaves. According to Gorham *et al.,* (1997) Sodium gains entry into root cell cytosole through cation channels or transporters (selective and nonselective) or into the root xylem stream via an apoplastic pathway depending upon the plant species. The genotypes NIFA-raya and Dunckled could not restrict the entry of Sodium in the upper plants part (Table 6), which confirmed the previous findings (Abbas *et al*., 2013; Rahman *et al*., 2014; Hasan *et al*., 2015). Increased Na^+ entry inside the plant might be due to increased permeability of cell membrane in these genotypes. It is reported that salt stress induces production of reactive oxygen species (ROS) and leads to oxidative damages. These toxic oxygen species may react with macromolecules and lipid components of membranes causing damage through lipid peroxidation, resulting in increased permeability of the membrane. It is widely accepted that sufficient K^+ uptake coupled with restricted Na^+ accumulation is necessary to tolerate under salinity. Regulation of K^+ uptake and/ or prevention of Na^+ entry, efflux of Na^+ from the cell and utilization of Na^+ for osmotic adjustment are the strategies commonly used by plants to maintain desirable K^+/ Na^+ ratios in the cytosole. In the present study tolerant genotypes are also showing the same trend. The K^+/Na^+ ratio in the tolerant genotypes (i.e. Early raya and Waster), was comparatively higher than the sensitive ones (Table 6). Although some other genotypes (i.e. Dunckled and P-78) also had high K^+ content but due to higher accumulation of Na^+ they could not maintain desirable K^+/Na^+ ratio inside the cell. According to (Zhu, 2002), Na^+ competes with K^+ uptakes through Na^+-K^+ co-transporters, and may also block the K^+ specific transporters of root cell under salinity. Hence it is concluded that the adaptability of tolerant Brassica genotypes might be due to low accumulation of Na^+, resulting in high K^+/Na^+ ratio for turgor maintenance. The results are in agreement with the findings of Ashraf & McNeilly (2004) who suggested the maintenance of high tissue K^+/Na^+ ratio as important selection criteria for salt-tolerance in *Brassica* species.

Based on these studies (seedling stage, crop maturity stage under saline field condition and solute accumulation), it is concluded that Early raya (*Brassica juncea*) and Waster (*Brassica napus*) are the suitable genotypes to perform better under saline field conditions.

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