STUDY OF SOMACLONAL VARIATION IN WHEAT FOR THE INDUCTION OF SALINITY TOLERANCE

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ABSTRACT

This study was conducted in the Department of Botany, Government College University, Faisalabad and partially at the Agricultural Biotechnology Research Institute, AARI, Faisalabad during the year 2005-2011. To induce the genetic variability in wheat for salt tolerance, calli of 20 wheat varieties *i.e.* Ufaq-2002, SA-42, Parwaz-94, LU-26S, Pothowar, Punjab-76, Barani-83, Kohinoor-83, Faisalabad-85, Chakwal-86, Pasban-90, Inqalab-91, Punjab-96, Uqab-2000, Chenab-70, Iqbal-2000, AS-2000, Bhakkar-2002, V-03079 and V-04189 were cultured on MS medium under four salinity levels (0, 50, 100 and 150 mM NaCl). The developed plantlets were transferred on rooting medium salinized with same salinity levels (MS+1mgL^{-1} IAA). Plants of ten wheat varieties (Ufaq-2002, Parwaz-94, LU-26S, Punjab-76, Pasban-90, Inqalab-91, Uqab-2000, Chenab-70, AS-2000, and Bhakkar-2002) developed roots and turned into complete plants. Then these complete plants having both shoots and roots were shifted to sand culture in pots to obtain regenerator seed (R_0). The R_0 seeds of these varieties were further evaluated for different growth parameters (shoot and root weights) to evaluate salt tolerance of wheat somaclones under same salinity levels. The results indicated that somaclones of genotype Ufaq-2002 developed tolerance to salinity at almost all salinity levels having 10.20 g fresh shoot weight, 0.945 g dry shoot weight, 9.783 g fresh root weight and 1.133 g dry root weight.

KEYWORDS: ***Triticum aestivum;*** **genotypes; tissue culture; salinity tolerance; Pakistan.**

INTRODUCTION

Salinity in agricultural lands is a major problem in many countries of the world. The extent, distribution and nature of salt-affected lands are very imperfectly known in most of the countries due to lack of standardization of characterization criteria. Over 800 million hectare area in the world is suffering from both salinity and sodicity (30). Abiotic stresses such as salinity,

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water logging, temperature, etc. have largely affected crop production at global level (39). Among these abiotic stresses, salinity is major factor that affects the plant growth (9, 36). In Pakistan 6.3 million hectares are salt-affected (2); 2.4 million hectares in Punjab, 2.1 million hectares in Sindh, 0.5 million hectares in KPK and 1.3 million hectares in Balochistan. Out of this area, 3369.7 thousand hectares have become totally unproductive due to soil salinity and productive lands are still continuously becoming uncultivable due to salinity problem (13). High salt concentration and water logging of soils are serious problems affecting the agricultural production in Pakistan and reducing yield by 25 percent (37). Since many of these soils are beyond the reach of conventional reclamation techniques, either for economic reasons or for lack of fresh water, a major scientific thrust has been aimed at developing suitable salt and water logging tolerant crops to bring these lands into agricultural productivity (22). In this regard, an understanding of plant responses to various stresses and mechanisms that make some species or genotypes more tolerant than others, seems essential. The accumulation of toxic ions in rhizosphere initially causes injury to plant roots and their presence in aerial parts causes damage to plant metabolism by reducing growth and yield (18).

Sodium chloride significantly reduces fresh and dry root/shoot masses, root length and shoot length (9, 44). Growing leaf cells maintain their turgor pressure in response to the salt stress by accumulating the osmotically active solutes (24). Root growth is almost always less affected than shoot growth by increasing soil salinity, so root-shoot ratio generally increases (28). Soil and water salinity directly affects the wheat production. The reduction in growth of many crop plants by salinity may be due to its effect on dry matter production, ionic relations, water potential, physiological disorders, metabolic reactions or combination of all these factors (5, 6, 7).

Two approaches are being used to tackle soil stress problem. The first is to amend the soil conditions to favour crop plants. The second one is to use the genetic ability of plants for their adaptability to adverse soil conditions. The first approach is a long term process and a few success has been seen in our country even after spending Rs. 21 billion upto the year 1988 (8). However, the latter is short term strategy and includes the crop cultivation on salt affected fields. Different varieties of wheat do not equally respond to soil salinity. Some varieties have been observed very sensitive to soil salinity while others have shown high salt tolerance (4). Thus there is a need to screen out wheat cultivars suitable for cultivation in saline areas. During the last few years, *in vitro* selection for salt tolerance has been adopted as a technological solution to salinity problem (10). Cell lines of major crops

having desirable characters, such as salt resistance, can provide base for the regeneration of plants and provide an insight into the mechanism of tolerance at cellular level.

It is very difficult, time consuming and tedious to breed salinity tolerant varieties through conventional means. In contrast exploitation of somaclonal variation in plants to develop salt tolerant cultivars through tissue culturing is advantageous over conventional methods. Exploitation of somaclonal variation is an efficient and potent method for producing novel and useful varieties (26).

In vitro somatic cell and tissue culture technologies have been developed to assist plant breeding. During the last few years tissue culture methods have been emerged that can improve the field crops. The use of cell, tissue and anther culture as tools for the betterment of crop plants has now been recognized (20, 41). The regeneration of a complete plant is possible today from major cereal crops; such as bread wheat, maize, rice and barley (15, 27, 35, 42, 43).

There are significant differences in salinity tolerance of plants and the way in which they partition excess of salts in various parts to sustain growth. Hu *et al.* (23) examined the short term effects of salinity and drought on nutrient imbalance in wheat seedlings. After harvesting, it was observed that reduction in plant height and shoot biomass under drought was quite similar to that under salinity as compared to control plants.

Keeping in view the importance of wheat crop and increasing problem of salinity, the present study was undertaken to develop wheat somaclones with a better potential to grow in saline areas where wheat is either grown inefficiently or not at all.

MATERIALS AND METHODS

This study was conducted in the Department of Botany, Government College University, Faisalabad, and partially at Agricultural Biotechnology Research Institute, AARI, Faisalabad, Pakistan during the year 2005-2011. Calli developed after four weeks of seed culture were proliferated by sub- culturing 4-5 times on the same salinity levels + 3mg L^{-1} 2,4-D. After proliferation, calli of 20 wheat varieties (Ufaq-2002, SA-42, Parwaz-94, LU-26S, Pothowar, Punjab-76, Barani-83, Kohinoor-83, Faisalabad-85, Chakwal-86, Pasban-90, Inqalab-91, Punjab-96, Uqab-2000, Chenab-70, Iqbal-2000, AS-2000,

Bhakkar-2002, V-03079 and V-04189) were subjected to regeneration under same salinity levels. The regeneration medium comprised MS media without having any auxin (2, 4-D). Ten test tubes of each varieties were maintained in each somaclone under the control led environmental conditions. Surviving calli under salt stress were shifted to regeneration medium for the development of plantlets. MS basal medium without any addition of 2,4-D was used for the regeneration of plants under same salinity levels (25). Thus these developed plantlets were transferred on rooting medium salinized with the same salinity levels (MS+1mgL^{-1} IAA). Plants of ten wheat varieties (Ufaq-2002, Parwaz-94, LU-26S, Punjab-76, Pasban-90, Inqalab-91, Uqab-2000, Chenab-70, AS-2000 and Bhakkar-2002) developed roots and turned into complete plants. The plantlets developed by this method were shifted to the pots filled with sand and gravel under same salinity levels and controlled conditions upto maturity to produce R_o seeds. R_0 seeds of these somaclones were germinated and grown in plastic buckets containing vermiculite and gravel (1:1 by volume) in a wire house to avoid the birds attack. Four levels of salinity, non-saline and saline (0, 50, 100, and 150mM NaCl) were used. The control and saline buckets were filled and drained daily. Full strength Hoagland nutrient solution (21) was used as nutrient solution. The buckets were recycled with the same solution throughout the week. Salt was mixed to the nutrient solution to develop the required concentration and salinity levels. Ten seeds of each somalone were grown in each treatment with five replications having two plants each. Plants were harvested seven weeks after sowing. Four parameters viz. fresh shoot weight, dry shoot weight, fresh root weight and dry root weight were studied at seedling stage.

The experiment was conducted in a completely randomized design (CRD) in factorial arrangement (38) with five replications in each treatment. The least significant differences (LSD) test was applied to assess the significant differences between the mean values.

RESULTS AND DISCUSSION

Regeneration studies

The data (Table 1) on plantlet development indicated that number of plantlets developed varied from 1 to 48 plants in all varieties for all treatments. Maximum number of shoots (48) were developed by LU-26S followed by Pasban-90 (40), Ufaq-2002 (31), AS-2000 (31) Inqalab-91 (29) and Uqab-2000 (26). Minimum number of shoots developed in SA-42(1). The data showed that shoot development was suppressed with increase in salinity indicating the deleterious effect of NaCl on morphogenetic process in wheat.

Table 1. Number of plants regenerated in 20 different wheat varieties under various salinity levels.

Name of variety	Salinity levels (mM NaCl)				Total
	0	50	100	150	
Ufaq-2002	15	10	4	2	31
SA-42	1	0	0	0	1
Parwaz-94	7	6	5	2	20
LU-26S	21	10	10	7	48
Pothowar	4	1	1	0	6
Punjab-76	9	3	2	1	15
Barani-83	2	0	0	0	2
Kohinoor-83	1	1	0	0	2
Faisalabad-85	4	2	0	0	6
Chakwal-86	7	1	0	0	8
Pasban-90	17	10	8	5	40
Inqalab-91	12	8	6	3	29
Punjab-96	4	1	1	0	6
Uqab-2000	13	7	5	1	26
Chenab-70	6	4	2	1	13
Iqbal-2000	6	2	2	0	10
AS-2000	12	9	7	3	31
Bhakkar-2002	10	6	6	2	24
V-03079	2	1	0	0	3
V-04189	1	1	1	0	3
Total	154	83	60	27	324

On overall basis, maximum plants developed (154) were observed where no salt was applied, followed by salinity levels of 50 mM (83) and 100 mM NaCl (60). Minimum plants (27) were produced at higher salinity level of 150 mM NaCl. In control treatment LU-26S excelled (21) while SA-42, Kohinoor-83 and V-04189 remained in bottom. At 50mM NaCl level variety LU-26S again topped and was at par with Pasban-90 and Ufaq-2002 having 10 plants each. Minimum response to regeneration was exhibited by Pothowar, Kohinoor-83, Chakwal-86, Punjab-96, V-03079 and V-04189 having one plant each. At 100 mM NaCl, LU-26-S once again showed better performance (10 percent) while SA-42, Barani-83, Kohinoor-83, Faisalabad-83, Chakwal-86, and V-03079 failed to develop any plant. At 150 mM level of salinity, ten varieties viz. SA-42, Pothawar, Barani-83, Kohinoor-83, Faisalabad-85, Chakwal-86, Punjab-96, Iqbal-2000, V-03179 and V-04189 did not produce any plant, while LU-26S ranked first (7 plants) at this salt level as well.

The effect of salts was quite visible on plant development which decreased as the salinity level increased. Ozgen *et al.* (32) and Poustini and Salmasi (34) supported these results who observed that plant regeneration was apparently influenced by culture medium with significant reduction in total dry production.

Thus these developed plantlets were transferred on rooting medium salinized with same salinity levels (MS+1mgL^{-1} IAA). Plants of 10 wheat varieties (Ufaq-2002, Parwaz-94, LU-26S, Punjab-76, Pasban-90, Inqulab-91, Uqab-2000, Chenab-70, AS-2000 and Bhakkar-2002) developed roots and turned into complete plants.

Fresh shoot weight

The data (Table 2) indicated that salinity significantly decreased the shoot fresh weight at all salt levels. Comparison of means showed significant differences among treatments, variety and salinity x varieties interaction. On treatments mean basis, maximum fresh shoot weight (9.94g) was observed in control followed by 50, 100 and 150 mM NaCl concentration with significant differences.

Table 2. Effect of salinity on fresh shoot weight (g) of wheat somaclones.

Name of variety	Salinity levels (mM NaCl)				Mean
	0	50	100	150	
Ufaq-2002	13.40b	14.26a	7.20m	5.92p	10.20a
Parwaz-94	12.94c	13.90a	5.32qr	3.46v	8.90b
LU-26S	9.04h	8.84hi	5.04r	3.98tu	6.72f
Punjab-76	7.42lm	6.52o	5.22qr	3.84u	5.75g
Pasban-90	9.62f	9.42fg	7.36lm	5.46q	7.96c
Inqalab-91	11.32e	12.12d	8.98hi	7.84jk	10.06a
Uqab-2000	8.14j	8.00jk	7.66kl	6.2op	7.50 cde
Chenab-70	9.18gh	8.64i	7.40lm	4.26t	7.37de
AS-2000	8.94hi	8.90f	7.84jk	4.98rs	7.66cd
Bhakkar-2002	9.40fg	8.16j	6.924n	4.66s	7.28e
Mean	9.94a	9.87a	6.90b	5.06c	

Mean sharing the same letter do not differ significantly at P = 0.05
LSD: Variety = 0.55, Salinity = 0.35, Salinity x Variety = 0.35

Minimum fresh shoot weight (5.06g) was noted in the highest salinity level. On the basis of mean for varieties, maximum shoot fresh weight was recorded in somaclones of Ufaq-2002 (10.20g) followed by Inqalab-91 (10.06g) and Parwaz-94 (8.90g). Minimum shoot fresh weight (5.75g) was produced by somaclone of Punjab-76. Interaction of salinity x varieties was also significant. Somaclones of Ufaq-2002 gave maximum shoot fresh weight (14.26 and 13.4g) at control and 50 mM NaCl while somaclone of Punjab-76 produced minimum fresh shoot weight (7.42 and 6.52g) at these salt levels. In case of 100 mM NaCl, variety Inqalab-91 produced maximum shoot fresh growth weight (8.98g) and LU-26S yielded minimum (5.04 g). At higher salinity level (150mM NaCl) Inqalab-91 excelled (7.84g) and Parwaz-94 ranked last (3.46g). The data further revealed that Ufaq-2002 was more

tolerant at salinity levels of 0 and 50 mM salt levels while Pasban-90, LU-26S, AS-2000 and Bakkar-2002 performed better at 50mM NaCl than control. Gorham and Jones (19) have reported similar results. It was probably because of the fact that low amount of salts might have rather stimulating effect on biological activities within the soil, especially the micro-organisms. Another reason could be that salts might have served as nutrients at low concentrations.

Dry shoot weight

The data (Table 3) revealed that average shoot dry weight decreased from 1.09g in control (0 NaCl) to 0.40g at the highest salinity level i.e. 150 mM NaCl. Differences among varietal means were also significant. The highest dry shoot weight (0.97g) was noted in LU-26S followed by Ufaq-2002 (0.94g) and Parwaz-94 (0.814g) while Uqab-2000 produced the lowest (0.43 g).

Salinity and varietal interaction also showed significant differences, where somaclones and salinity levels were negatively correlated with each other. Minimum dry shoot weight (0.20g) was found in Bhakkar-2002 at maximum salinity level and maximum dry weight (1.40g) was noted in Ufaq-2002 in control.

Table 3. Effect of salinity on dry shoot weight (g) of wheat somaclones.

Name of variety	Salinity levels (mM NaCl)				Mean
	0	50	100	150	
Ufaq-2002	1.40b	1.01cd	0.86efg	0.50kl	0.94b
Parwaz-94	1.09cd	0.87f	0.78fg	0.50kl	0.81c
LU-26S	1.47a	0.93e	0.79fg	0.71fgh	0.97a
Punjab-76	0.84f	0.83fg	0.65ghi	0.49lm	0.70f
Pasban-90	1.17c	0.80fgh	0.76fgh	0.32n	0.76de
Inqalab-91	1.36bc	0.82fg	0.60hi	0.38mn	0.79d
Uqab-2000	0.65hi	0.53k	0.32n	0.25pq	0.43gh
Chenab-70	1.06cd	0.76gh	0.90e	0.35mn	0.77de
AS-2000	1.11c	0.86f	0.56jk	0.30no	0.71e
Bhakkar-2002	0.72gh	0.62hi	0.29op	0.20qr	0.46g
Mean	1.09a	0.80b	0.65c	0.40d	

Means sharing the same letter do not differ significantly at P = 0.05
LSD: Variety = 0.27, Salinity=0.13, Salinity x Variety=0.13

Fresh root weight

Among salinity levels, maximum fresh root weight (9.77g) was obtained in control which decreased significantly as the salinity level increased and was minimum (5.33g) in treatment of 150 mM NaCl (Table 4). Comparison of means among genotypes indicated that Ufaq-2002, Parwaz-94, Inqalab-91 and Pasban-90 had significantly higher fresh root weight (9.78, 8.41, 8.27

Table 4. Effect of salinity on fresh root weight of wheat somaclones (g).

Name of variety	Salinity levels (mM NaCl)				Mean
	0	50	100	150	
Ufaq-2002	12.14a	10.46c	8.92hi	8.06k	9.785a
Parwaz-94	11.3b	9.4fg	6.76m	6.2no	8.415b
LU-26S	8.02k	7.28l	5.54p	4.84rs	6.420e
Punjab-76	6.14o	8.08k	3.14w	4.02uv	5.345f
Pasban-90	11.38b	9.18gh	5.28pq	4.18qr	7.745cd
Inqalab-91	9.76de	9.72ef	7.12l	6.5mn	8.275bc
Uqab-2000	11.3b	9.42efg	4.06uv	4.36tu	7.285d
Chenab-70	9.24gh	8.9hi	4.48st	4.12uv	6.695e
AS-2000	9.9d	9.72ef	5.3pq	5.52pq	7.660d
Bhakhar-2002	8.8ij	8.52j	3.8v	4.52st	6.410e
Mean	9.770a	9.07b	5.44c	5.33c	

Means sharing the same letter do not differ significantly at P = 0.05
LSD: Variety = 0.54 Salinity = 0.34, Salinity x Variety = 0.34.

and 7.74g) than other wheat somaclones while Punjab-76 ranked last (5.34g) (Table 4). At salinity level of 100 and 150 mM NaCl, Ufaq-2002 excelled while Bhakkar-2002 and Punjab-76 produced the lowest.

Dry root weight

A similar trend for dry root weight was observed (Table 5). Comparison of treatment means indicated that increase in salinity significantly decreased the dry root weight of wheat somaclones under all salinity levels. The highest dry root weight was noted in control (0.724g) which was was at par with 50mM NaCl level (0.720g). The differences among the treatments were significant. In case of varieties, the highest dry root weight was recorded in Ufaq-2002, (1.33g) against minimum in Punjab-76 (0.373g).

Table 5. Effect of salinity on dry root weight of wheat somaclones.

Name of variety	Salinity levels (mM NaCl)				Mean
	0	50	100	150	
Ufaq-2002	1.266a	1.326a	0.960b	0.940b	1.133a
Parwaz-94	0.916b	0.792b	0.480b	0.424b	0.656b
LU-26S	0.67b	0.810b	0.450b	0.354b	0.571b
Punjab-76	0.424b	0.522b	0.260b	0.276b	0.373b
Pasban-90	0.766b	0.654b	0.434b	0.302b	0.539b
Inqalab-91	0.608b	0.682b	0.578b	0.468b	0.585b
Uqab-2000	0.746b	0.554b	0.326b	0.308b	0.508b
Chenab-70	0.604b	0.614b	0.362b	0.228b	0.452b
AS-2000	0.66b	0.672b	0.418b	0.374b	0.531b
Bhakkar-2002	0.582b	0.582b	0.300b	0.280b	0.438b
Mean	0.724a	0.720a	0.460b	0.405b	

Means sharing the same letter do not differ significantly at P = 0.05
LSD: Variety = 3.05, Salinity = 1.93, Salinity x Variety = 1.93.

The interaction of treatments x somaclones also showed significant differences. Maximum dry root weight (1.326g) was produced by Ufaq-2002 at 50 mM salt level while Chenab-70 somaclones showed minimum root dry weight (0.228g) at salinity level of 150 mM.

At 50mM NaCl a slight increase in dry root weight (1.326g) was observed in Ufaq-2002 as compared to control (1.266g) while Punjab-76 showed poor response (0.522g). Ufaq -2002 also produced better dry root weight at salinity level of 100 and 150 mM NaCl but it was less than control. Somaclones of Punjab-76 produced the lowest for dry root weight also at higher salinity levels.

In saline soils where salts are present in great concentration The growth of plant is adversely affected due to osmotic effects, specific ion effect and nutritional imbalance; all occurring probably simultaneously (16). Initial retarded growth in saline soils is induced by the decreased water potential of medium due to higher salt presence (29). A major effect of high concentration of Na^{+} and Cl^{-1} in the rooting medium is the suppression of uptake of essential nutrients such as K^{+}, Ca^{++}, Na^{+}, etc. (18). The results revealed that slow increase in salinity in rooting medium reduced while shoot and root length was increased. Akhter *et al.* (1) and Pervaiz *et al.* (33) have also observed similar adverse effects of salts. The reduction in shoot and root yield of all genotypes with the addition of salt may be due to decrease in availability of water with increasing solute suction from saline media or presence of toxic ions in plants. These results are similar to those of Ehret *et al.* (16) and Pervaiz *et al.* (33). Less reduction in shoot length is because of the toxic effect of salts, added to the medium.

CONCLUSION

The study concludes that salinity adversely affected the plant growth of wheat However, somaclones of wheat varieties having salt tolerance produced higher root and shoot weights at different salinity levels and can be sown on marginal lands where no other crop is grown.

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