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INCONSISTENCY IN SALT TOLERANCE OF SOME WHEAT (*TRITICUM AESTIVIUM* L.) GENOTYPES EVALUATED UNDER VARIOUS GROWING ENVIRONMENTS

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Abstract

Wheat genotypes were evaluated for their salt tolerance under water culture at early seedling stage, gravel culture at vegetative & maturity stage and under natural saline field condition at maturity. Ten newly developed wheat genotypes were included along with local check (LU-26s). The tested wheat genotypes were found more sensitive at vegetative and maturity than at early seedling stage. At early seedling stage 4 genotypes (NIA-AS-14-2, NIA-AS-14-5, NIA-AS-14-10 and LU-26s) fell into the tolerant category (i.e., STI > 50%) and one (NIA-AS-14-1) as sensitive (S) (STI < 30%). The response of wheat genotypes was bit different under gravel culture at maturity stage, where the genotypes LU-26s, NIA-AS-14-2 and NIA-AS-14-8 performed well. The genotype LU-26s, NIA-AS-14-1, NIA-AS-14-2, NIA-AS-14-4, NIA-AS-14-7 and NIA-AS-14-8, showed better performance in all the parameters and categorized as tolerant (T). Least performance under gravel culture was observed by NIA-AS-14-6. The results with respect to the performance under natural saline field conditions showed that the genotypes NIA-AS-14-1, NIA-AS-14-4, NIA-AS-14-9 and NIA-AS-14-10, along with local check (LU-26s), had better tolerance at medium to highly saline patches. The genotypes were also evaluated for their physiological performance at early seedling and vegetative (at the time of flowering) stage. The physiological traits studied were solute contents (organic and inorganic). Tolerant genotypes showed dual types of behavior in case of Na^+ accumulation (i.e. NIA-AS-14-1, NIA-AS-14-2 and LU-26s (local check) showed low Na^+ accumulating pattern, where as NIA-AS-14-8 and NIA-AS-14-9 showed higher accumulating trend under saline environments. Accumulation of proline was also low in low Na^+ accumulating genotypes. The overall investigations showed that screening at early growth stage (2–4 weeks) was more convenient than at vegetative or at maturity, but its reliability might be questioned as most of the genotypes were tolerant at early seedling stage but showed sensitivity during vegetative and at maturity during grain filling stage. Therefore, it necessitates that selection of salt tolerant genotypes under different environments before recommending a genotype as tolerant. In the present investigation three genotypes (NIA-AS-14-2 NIA-AS-14-4 and NIA-AS-14-10) and a local check LU-26s were indentified to have the potential to perform economically under medium to high saline soils.

Key words: Wheat, Salt tolerance, Growth stages, Screening techniques.

Introduction

Since the adaptation of concept "biological approaches" for the management of salt affected soils, evaluation of genetic potential of crops for salt tolerance has become a challenge for plant physiologists and breeders. Improvement of salt tolerance in crops and pasture species requires efficient techniques for identifying salt-tolerance in the existing germplasm and access to new genetical diversity. Significant attempts have been made throughout the world to identify salt tolerance in the existing germplasm and natural races. However, the majority of the work was carried out to develop selection criteria for salt tolerance using solution culture techniques at early seedling stage or hydroponic systems (Munns *et al*., 2002; Genc *et al*., 2007) and gravel/ sand-culture technique (Khan *et al*., 2009, Munns *et al*., 2002). Testing of crop plants at early seedling stage is less time consuming, provides good information about their genetical potential and also helps to short list a large number diversified genetic material during initial stages of screening. However, the authentication of results always depends on the evaluation of this diversified material at later stages of growth under natural saline field conditions at vegetative and reproductive stages. It has been suggested that longer-term experiments are necessary to detect genotypic differences on growth by exposing plants to salinity for several months (Kingsbury & Epstein, 1984; Francois *et al*., 1986; Fortmeier & Schubert, 1995; Munns *et al*., 1995).

Wheat as an important cereal crop of Pakistan (Rao, 2013), also needs to be explored for salt tolerance. Sayed (1985), reported a substantial genetic diversity in both tetraploid and hexaploid wheat, while screening was done on the basis of survival at seedling stage (Din *et al*., 2008) and the performance in the field. Munns *et al*., (2002) also reported little relations to overall performance with early screening, under saline conditions. The lack of reliable large scale screening technique is still a great problem in genetic improvement of salt tolerance of crop plants (Mehmood, 2009). Screening field crops under natural saline field conditions is difficult due to non uniformity and temporal fluctuations of soil salinity. Further, the abiotic stresses like fluctuations in soil salinity, extreme temperature and high light intensity may occur simultaneously under field conditions; therefore a large degree of heterogeneity among the stress levels that impact different plants growing in the same vicinity may occur (Tavakkoli *et al.*, 2012). This heterogeneity, in turn, can affect plant performance and yield. Additional field tests of these plants under stress conditions will help to verify their potential utility in crop-improvement programmes. Keeping in view the inequality of different growing environments, some newly developed wheat genotypes were evaluated under water culture in laboratory, gravel culture in glass house and under natural saline field conditions to observe the degree of salt tolerance.

Materials and Methods

Plant materials: To compare the variability in salt tolerance of wheat under different growing environment/stages, some newly developed wheat genotypes were tested at i) early seedling stage in water culture, ii) gravel culture and iii) natural saline field condition. Ten wheat genotypes (advance lines) and a salt tolerant check (LU-26s) were collected from plant breeding and genetics (PBG) division, Nuclear Institute of Agriculture, Tandojam (Table 1). The details of experimental studies conducted using different growing techniques are presented as under.

Table 1. Pedigree details of wheat genotypes under study

S. #	Genotypes/ varieties	Pedigree
1.	NIA-AS-14-1	(TJ-83 x VASCO) x INQILAB-91
2.	NIA-AS-14-2	(TJ-83 x 4085/3) x INQILAB-91
3.	NIA-AS-14-3	TJ-83 x4085/3
4.	NIA-AS-14-4	SUNCO x TJ-83
5.	NIA-AS-14-5	CIMMYT-6055 x AS-2002
6.	NIA-AS-14-6	TD-1 x D-108
7.	NIA-AS-14-7	CIMMYT-6007 x TD-1
8.	NIA-AS-14-8	CIMMYT-6009 x TD-1
9.	NIA-AS-14-9	SD-88 x AS-2002
10.	NIA-AS-14-10	No.B3 mot x TD-1
11.	LU26s	BLUE SILVER x KHUSHALl-69

Water culture studies: The study on salt tolerance in wheat genotypes at early seedling stage was conducted in water culture (in plastic bowls containing molded plastic sieves), in growth cabinets (Vindon, England), using 1/4th strength Hoagland solution (Hoagland & Arnon, 1950). Twenty seeds of each genotypes (pretreated with 5% sodium hypochlorite for 10 ten minutes to surface sterilized) were placed on each sieve. Two salinity treatments (12 and 16 dS/m NaCl) along with non saline control were imposed using three replication. The bowls were placed in growth cabinets maintained at 25/20°C day/night temperature and were kept in dark for 48 hours for smooth germination, later on 12 hours photoperiod (irradiance 22Wm^{-2}) was given up to termination of experiment. The bowls were arranged, in randomized manner using completely randomized design (CRD). Sufficient solutions (approximately 30 ml) of respective salinity were poured in each bowl and level was maintained on regular basis. Seedlings were harvested after 10 days and growth observations (shoot & root length, shoot & root fresh weight, shoot & root dry weight), were recorded. Sodium (Na) and potassium (K) was analyzed in shoot samples after extracting with 0.1M acetic acid (Ansari& Flowers, 1986), using flame photometer (PFP-7).

Gravel culture studies: The study was conducted in glass house in cemented beds (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. Two treatments (i.e. control (1.56 dSm^{-1}) and saline (12.0 dSm^{-1}) were imposed. Salinity treatment was imposed gradually through irrigation after two weeks of germination, by commercial sodium chloride (NaCl) salt. The beds were irrigated with modified Hoagland solution (1/4th strength), of respective salinity. The experiment was terminated at the time of crop maturity. Growth observations were recorded in terms of plant height, biomass/ plant, productive tillers, number of grains/ spikelet and grain yield / plant. Plant samples (next to flag leaf) were collected at the time of flowering stage (ear head emergence) and were subjected for the analysis of organic (proline) and inorganic (Na^+, K^+ and K^+/Na^+ ratio) solutes. Proline was estimated in fresh leaf samples according to the method of Bates *et al*., (1973), after extracting by 0.5% (v/v) acqueous solution of toluene (Weimberg *et al*., 1981). Sodium (Na^+) and Potassium (K^+) contents were analyzed as described earlier.

Field studies: Screening studies were also conducted under field conditions at NIA, experimental farm. In this regard two sites (normal and saline) 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} in saturated soil extract. 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 between 12-16 dSm^{-1}. To maintain the uniformity of soil salinity, sowing was done on small plots. The size of small sub plots was 2.0 m^2. Four rows of 2.0 meter length at the spacing of 30 cm were planted in each sub plot. The experiment was laid out according to randomized block design (RBD), with three replicates. Growth parameters (i.e. Population percentage, leaf area, biological and grain yield.) were recorded at crop maturity.

Statistical analysis: Analysis of variance (ANOVA) and correlations studies among different growth parameters were performed (steel *et al.*, 1997), using Statistix-08 computer package. Wheat genotypes were categorized on the basis of salinity traits tolerance index (STTI) according to Ali *et al*., (2007).

$$\text{Salinity traits tolerance index (STTI)} = \frac{\text{Value of trait under stress condition}}{\text{Value of trait under normal condition}} \times 100$$

Salinity tolerance trait indexes (STTI) were mean as salinity tolerance index (STI). Wheat genotypes were classed as tolerant (T), have STI values > 50%, medium tolerant (MT) = 40-49%, medium sensitive (MS) = 30-39% and sensitive (S) = <30%. However under field condition the scale was bit changed as genotypes were classed as tolerant (T) having STI values > 70%, medium tolerant (MT) = 60-69%, medium sensitive (MS) = 50-59% and sensitive (S) = <50%.

Results

Water culture studies: The tested genotypes showed significant ($p<0.05$) reduction in all growth parameters at 12 & 16 dS/cm salinity treatments. The major source of variation was salinity levels, while wheat genotypes exhibited significant effects ($p<0.05$) due to their genetic

behavior and also showed significant ($p<0.05$) interaction with salinity treatments except in root fresh wt. and shoot dry wt. (Table 2). Minimum and maximum values for each parameter and means square (ANOVA) are presented in table 2. The data at maximum salinity treatment (16 dS/m) is transformed in terms of salinity tolerance trait index (STTI) for each parameter (Table 2). It was observed that the genotype LU-26s had maximum STTI values both for shoot and root length. At 16 dS/m NaCl stress, STTI of shoot length ranged from 32 to 81% and for root length from 26.0 to 92%. These wide differences in STTI range indicate that genotypes had broad genetic base for shoot & root length. Irrespective to tolerant or susceptible, genotypes showed greater shoot growth than root growth (data not shown). There was also decline in shoot and root fresh weight due to salinity. The STTI values for shoot fresh weight ranged from 24 to 60.0% and for root fresh weight from 18 to 64%. In case of shoot and root fresh weight the genotype NIA-AS-14-5 had the maximum STTI values. STTI values for shoot and root dry weights also varied widely among the genotypes at 16 dS/m salinity treatment. The STTI of shoot dry weight ranged from 30 to 86% and for root dry weight ranged from 42 to 78%. Maximum STTI values were recorded in genotypes NIA-AS-14-2 for shoot and root dry weight. On the basis of mean STI from the 11 tested wheat genotypes only 4 genotypes (NIA-AS-14-2, NIA-AS-14-5, NIA-AS-14-10 and LU-26s) fell into the tolerant category (*i.e*. STI> 50%), three (NIA-AS-14-4, NIA-AS-14-6 and NIA-AS-14-9) fell in MT (STI 40-49%,) three (NIA-AS-14-3, NIA-AS-14-7 and NIA-AS-14-8,) fell in medium sensitive MS (STI= 30-39%) and one (NIA-AS-14-1) as sensitive S (STI < 30%) category at early seedling stage (Table 3).

Table 2. Ranges and mean square (salinity, genotypes and Sal x Gen) of different growth parameters at early seedling stage under different salinity levels.

Parameters	Control		12 dS/m		16 dS/m		Salinity	Gen.	Sal. x Gen.
	Min	Max	Min	Max	Min	Max	DF =2	DF=10	DF =20
Shoot length (cm)	11.28	19.1	6.89	7.02	2.5	14.8	545.2**	28.78**	9.66 **
Root length (cm)	5.29	15.3	4.94	1.86	0.86	10.83	294.50**	16.39 **	13.87 **
Shoot Fresh wt. (g)	1.74	3.78	1.13	2.24	0.29	1.71	21.34**	0.482 **	0.152 *
Root Fresh wt. (g)	0.82	2.47	0.63	1.38	0.14	1.07	7.82**	0.289 **	0.093 NS
Shoot Dry wt. (g)	0.1	0.36	0.09	0.32	0.04	0.22	0.11**	0.006 **	0.0024 NS
Root Dry Wt. (g)	0.06	0.15	0.03	0.11	0.03	0.09	0.019**	0.0015 **	0.0004 **
Potassium (%)	1.68	4.6	1.68	4.1	0.85	4.85	0.692**	2.715**	0.974 *
Sodium (%)	0.32	0.98	1.3	3.83	1.45	4.75	52.90**	1.504 **	0.458 **
K/Na ratio	2.77	7.73	0.74	2.96	0.33	2.77	154.09**	1.866 **	0..537 **

** = Significant @0.01, * = Significant @0.05 and NS = Non-significant

Table 3. Salinity tolerance trait index (STTI) of different growth parameters at early seedling stage (EC= 16dS/m).

Genotypes	Shoot length	Root length	Shoot fresh weight	Root Fresh weight	Shoot dry weight	Root dry weight	Mean STI	Category
NIA-AS-14-1	32.3	26.0	23.8	18.2	30.0	42.9	28.9	S
NIA-AS-14-2	68.5	43.6	53.6	64.3	86.4	77.8	65.7	T
NIA-AS-14-3	33.3	31.4	30.0	25.0	46.4	46.2	35.4	MS
NIA-AS-14-4	54.0	27.2	36.7	43.8	48.3	70.0	46.7	MT
NIA-AS-14-5	76.3	80.0	60.0	62.5	83.3	72.7	72.5	T
NIA-AS-14-6	45.1	52.2	26.1	37.5	47.8	45.5	42.4	MT
NIA-AS-14-7	31.0	61.9	25.9	33.3	35.7	41.7	38.3	MS
NIA-AS-14-8	34.2	27.8	37.0	31.6	50.0	50.0	38.4	MS
NIA-AS-14-9	58.2	36.4	33.3	35.0	56.7	50.0	44.9	MT
NIA-AS-14-10	59.1	54.6	42.3	50.0	61.5	63.6	55.2	T
LU-26s	80.9	92.1	59.2	51.6	75.0	50.0	68.2	T

Tolerant (T), STI > 50%, Med. tolerant (MT) = 40-49%, Med. sensitive (MS) = 30-39% and Sensitive (S) = <30%

Table 4. Correlation studies among different parameters at early seedling stage.

Genotypes	Shoot length	Root length	Shoot fresh wt.	Root fresh wt.	Shoot dry wt.	Root dry wt.	Sodium (Na^+)	Potassium (K^+)
Root length	0.85**							
Shoot fresh wt.	0.83**	0.73**						
Root fresh wt.	0.70 *	0.55 *	0.84**					
Shoot dry wt.	0.81 **	0.69*	0.94**	0.91**				
Root dry wt.	0.63 *	0.42 NS	0.83**	0.93**	0.91**			
Sodium (Na)	-0.54 *	-0.55 *	-0.41 NS	-0.12 NS	-0.36 NS	-0.06NS		
Potassium (K)	0.12 NS	0.22 NS	0.05 NS	0.37 NS	0.28 NS	0.54 NS	0.43 NS	
K/Na ratio	0.56 NS	0.26 NS	0.37 NS	0.44NS	0.56 NS	0.57 NS	-0.41 NS	0.64*

** = Significant @ 0.01, * = Significant @ 0.05 and NS = Non-significant

Table 5. Ionic contents (Na, K) and K/Na ratio in shoot samples at early seedling stage.

Genotypes	Sodium (Na) %			Potassium (K) %			K/Na Ratio		
	Cont.	16 dS/m	Relative Inc./ Dec (fold)	Cont.	16 dS/m	Relative Inc./ Dec (fold)	Cont.	16 dS/m	Relative Inc./ Dec (fold)
NIA-AS-14-1	0.68j	3.13bcd	(+) 4.6	2.93e-f	1.78 l	(-) 1.65	4.31bc	0.57f	(-)7.6
NIA-AS-14-2	0.50j	2.85cdef	(+) 5.7	2.00 kl	2.08c-i	(+) 0.96	4.00c	0.73ef	(-)5.5
NIA-AS-14-3	0.49j	3.78bc	(+) 7.7	1.95kl	2.7 hij	(+) 0.72	3.98c	0.71f	(-)5.6
NIA-AS-14-4	0.49j	3.03bcde	(+) 6.2	1.98kl	3.44b-f	(+) 0.58	4.04c	1.14def	(-)3.6
NIA-AS-14-5	0.38j	2.73fg	(+) 7.2	2.05kl	2.75g-j	(+) 0.75	5.39bc	1.01def	(-)5.4
NIA-AS-14-6	0.65j	3.16bcd	(+) 4.9	3.98ab	2.83f-j	(-) 1.41	6.12a	0.90ef	(-)6.8
NIA-AS-14-7	0.76j	3.13bcd	(+) 4.1	3.37b-g	2.03kl	(-)1.66	4.43bc	0.65f	(-)6.8
NIA-AS-14-8	0.83j	2.95cde	(+) 3.6	3.17c-h	2.42jk	(-) 1.31	3.82c	0.82f	(-)4.7
NIA-AS-14-9	0.51j	2.68def	(+) 5.3	3.07d-i	4.13a	(+) 0.74	6.02a	1.54de	(-)3.9
NIA-AS-14-10	0.48j	4.10a	(+) 8.5	2.69hij	3.97ab	(+) 0.68	5.60a	0.97ef	(-)5.8
LU-26s	0.34j	1.47h	(+) 4.3	1.78l	1.73l	(+) 1.03	5.26ab	1.21def	(-)4.3
LSD (0.05) Sal x Gen	0.601			0.632			0.956		

Rel. Inc./ dec. = (+) / (-)

Ionic contents: Shoot samples at early seedling stage were analyzed for Na^+ and K^+ contents. Wheat genotypes revealed significant increase in Na^+ in shoot under both salinity treatments (*i.e.* 12 and 16 dS/m). Almost all the growth parameters were negatively correlated with Na contents. The relations were significant in case of shoot and root length and non-significant with fresh and dry weights. Whereas, the growth parameters were positively (non-significant) related with K contents (Table 4). Least Na^+ accumulation was observed in LU-26s, however, the relative increase was bit higher (4.3 folds) than NIA-AS-14-8 and NIA-AS-14-7 (3.6 and 4.1 folds, respectively) (Table 5). Maximum Na^+ contents were observed in genotype NIA-AS-14-10, also, had maximum relative increase (8.5 folds). In contrast to this the genotype NIA-AS-14-1, which was categorized as sensitive comparatively had less Na contents, showing less relative increase (4.6 folds) (Table 5). The data with respect to K^+ contents in shoot revealed that almost all tolerant genotypes maintained K content quite successfully at 16 dS/m salinity treatment. On the other hand there was a decrease in K^+ in genotypes which countered sensitivity at 16dS/m salinity treatment. Among the tested genotypes maximum K accumulation was observed in NIA-AS-14-9, followed by NIA-AS-14-10. The genotype NIA-AS-14-9 also had maximum K/Na ratio followed by LU-26s. The genotype NIA-AS-14-1, which was categorized as sensitive, had minimum K^+ accumulation at 16 dS/m salinity treatment, resulted in minimum K^+/Na^+ ratio (Table 5).

Gravel culture studies: Wheat genotypes evaluated under controlled environment (gravel culture) using saline (12dS/cm) irrigation water. The genotypes were evaluated on the basis of growth performance (Plant height, biomass/ plant, productive tillers, number of grains / plant and grain yield / plant). Mean square values indicated that salinity was a major source of variation showing significant ($p=<0.01$) effect on all the growth parameters (Table 6). Significant differences were recorded among the genotypes for plant height, productive tillers and number of grains. However, the interaction between wheat genotypes and salinity treatment were only significant in case of plant height and productive tillers (Table 6). The growth performance were pooled and categorized on the basis of salt tolerance trait index (STTI) (Table 7). The result showed that plant height was least effected by salinity and displayed relatively better STTI values, ranging from 78 to 99. Maximum STTI was observed in NIA-AS-14-2 and NIA-AS-14-7. Effect of salinity was more prominent in case of tillering capacity of wheat genotypes. The STTI values for productive tillers were ranged from 22 to 64. Least effect of salinity on tillering capacity was observed in genotypes LU-26s. Reduction in biomass was also evident, almost all the genotypes showed reduction in plant biomass under salinity. STTI values for plant biomass ranged from 38 to 72 with highest values for genotype LU-26s. Likewise, salinity has considerable effect at reproductive stage of all tested genotypes, showing significant decrease in number of grains per plant presenting STTI values below 50, except LU-26s. The STTI values for number of grains ranged from 32 to 63 (Table 7). Salinity also resulted in reduction of grain weight per plant; however, only two genotypes showed STTI values above 50% (i.e. LU-26s and NIA-AS-14-4). Wheat genotypes were categorized on the basis of mean STI values. The genotype LU-26s, NIA-AS-14-1, NIA-AS-14-2, NIA-AS-14-4, NIA-AS-14-7 and NIA-AS-14-8, showed better performance in all the parameters and hence categorized as tolerant (T). The genotypes (NIA-AS-14-3, NIA-AS-14-5, NIA-AS-14-6, NIA-AS-14-9 and NIA-AS-14-10) displayed intermediate performance, therefore classified as medium tolerant (MT) at 12 dS/cm salinity level. Wheat genotype NIA-AS-14-6 exhibited least performance under gravel culture, hence categorized as medium sensitive (MS).

Solute accumulation: Inorganic (Na and K) and organic solutes (proline), were estimated in leaf samples at flowering stage under gravel culture (Figs. 1a, 1b, 1c). The results regarding Na showed that wheat genotypes accumulated high Na in leaf samples on exposure to salinity (Fig. 1a). The relative increase was 2 to 5 folds greater in leaves under salinity than non saline control. Irrespective of tolerant and sensitive genotypes, high Na contents were observed in NIA-AS-14-3, NIA-AS-14-5, NIA-AS-14-6 (sensitive) and NIA-AS-14-4, NIA-AS-14-8, NIA-AS-14-9 (tolerant or medium tolerant).The genotypes NIA-AS-14-1, NIA-AS-14-2 and LU-26s exhibited comparatively less Na accumulation under 12 dS/m salinity treatment.

As a general trend potassium contents in leaf samples decreased at 12 dS/cm salinity, in all the wheat genotype, except LU-26s which showed a bit increasing trend (Fig. 1b). Among the tested genotypes potassium contents in NIA-AS-14-1 was comparatively higher under both environments. The results with respect to the relative decrease/ increase showed higher decrease in NIA-AS-14-1, NIA-AS-14-3 and NIA-AS-14-6 (i.e. 0.82, 0.86 and 0.83 folds, respectively). On the other hand the genotype LU-26s showed 1.36 folds increase.

The results for the accumulation of proline also showed similar trend like in Na accumulation i.e. comparatively less accumulation of proline in genotypes which had less Na contents (Fig. 1c). The genotypes NIA-AS-14-1, NIA-AS-14-2, NIA-AS-14-3 and LU-26s had lower proline accumulation at 12 dS/cm salinity treatments. On the other hand comparatively high proline accumulation was observed in NIA-AS-14-9 and NIA-AS-14-10 (14.17 and 14.60 µmole/ g. F.wt. respectively). However, the relative increase in proline accumulation was maximum in genotypes NIA-AS-14-6 followed by NIA-AS-14-9 (35 and 32 folds, respectively).

Field studies: Wheat genotypes were also evaluated under natural saline field conditions, where salinity ranged between 12 to 16 dS/m. Under field condition growth performance were recorded in terms of survival (% population), leaf area, biological yield and grain yield. Significant effect of salinity was observed on all the parameters studied (Table 8). As the sowing was done at field capacity, some genotypes showed delayed germination. However, the germination improved later on, when field was irrigated after 10-12 days of sowing. In some genotypes improved germination did not result in better survival. The population was decreased after two to three weeks of irrigation. Almost all the genotypes showed better survival (STTI values > 70%) except in genotypes NIA-AS-14-2, NIA-AS-14-5 and NIA-AS-14-7. Salinity tolerance trait index (STTI) values for population density were ranged from 63 to 98%. The genotype LU-26s had maximum STTI values for population density (i.e. 98%). The STTI values for population density were comparatively less in NIA-AS-14-2, NIA-AS-14-5 and NIA-AS-14-7, than the other tested genotypes. Salinity in the field also resulted in reduction in leaf area (next to flag leaf). However reduction was not significant as almost all the genotypes showed >70% STTI values for leaf area, except in genotypes NIA-AS-14-2 and NIA-AS-14-4, where a bit less values for STTI were observed (i.e. 69% each). The genotypes differed significantly in their biological yield. Under field condition the genotypes NIA-AS-14-1, NIA-AS-14-2, NIA-AS-14-4, NIA-AS-14-10 and LU-26s performed extremely well with STTI values > 70% (Table 9). The genotypes (NIA-AS-14-5, NIA-AS-14-6, NIA-AS-14-8 and NIA-AS-14-10) showed intermediate performance with bit less STTI values (>60%). The other genotypes which had low STTI values (<60%) for biological yield under field condition were NIA-AS-14-3 and NIA-AS-14-7. Effect of salinity on grain yield was significant, where number of genotypes showed reduction in grain yield. The STTI values of these genotypes were also < 50%. The STTI values for grain yield showed that there were five genotypes which had STTI value > 70%. (i.e. NIA-AS-14-1, NIA-AS-14-4, NIA-AS-14-9, NIA-AS-14-10 and LU-26s). Like other parameter the genotypes NIA-AS-14-7 had minimum STTI values for grain yield (i.e. only 27%). On the basis of mean STI values four genotypes (NIA-AS-14-1, NIA-AS-14-4, NIA-AS-14-9 and NIA-AS-14-10) along with local check (LU-26s), were found tolerant(T), five (NIA-AS-14-2, NIA-AS-14-3, NIA-AS-14-5, NIA-AS-14-6 and NIA-AS-14-8) as medium tolerant (MT) and one (NIA-AS-14-7) as sensitive (S).

Table 6. Ranges and mean square (salinity, genotypes and sal x gen) of different growth parameters at crop maturity in gravel culture studies.

Parameters	Control		12 dS/m		Salinity	Gen.	Sal. x Gen.
	Min	Max	Min	Max	DF =1	DF=10	DF =10
Plant height (cm)	69.0	88.0	60.4	80.4	514.10**	72.089 **	31.177 **
Plant biomass (g)	2.8	11.4	2.2	6.3	182.05**	2.867 NS	2.251 NS
Productive tillers (Nos.)	2.0	4.0	1.0	2.0	35.10**	0.3092**	0.261*
No. of grains (Nos.)	80	124	24	67	35002**	213.2*	174.7 NS
Grain weight/ plant (g)	2.7	4.9	0.8	2.1	51.71**	0.3662 NS	0.3471 NS

** = Significant @ 0.01, * = Significant @ 0.05 and NS = Non-significant

Table 7. Salinity tolerance trait index (STTI) of different growth parameters at maturity stage in gravel culture studies (EC = 12 dS/m).

Genotypes	Plant height	Productive tillers	Plant biomass	No. of grains/p	Grain wt./ plant	Mean STI	Category
NIA-AS-14-1	96	22	64	45	39	56	T
NIA-AS-14-2	99	38	50	45	39	54	T
NIA-AS-14-3	84	39	40	32	32	45	MT
NIA-AS-14-4	97	35	54	48	50	57	T
NIA-AS-14-5	88	31	42	37	32	46	MT
NIA-AS-14-6	78	50	38	34	23	38	MS
NIA-AS-14-7	99	40	47	49	45	56	T
NIA-AS-14-8	85	50	47	49	44	55	T
NIA-AS-14-9	90	33	47	39	35	49	MT
NIA-AS-14-10	98	37	42	35	35	49	MT
LU-26s	91	64	72	63	67	72	T

Tolerant (T), STI values > 50%, Medium tolerant (MT) = 40-49%, Medium sensitive (MS) = 30-39% and Sensitive (S) = <30%

Table 8. Ranges and mean square (salinity, genotypes and Sal x Gen) of different growth parameters field condition at (12-16 dS/m) salinity levels

Parameters	Non - saline		Saline		Salinity	Gen.	Sal. x Gen.
	Min	Max	Min	Max	DF =1	DF=10	DF =10
Population density (%)	93.0	100	63	88	7089 **	120. NS	139 NS
Leaf area (cm^2)	12.69	28.73	8.73	24.14	74.24 *	95.9 **	22.3 NS
Biological yield (kg/plot)	0.91	1.45	0.50	1.44	1.046 *	0.19 NS	0.26 NS
Grain yield (kg/plot)	0.37	0.66	0.13	0.48	1.046 **	0.19 NS	0.28 *

** = Significant @ 0.01, * = Significant @ 0.05 and NS = Non-significant

Table 9. Salinity tolerance index (STI) of population density, biological and grain yield under natural saline field conditions.

Genotypes	Pop %	Leaf area	Biological yield	Grain. yield	Mean STI	Category
NIA-AS-14-1	87	97	98	83	91	T
NIA-AS-14-2	65	69	73	59	66	MT
NIA-AS-14-3	70	89	55	62	69	MT
NIA-AS-14-4	77	69	84	91	80	T
NIA-AS-14-5	67	79	62	48	64	MT
NIA-AS-14-6	93	80	62	44	70	MT
NIA-AS-14-7	63	87	37	27	53	S
NIA-AS-14-8	82	84	63	42	68	MT
NIA-AS-14-9	80	90	91	85	86	T
NIA-AS-14-10	83	98	64	76	80	T
LU-26s	98	84	71	83	84	T

Tolerant (T) = STI values > 70%, Medium tolerant (MT) = 60-69%, Medium sensitive (MS) = 50-59% and Sensitive (S) = <50%.

Table 10. Comparison of wheat genotypes for the growth performance for salt tolerance under three different growth environments.

Genotypes	Early seedling studies	Hydroponics studies	Saline field studies	Category
NIA-AS-14-1	S	T	T	MT
NIA-AS-14-2	T	T	MT	T
NIA-AS-14-3	MS	MT	MT	MT
NIA-AS-14-4	MT	T	T	T
NIA-AS-14-5	T	MT	MT	MT
NIA-AS-14-6	MT	MS	MT	MT
NIA-AS-14-7	MS	T	S	S
NIA-AS-14-8	MS	T	MT	MT
NIA-AS-14-9	MT	MT	T	MT
NIA-AS-14-10	T	MT	T	T
LU-26s	T	T	T	T

The results of the three studies were pooled to compared the variability among different environments (Table 10), showed that the genotypes NIA-AS-4-2, NIA-AS-14-4, NIA-AS-14-10, and LU-26s confirmed the tolerance in two studies, thus classed as tolerant (T). Six genotypes (i.e. NIA-AS-14-1, NIA-AS-14-3, NIA-AS-14-5, NIA-AS-14-6, NIA-AS-14-8 and NIA-AS-14-9), showed medium tolerance in two studies therefore was categorized as medium tolerant (MT). On the other hand the genotype NIA-AS-14-7 found either medium sensitive (MS) or sensitive (S) in two studies thus may be categorized sensitive (S) genotypes.

Discussion: In the present investigations considerable variation in salt tolerance was observed among wheat genotypes under diverse growth environments (water culture, gravel culture and natural saline and non saline field) at different stages of growth. During early screening trial at seedling stage, four genotypes were identified as tolerant at 16 dS/m salinity. Better tolerance of these genotypes might be due to higher shoot & root dry weights (as evident from high STTI values) and low Na^+ accumulation in shoot. Ahmadi & Ardekani (2006) were of the opinion that wheat genotypes having greater plant biomass at the seedling stage demonstrate better salt tolerance at maturity. Least Na^+ accumulation was observed in LU-26s, followed by NIA-AS-14-5 and NIA-AS-14-2. The genotype NIA-AS-14-10, which also categorized as tolerant had maximum Na accumulation in shoot (Table 5). Significantly negative relations of Na^+ content with shoot length (r =- 0.54) & root length (r = -0.55) and negatively non-significant with shoot fresh (r = -0.41) & dry weights (r = -0.36) were observed. Significant negative correlation between shoot dry matter and leaf sodium concentrations under salinity stress were also reported in earlier studies (Azadi *et al.*, 2011, Tavakkoli *et al.*, 2012). Munns & James (2003) considered Na exclusion as a robust trait for salinity tolerance in wheat. Husain (2002), while conducting glass house experiments also reported that low sodium accumulating landraces yield better than high sodium accumulating genotypes under moderate salinity. On the other hand Munns & James (2003) reported that at early seedling stage the genotypic differences in salinity tolerance are due to osmotic effects of salts out the roots. While salt-specific effects appear later on with time when these salts accumulate inside the plant.

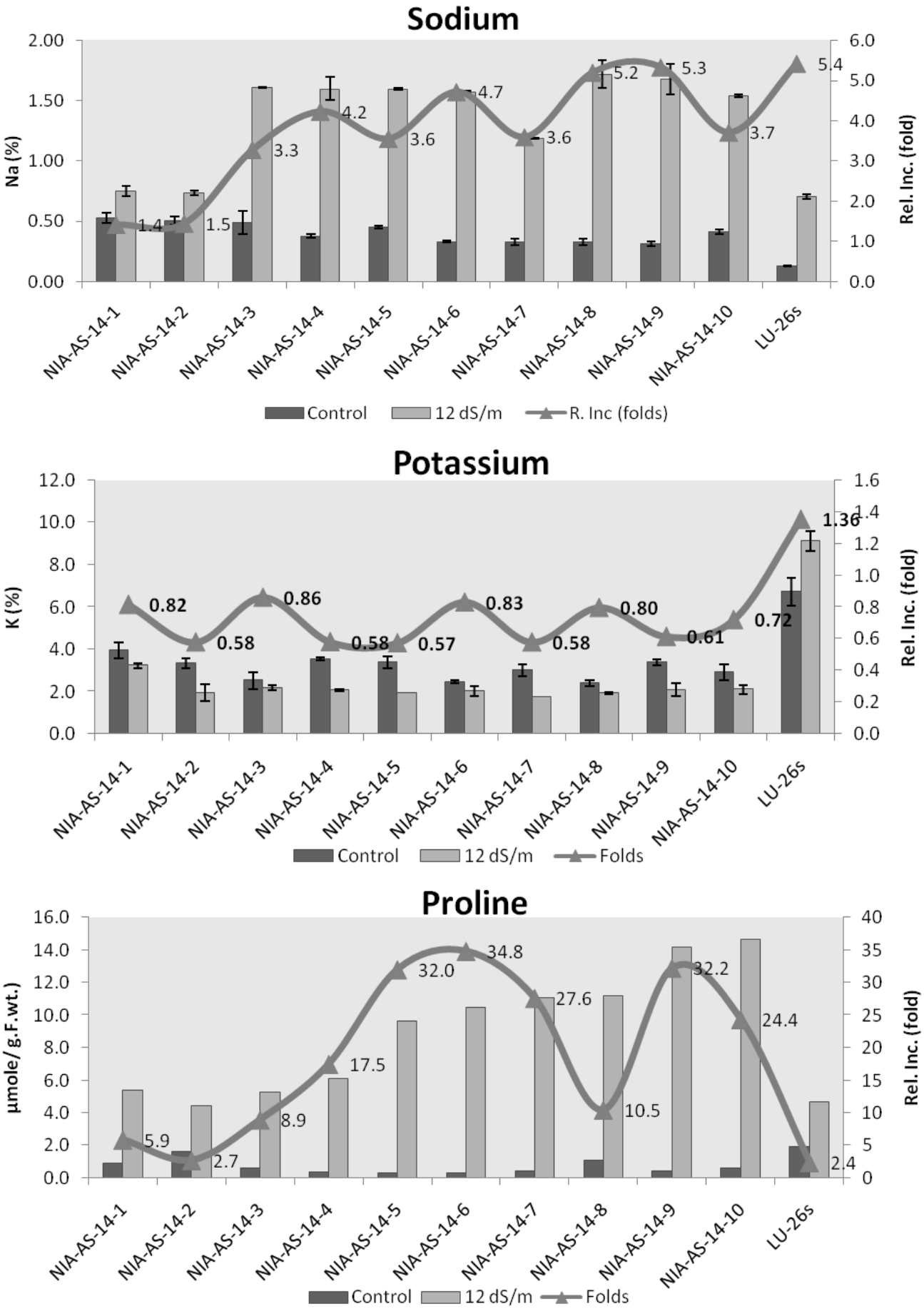

Fig. 1. Effect of salinity on sodium, potassium and proline contents in wheat genotypes at vegetative stage under gravel culture.

Wheat genotypes were also evaluated at vegetative and maturity stage under gravel culture as screening at seedling stage only offers the possibility of pre selection of breeding lines or cultivars before large-scale field evaluation. Krishnamurthy *et al*., (2007) also suggested that tolerance to salinity is necessary at the whole plant level through the complete life cycle in grain-producing species. Under gravel culture environments at maturity stage fairly different response was observed among wheat genotypes, where five genotypes (NIA-AS-14-1, NIA-AS-14-2, NIA-AS-14-4, NIA-AS-14-7, and NIA-AS-14-8) showed tolerance along with local salt tolerant check (LU-26s) (Table 7). Beside this, when the performance were interrelated with early seedling studies, there were only two genotypes (i.e. NIA-AS-14-2 and LU-26s), which were categorized as tolerant and showed consistency under both environments (Table 10). Ayers & Hayward, (1948) also concluded that there may not be a positive correlation between salt tolerance at germination stage and during later phases of growth. Better response of these two genotypes might be due to less accumulation of Na^+ at vegetative stage. Akhtar *et al.,* (2003) concluded that maintenance of low Na in leaf was mainly by efficient exclusion of Na at root or leaf level. In contrast to this, the genotypes NIA-AS-14-4, NIA-AS-14-7, and NIA-AS-14-8, regardless of their better tolerance, had high leaf Na accumulation. Despite having very high leaf Na^+ levels, higher degree of salt tolerance in non durum tetraploid was also observed by Munns & James (2003). This might be due to their ability to tolerate higher levels of Na^+ inside the cell. It was also observed that the genotype NIA-AS-14-1, which performed poorly at seedling stage found tolerant at maturity under gravel culture studies and under field conditions as well. Better performance of NIA-AS-14-1 may be due its low Na accumulation at later growth stages. The genotypes also illustrated divergent behavior in case of K^+ accumulation in leaf, where LU-26s showed significant increase in K contents, while other genotypes, either tolerant or sensitive, had decreased K contents in leaves. Tavakkoli *et al*., (2012), while conducting screening under different environments reported that for plants grown in hydroponics (ECe; 15.3 dS/m) and in the field had no significant relationship between salt tolerance and/or grain yield and the shoot concentrations of K^+. They also observed that maintenance of high K^+ concentrations in salt-tolerant genotypes was only among plants grown in soil at ECe 7.2 dS/m in saline soil, which may be one of the mechanisms underlying their higher salt tolerance. There was increase in proline accumulation under salinity (Fig. 1c). Increased production of compatible solutes under salt stress has already been reported in wheat (Din *et al*., 2008; Mahboob *et al*., 2016). Genotypes also showed contrasting behavior in the accumulation of proline. Lower Na accumulating genotypes also accumulated less proline under salinity. The genotypes NIA-AS-14-1, NIA-AS-14-2 and LU-26s showed low proline accumulation. On the other hand the genotypes NIA-AS-14-9 and NIA-AS-14-10 had high proline accumulation. This indicates that higher accumulation of proline may be to combat the toxic effects of high Na accumulation in shoot.

The genotypes also performed differently under field conditions. Salinity in the field does not remain same; it may vary with time and with soil moisture contents. Some consistencies as well as inconsistency in salinity tolerance of wheat genotypes were observed. The genotypes (NIA-AS-14-1, NIA-AS-14-3, NIA-AS-14-4, NIA-AS-14-5 and LU-26s), showed stability in their salt tolerance (Table 9). Among them three genotypes (NIA-AS-14-1, NIA-AS-14-4 and LU-26s) showed tolerance (T) and two (NIA-AS-14-4 and NIA-AS-14-5) showed medium tolerance (MT) during gravel culture and field evaluation. On the other hand the genotypes NIA-AS-14-7 and NIA-AS-14-8 showed inconsistency, as these genotype were medium sensitive at early seedling stage, showed tolerance under gravel culture studies and then again found sensitive or medium tolerant during field evaluation, respectively. Such type of inconsistency might be due to heterogeneity in salinity under field condition or genetic variation in salinity tolerance among the genotypes.

The overall investigations showed that screening at early growth stage (2–4 weeks) was more convenient than at vegetative or at maturity. However, its reliability may be questioned as most of the cereals are tolerant at early seedling stage but become sensitive during vegetative and early reproductive stages and less sensitive during flowering and grain filling stage (Mass & Poss, 1989). Munns & James, (2003) reported that genotypic differences in salinity tolerance at early seedling stage was due to the osmotic effects of the salts. Relatively high solute concentration in the growing media/soil creates water shortage due to more negative water potential resulting in delayed germination and stunted plant growth at early seedling stage. It takes more time for the salt-specific effects to show up, i.e., the effects of the salt inside the plant. Specific effect of salts inside the plant may become toxic during vegetative stage. Similarly salinity stress increases gradually when there is drying of soil at maturity which may cause shrinkage of grain resulting in lower biomass and grain yield. It is therefore necessary that selection of salt tolerant genotypes should be done under different environments before recommending a genotype as tolerant. In the present investigation three genotypes (NIA-AS-14-2, NIA-AS-14-4 and NIA-AS-14-10) and local check LU-26s showed potential for salt tolerance due to consistency in various growing environments.

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