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PRODUCTION AND EVALUATION OF SALT TOLERANT WHEAT GERMPLASM DERIVED THROUGH CROSSES BETWEEM WHEAT (*TRITICUM AESTIVUM* L.) AND *AEGILOPS CYLINDRICA*. I. PRODUCTION OF SALT TOLERANT WHEAT GERMPLASM.

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Abstract

To produce salt-tolerant wheat germplasm through wide hybridization, 8 different salt-tolerant accessions of *Ae. cylindrica* were crossed with 3 hexaploid wheat cultivars: Pak-81, LU-26, and Shalimar-88. Frequency of seed set ranged between 1.72% (Shalimar-88 x *Ae. cylindrica* acc. 38-53) and 52.6% (Pak-81 x *Ae. cylindrica* acc. G.). The performance of wheat cultivars LU-26 and Shalimar-88 as female parent for F hybrid production was poor. Frequency of BC_1 seed production ranged between 0.53% (Pak-81 x *Ae. cylindrica*// LU-26) and 8.92% (Shalimar-88 x *Ae. cylindrica*// LU-26). BC_1 plants with different chromosome numbers were selfed to produce BC1F1 derivatives. Seven BC_1 combinations produced selfed seeds ranging between 1 and 200. Six BC1 combinations did not produce selfed seeds and were backcrossed again with LU-26 to produce BC_2 seeds, which ranged between 1 and 12 seeds per BC_1 combination. Of the 30 different BC_2 seeds produced in different combinations, only 19 produced BC_2F_1 selfed seeds. All the BC_2F_1 and BC_1F_2 seeds were tested for germination under saline solutions of EC 2.5 (control) 15, 20, and 25 dS/m. Salinity induced reduction and delay in seed germination; reduction in shoot and root length and in seedling fresh weight was observed with considerable variation among different salinity levels and among different genotypes at one salinity level. The results have been discussed with reference to the wide hybridization approach and its potential use in the production of genetic variability for polygenically controlled characters such as salt-tolerance.

Introduction

Hexaploid wheat (*Triticum aestivum*) is reported as moderately tolerant to salinity (Kingsbury & Epstein, 1984), with adequate genetic variability for salt-tolerance (Qureshi *et al*., 1980; Kingsbury & Epstein, 1984). However, intervarietal differences in salt-tolerance in wheat generally fall within a narrow range of EC 6-10 dS/m (Rana, 1986). Therefore, improvement in the tolerance level of cultivated wheat beyond this tolerance limit may not be achieved through any varietal combination. However, such tolerant germplasm has been produced by transferring salt-tolerant genes from perennial and annual salt-tolerant wild wheat grasses (Dewey, 1960; McGuire & Dvorak, 1981; Farooq *et al*., 1988, 1989a) to cultivated wheat. The transfer of salt-tolerant genes from perennial wild wheat grasses to cultivated wheat has generally been achieved through production of amphiploids (Storey *et al*., 1985; Foster *et al*., 1987); or addition lines (Dvorak *et al*., 1985; Foster *et al*., 1988; Farooq *et al*., 1993a), whereas gene(s) from annual goat grass species (*Ae. squarrosa* or *Ae. cylindrica*) are transferred through direct crossing over between chromosomes of

Table 1. Production of F_1 hybrids between different wheat varieties and various salt tolerant accessions of *Aegilops cylindrica*

Male Parent *Ae. cylindrica*/accession	Florets pollinated			Seed Set		
	Pak-81	LU-26	Shalimar	Pak-81	LU-26	Shalimar
Ae. cylindrica 50-42	386	20	0	138(35.7)	0	0
Ae. cylindrica 50-43	150	20	0	27(18.0)	0	0
Ae. cylindrica 38-53	254	24	58	34(13.4)	1(4.2)	1(1.7)
Ae. cylindrica 17-59	272	0	0	18(6.6)	0	0
Ae. cylindrica 42-61	0	34	16	0	0	1(6.3)
Ae. cylindrica 34-35	0	0	22	0	0	1(4.5)
Ae. cylindrica G	97	0	0	51(52.6)	0	0
Ae. cylindrica 21-87	118	56	0	2(1.7)	0	0

Figures in parenthesis are % seed set.

wheat and the alien species (Farooq *et al.*, 1992). Salt-tolerance evaluation of the germplasm produced through crosses of wheat with *Ae. cylindrica*, conducted under saline fields, indicated its salt-tolerance potential (Farooq *et al.*, 1993b). Results of the hybridization of salt-tolerant accessions of *Ae. cylindrica* (Farooq *et al.*, 1989b) as donors of salt-tolerance gene(s) to cultivated wheat are presented here.

Materials and Methods

Materials used in this study comprised of 8 salt-tolerant accessions of *Ae. cylindrica* (Farooq *et al.*, 1989a) and 3 different cultivars of hexaploid wheat viz., Pak-81: a high yielding variety which is salt tolerant at seedling stage but salt sensitive at adult plant stage as grain yield reduces significantly under saline conditions (Farooq *et al.*, 1992), LU-26: a salt-tolerant wheat cultivar while Shalimar-88: a salt sensitive but high yielding variety. Pak-81 and Shalimar-88 were selected to incorporate into them gene(s) for salinity tolerance in order to get their full yield potential under saline conditions and LU-26 was used to improve its tolerance. Plant material was planted in plastic pots filled with 6 kg normal soil to which nitrogen and phosphorus fertilizer was mixed in the form of ammonium nitrate and Superphosphate in the ratio of 100:75 kg/ha (Anon., 1993). The pots were kept in the net house in November 1990 (average day and night temperature $20\pm5^{o}C/10\pm5^{o}C$, 8-10 h natural light, and 50-60% R.H.). At flowering, cultivated wheat varieties were emasculated and pollinated with pollens from different accessions of *Ae. cylindrica*. F_1 seeds were left on the plant and harvested at maturity. F_1 hybrids, confirmed through mitotic cytology, were backcrossed with wheat cultivars Pak-81 and LU-26 during 1991-92. Before backcrossing, immature spikes of all the F_1 hybrid combinations were fixed in a mixture of alcohol, acetic acid and chloroform (6:3:1). Aceto-carmine squash preparations were made and analyzed for the meiotic chromosome associations at metaphase-I.

BC_1 plants produced from different BC_1 seeds were selfed to produce BC_1F_1 self-fertile derivatives. Some of the combinations that did not produce selfed seeds were again backcrossed with the same wheat varieties to produce BC_2 seeds. All the BC_1 and BC_2 combinations that produced more than 100 selfed seeds were tested for germination in Petriplates lined with filter papers. The filter papers were soaked in saline solutions of EC 2.5 (control), 15, 20, and 25 dS/m by mixing NaSO4, $MgCl_2$, $CaCl_2$, and NaCl (Qureshi *et al.*, 1977) in a ratio of 10:1:5:4 in Hoagland nutrient solution (Hoagland & Arnon, 1950). Five seeds per treatment per combination were tested in 3 replications. Data on root length, shoot length and seedling fresh weight were taken, 2 weeks after seed germination. After 2 weeks, seedlings were removed from the Petri plates washed in 3 changes of distilled water to remove any salt crystal attached on the leaf surfaces. The seedlings were dried at $70^{o}C$ for 72 h., ground, and digested with concentrated nitric acid (HNO_3). The concentration of K and Na in the digest was analyzed by flamephotometer (Anon., 1954). All the data were subjected to statistical analysis using Duncan's Multiple Range Test.

Results

Of the 8 different salt-tolerant accessions of *Ae. cylindrica* used as pollen sources, only 6 could be crossed with wheat cultivar Pak-81, 3 with Shalimar-88, and 2 with LU-26 (Table 1). Observed seed set in all the combinations ranged between 1.69% (Pak-81 x *Ae. cylindrica* acc. 21-87) and 52.6% (Pak-81 x *Ae. cylindrica* acc. G). As female parent in F_1 hybrid production, LU-26 performed poorly as compared with Pak-81 and produced only two F_1 seeds.

Significant variations were observed in mean meiotic chromosome associations at metaphase-I in different hybrid combinations (Table 2). Minimum chiasma frequency (10.0/cell) was observed in the hybrid of Pak-81 x *Ae. cylindrica* acc 50-42. Hybrids of

Table 2. Mean meiotic chromosomal associations at metaphase-I of wheat cultivars Pak-81, LU-26 and Shalimar-88 with different salt-tolerant accessions of *Ae.cylindrica*.

F1 Hybrid combinations	No.of plants analyzed	No.of cells analyzed	Mean meiotic association					chiasmata/cell
			I	II	(II)	III	IV	
Pak 81 x *Ae.cylindrica* 50-425		500	21.00	4.00	3.00	0	0	10.00a
Pak 81 x *Ae.cylindrica* 50-433		450	19.02	4.01	3.98	0	0	11.97b
Pak 81 x *Ae.cylindrica* 21-878		600	18.00	4.00	3.00	1.0	0	12.00b
Pak 81 x *Ae.cylindrica* 38-535		300	20.00	3.5	4.00	0	0	11.5ab
Shalimar 88 x *Ae.cylindrica* 6		400	20.00	4.5	3.00	0	0	10.5a
LU-26 x *Ae.cylindrica* 38-535		500	15.00	3.97	6.03	0	0	16.03c

Figures followed by the same letter are not significantly different from each other at 5% level of significance according to DMRT.

Pak-81 with two other accessions (50-43 and 21-87) showed significantly high chiasma frequency (12.0/cell) as compared with accession 50-42. Hybrids of three wheat cultivars (Pak-81, LU-26, and Shalimar-88) with one accession of *Ae. cylindrica* (38-53) also produced different chiasma frequencies, with the maximum (16.0/cell) being observed in LU-26 x *Ae. cylindrica* acc. 38-53.

All the F_1 hybrid combinations except Shalimar-88 x *Ae. cylindrica* acc. 39-55 produced backcross seeds which ranged between 1 and 15 seeds per combination. LU-26 showed excellent performance as a backcross parent, producing 57 BC_1 seeds as compared with 16 in the case of Pak-81 (Table 3). The F_1 hybrid of Shalimar-88 and *Ae. cylindrica* acc. 34-35 did not produce seeds with either of the backcross parents.

Of the 73 BC_1 seeds, progenies of 25 seeds belonging to 8 different hybrid combinations produced selfed seeds, the number ranging between 1 and 200 seeds per combination (Table 4). Progenies of six BC_1 plants did not produce selfed seeds, but produced BC_2 seeds upon second backcrossing (1-149 seeds per BC_1 progeny). Of the 261 seeds, only 6 produced BC_2 selfed seeds, with 4-150 seeds per BC_2 combination (Table 4).

Considerable reduction coupled with delay in seed germination was observed in all the combinations at 4 salinity levels. At EC 15 dS/m and in some of the combinations at EC 20 dS/m, germination started after the third day and was complete within 24 h. In some of the combinations at EC 20 and in all but one combination at EC 25 dS/m, germination started after 8 days and was complete within 10 days (data not presented). Reduction in seed germination was also observed in all the test material at the three salinity levels. At EC 20 and 25 ds/m, reduction in

Table 3. Production of backcross seeds in F_1 hybrid combinations of different wheat varieties with various accessions of *Ae.cylindrica*.

Female Parent (F1 combination)	Florets pollinated with		Seed Set in	
	LU-26	Pak-81	LU-26	Pak-81
Pak.81 x *Ae.cylindrica* G.	830	184	15(1.8)	5(2.7)
Pak.81 x *Ae.cylindrica* 50-42	1580	780	14(0.9)	2(2.1)
Pak.81 x *Ae.cylindrica* 50-43	188	132	1(0.5)	0
Pak.81 x *Ae.cylindrica* 38-53	298	142	2(0.7)	1(0.7)
Pak.81 x *Ae.cylindrica* 34-35	334	100	3(0.4)	2(2.0)
Shalimar-88 x *Ae.cylindrica* 38-53	168	40	15(8.9)	0
Shalimar-88 x *Ae.cylindrica* 34-35	64	10	0	0
LU-26 x *Ae.cylindrica* 50-43	186	186	1(0.5)	5(2.7)
LU-26 x *Ae.cylindrica* 38-53	302	34	6(2.0)	1(2.9)

Figures in parenthesis are % seed set.

Table 4. Frequency and production of BC_1 selfed seeds, BC_2 seeds and BC_2 selfed seeds in different hybrid combination of three cultivars with various salt tolerant accessions of *Ae.cylindrica*.

Hybrid combinations with pedigree	BC1 No.of combinations	BC1 Range of selfed seeds/ combination	BC2 No.of combinations	BC2 Range of seed/ BC1 plant	No.of seed	Range of selfed seeds/ BC2 combination
Shalimar 88 /*Ae.cylindrica*//LU 26 38-53	7	(4-65)				
pak 81 /*Ae.cylindrica*//Pak 81 50-42	5	(6-150)				
Pak 81 /*Ae.cylindrica* G //LU 26	4	(37-100)				
Pak 81 /*Ae.cylindrica*//LU-26 21-87	2	(1-200)				
Pak 81 /*Ae.cylindrica* //Pak 81 38-53	1	(37)				
Pak 81 /*Ae.cylindrica* //Pak 81 50-40	1	(1)				
LU-26 /*Ae.cylindrica* //Pak 81 38-53	2	(2-6)				
LU-26 /*Ae.cylindrica* //LU-26 38-53	3	(7-67)				
LU-26 /*Ae.cylindrica* //LU-26*2 38-53			1	(21)		
LU-26 /*Ae.cylindrica* //Pak 81/3/LU-26 50-43			2	(1-16)		
Pak 81 /*Ae.cylindrica* G //Pak 81/3/LU-26			2	(7-67)		
Pak 81 /*Ae.cylindrica* //LU-26/3/Pak 81 38-53			1	(149)		
Pak 81 /*Ae.cyl*: G// Pak 81/3/LU-26					4	(4-100)
Pak 81 /*Ae.cyl*: G// LU-26 /3/Pak 81					1	(17)
LU-26 /*Ae.cyl*: 38-53//Pak 81/3/LU-26					1	(150)

germination was higher in BC1 than in BC2 derivatives (Fig. 1a). In LU-26, reduction was the lowest at all salinity levels. In all the BC2 and in one of the BC1 derivatives, seed germination was higher as compared with salt-sensitive wheat cultivar Pak-81 at all salinity levels.

Like seed germination, reduction in seedling fresh weight was also observed. It was significantly higher ($P < 0.05$) in hybrid derivatives than in wheat cultivars LU-26 and Pak-81, particularly at EC 20 and 25 dS/m. Among the different hybrid derivatives, seedling fresh weight was higher in BC_2 than in BC_1 derivatives (Fig. 1b).

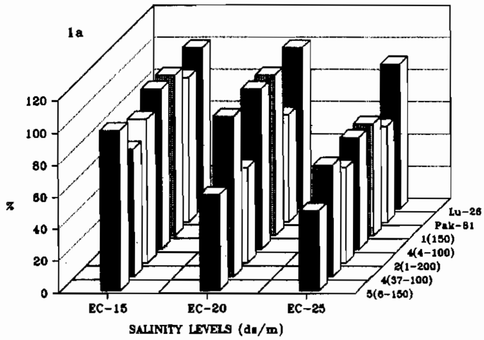

Fig. 1a. Mean seed germination at three salinity levels. (% of control in non-saline)

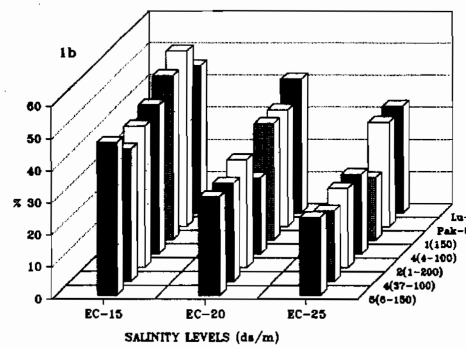

Fig. 1b. Plant fresh weight at three salinity levels. (% of control in non-saline)

Effects of salinity on root length and shoot length were also observed in all the test material (Fig. 2a and 2b). Between EC 15 and 20 dS/m, differences in the root and shoot length of all the test material were highly significant ($P < 0.01$), while between EC 20 and 25 dS/m, significant differences ($P < 0.05$) were observed in the reduction of root length (Fig. 2b). Root length and shoot length of wheat cultivar Pak-81 (salt-sensitive parent) were higher than in wheat cultivar LU-26 (salt-tolerant parent) at all salinity levels.

Considerable differences were observed in the concentrations of Na and K at all combinations (Table 5). Na concentration in salinized plants ranged between 15.00 (Pak-81; salt-sensitive wheat parent) and 2.75 mg/g dry weight (LU-26; salt-tolerant wheat parent). In all the hybrid combinations, Na concentration was in between that of the two parents and ranged between 3.5 and 13.25 mg/g dry weight. In three hybrid combinations: [4(37-100), 2(1-200) and 4(4-200)], K concentration in salinized plants was significantly higher as compared with the nonsalinized control. The K/Na ratio

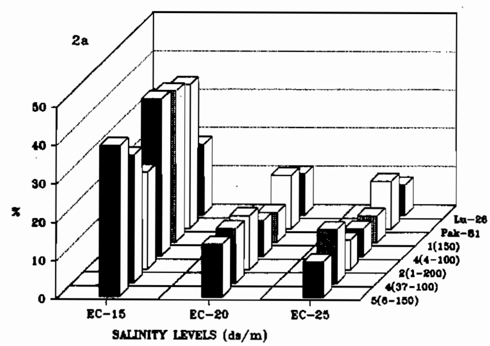

Fig. 2a. Mean shoot length at three salinity levels. (% of control in non-saline)

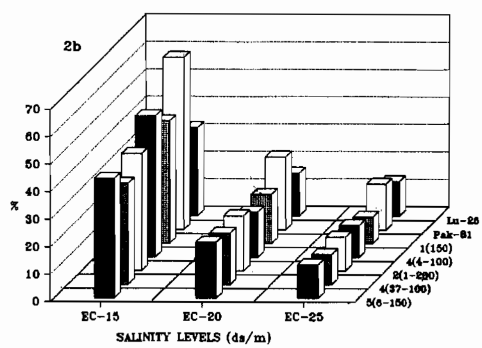

Fig. 2b. Mean root length at three salinity levels. (% of control in non-saline)

Table 5. Comparative mean values of concentration of Na and K and their ratio in different hybrid derivatives after two weeks growth in saline medium of EC 15 dS/m and non saline control.

Pedigree designation	Na conc: mg/g dry wt.			K conc: mg/g dry wt.			K/Na		
	S	N.S	Mean	S	N.S	Mean	S	N.S	Mean
5(6-15)	13.75	0.13	6.94[d]	9.00	14.62	11.81[a]	0.65	31.11	15.88[b]
4(37-100)	4.25	0.37	2.31[b]	21.87	12.25	17.60[d]	5.14	33.11	19.13[cd]
2(1-200)	7.87	0.32	4.10[c]	17.50	12.50	15.00[c]	2.22	39.60	20.91[d]
4(4-100)	7.50	0.45	3.98[c]	23.50	14.75	19.13[e]	3.13	32.78	17.96[c]
1(500)	3.50	0.50	2.00[b]	13.00	11.00	12.00[b]	3.70	22.00	12.85[a]
Pak 81	15.00	0.37	7.68[e]	12.50	10.00	11.25[a]	0.83	27.35	14.09[ab]
LU-26	2.75	0.32	1.54[a]	13.25	12.00	12.60[b]	4.82	37.50	21.16[d]
Mean	7.80[b]	0.35[a]		15.80[b]	12.44[a]		2.93[a]	31.92[b]	

S = Saline, NS = Non saline.

Figures followed by the same letters in each column and row are not significantly different from each other at 5% level of significance according to Duncan's Multiple Range Test.

was higher in the plants growing under nonsaline conditions than in those growing under the saline conditions. However, in one hybrid combination [4(37-100)] growing under saline conditions, the K/Na ratio was higher than in wheat cultivar LU-26. Generally, in all the hybrid derivatives and in salt-tolerant wheat cultivar LU-26 growing under saline conditions, the concentration of Na was low and of K high, while in salt-sensitive wheat cultivar Pak-81, the concentration of Na was high and of K low in salinized plants (Table 5).

Discussion

In our earlier attempt at transferring salt-tolerance gene(s) from salt-tolerant accession D of *Ae. cylindrica*, we found considerable crossability variations in different hybrid combinations (Farooq *et al.*, 1990a). In the present study, crossability values for different wheat cultivars were of the same order except for Shalimar-88, where crossability response was similar to that of LU-26 which was poor. Efforts were made to synchronize the flowering time of *Ae. cylindrica* and LU-26. Neverthless, crossability response was low, which negates our earlier hypothesis (Farooq *et al.*, 1989b) that the poor crossability performance of LU-26 with accessions of *Ae. cylindrica* was due to nonsynchronization of flowering of LU-26 (early flowering) and *Ae. cylindrica* (late flowering). Thus there are some other factors operating in these crosses that have not yet been identified. Crossability variations observed in different accessions of *Ae. cylindrica* however, confirmed the earlier findings of Sharma & Gill (1983) and Farooq *et al.*, (1989a) suggesting the presence of some crossability genes in *Aegilops* species that affected their crossability with wheat varieties.

Considerably high chiasma frequency was observed at meiotic metaphase-I in all the F_1 hybrids, which indicated pairing between D genome chromosomes of *Ae. cylindrica* and of wheat. This pairing signifies the transfer of salt-tolerance gene(s) to wheat through the exchange of genetic material, which is a stable transfer as compared with the transfer of gene(s) achieved with amphiploids and/or addition lines (Farooq *et al.*, 1992).

The hybrid of LU-26 with accession 38-53 produced maximum chiasmata/cell, which confirmed our earlier report (Farooq *et al.*, 1990b) on the presence of additional pairing promotor gene(s) on LU-26 that have not yet been identified. Differences in chiasma frequency observed in hybrids of one wheat variety with different accessions indicated that transfer of salt-tolerance gene(s) to cultivated wheats can be enhanced if selections are made for alien species that can produce maximum chiasmata.

Backcrossing is a routine procedure of advancing intergeneric hybrids. In the present study, the frequency of backcross seed production and selfing of BC_1 seeds was low, which indicated variation in the chromosome number of BC_1 combinations.

Reduced and delayed seed germination coupled with decreased seedling growth due to salinity stress has been reported in many crop plants (Abel & Mackenzie, 1964; Akber & Yabuno, 1974; Norylin & Epstein, 1984; Mozafar & Goodin, 1986; Azmi & Alam, 1990). In the present study also, reductions in seed germination, root and shoot length, and seedling fresh weight were observed in all the test material. The higher values of seed germination and seedling fresh weight of LU-26 as compared with BC_1 and BC_2 derivatives indicated that the newly produced salt-tolerant material may not have higher salt-tolerance than the tolerance level already existing in LU-26, while lower values of root and shoot length of LU-26 as compared with BC_1 and BC_2 derivatives indicated higher level of tolerance of the hybrid derivatives as compared with LU-26. Kingsbury & Epstein (1984) reported that salt-tolerance response at early growth stages is not an indication of the same response at later growth stages therefore, it is possible that the salinity response of the test material observed at seedling stages may not remain the same at later growth stages. Nevertheless, the results did show a variability in salt-tolerance that needs to be examined at later growth stages and under higher salinity levels.

Three hybrid combinations growing in saline medium exhibited significantly higher concentrations of K as compared with the nonsaline control. This is a rare observation and indicated the presence of K-Na discrimination phenomenon in these genotypes. Since Na is known to compete with potassium for the common absorption site, high potassium uptake can result only if the inherent mechanism of the test material can discriminate between K and Na and allow preferential uptake of the former. As reported by Gorham (1990), K-Na discrimination factor operates only at low salinities, while in the present study, this factor seems to have been operational at EC 15 dS/m, which is not very low for wheat. It would be good for breeding programs if the expression of K-Na discrimination operating at seedling stages could be detected during the screening of the same material at later growth stages, as selection for salt-tolerant plants would then be possible at seedling stages using the K-Na discrimination factor as a marker.

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