INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
11–728/AWB/2012/14–4–600–604
http://www.fspublishers.org

Full Length Article

Somaclonal Variation for Development of Salt Tolerance in Selected Wheat (*Triticum aestivum*) Cultivars

SALEEM AKHTAR, MUBASHIR NIAZ[1]†, SAJID-UR-RAHMAN, MUHAMMAD YOUNAS AND MUHAMMAD ZAFFAR IQBAL
Agricultural Biotechnology Research Institute, AARI, Faisalabad, Pakistan
†Department of Botany, Government College University, Faisalabad, Pakistan
[1]Corresponding author's e-mail: mniaz_prof@yahoo.com

ABSTRACT

The calli of twenty wheat varieties were cultured on MS medium under four salinity levels *i.e.,* 0, 50, 100 and 150 mM NaCl to develop salt tolerant wheat somaclones. The developed plantlets were transferred on rooting medium salinized with the same salinity levels (MS + 1 mgL^{-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 & Bhakhar-2002) developed roots and turned in to complete plants. These complete plants having both shoots and roots were shifted to sand culture in pots to obtain regenerator seed (R0). The R_0 seeds of these varieties were further evaluated for Na^+, K^+ and K^+:Na^+ ratio to evaluate the salt tolerance of wheat somaclones. Keeping in view Na^+, K^+ ion concentration and K^+:Na^+ ratio. Somaclones of genotype Inqulab-91 were found to be tolerant to salinity at almost all the salinity levels. © 2012 Friends Science Publishers

Key Words: Wheat; Somaclones; NaCl; K^+:Na^+ ratio

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the major staple foods in world. In Pakistan it is grown on an area of 8.41 million hectares with an annual production of 21.74 million tons (Anonymous, 2008-2009), which is very low as compared to world production. Various environmental stresses, particularly salt stress, are associated with low production potential of wheat in Pakistan.

Two approaches are being used to tackle soil stress problem. The first is to amend the soil conditions to favor crop plants. A variety of management practices are being used to ameliorate these salt affected soils such as provision of adequate drainage and use of amendment additives yet on account of setbacks like poor quality irrigation water, high cost of amendment additives and low exploitation ability of plants for their adaptability to adverse soil conditions. The first approach is a long term process and a little success has been seen in our country even after spending Rs.21 billion up to 1988 (Aslam *et al*., 1993). However, the latter is short term strategy and includes the crop cultivation on the salt effected fields.

In vitro somatic cell and tissue culture technology has been developed to assist plant breeding programs. During the last few decades tissue culture methods have been emerged that can be under taken to improve the field crops. The cell, tissue and anther culture as tools to use in the crop plants performance have now been recognized (Green, 1977; Vasil, 1987). The regeneration of a complete plant is possible today from major cereal crops; such as bread wheat, maize, rice and barley (Duncan *et al*., 1985; Yamada *et al*., 1986; Luhrs & Lorz, 1987; Redway *et al*., 1990; Vasil *et al*., 1990).

The first successful tissue culture in cereal from endosperm was done by La Rue in 1949. Since then a handful reports are there in literature for the production of agriculturally useful *in vitro* selected traits of agronomic values (Sibi & Fakiri, 2000; Khan & Ahmad, 2011). Regenerated plants varied in a wide range of agro-morphological characters. The variability associated with cell and tissue culturing has been somaclonal variation (Larkin & Scowcroft, 1981). This variation is due to heterogeneity between the cells and explants tissue, or a simple representation of spontaneous mutation and is due to the activation by cultural environmental transposition of genetic materials.

In saline soils, where salts are present in great concentrations, growth of plant is affected adversely through various ways such as osmotic effects, specific ion effect and nutritional imbalance; all occurring probably simultaneously (Flowers *et al*., 1991). Initial retarded growth in saline soils is induced by the decreased water potential of medium due to higher salt presence (Munns *et al*., 1995). A major effect of high concentration of Na^+ and Cl^- in the root medium is the suppression of uptake of essential nutrients such as K^+, Ca^{2+}, Na^+ etc. (Gorham &

To cite this paper: Akhtar, S., M. Niaz, S.U. Rahman, M. Younas and M.Z. Iqbal, 2012. Somaclonal variation for development of salt tolerance in selected wheat (*Triticum aestivum*) cultivars. *Int. J. Agric. Biol.*, 14: 600–604

Wyn Jones, 1993). Ghannadha *et al*. (2005) studied the relationship between different traits and salt tolerance in bread wheat through tissue culture and observed that with the increase of salt the callus size was decreased and Na^+ contents of the callus increased. Genotypes and genotypes x salt effects were significant for K^+ and K^+:Na^+ ratio. Similar findings were reported by Patade *et al*. (2008). There is an apparent proof that cultivar with high K^+ levels can tolerate the presence of high sodium (Caterina & Giuliani, 2007).

Keeping in view the importance of wheat crop and increasing problem of salinity, the present studies were 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

Experimental material and culture conditions: The research work presented in this paper was conducted at the Department of Botany, Government College University, Faisalabad and partially at the Agricultural Biotechnology Research Institute, AARI, Faisalabad, during the year 2005-2011. Seeds of twenty 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, Inqulab-91, Punjab-96, Uqab-2000, Chenab-70, Iqbal-2000, AS-2000, Bhakhar-2002, V-03079 and V-04189 were cultured on solidified MS medium (Murashige & Skoog, 1962) supplemented with 2,4-D @ 3 mg L^{-1} under four salinity levels *i.e.,* 0, 50, 100 and 150 mM NaCl under aseptic conditions to produced callus Then the material was placed in incubation room at 28±2°C with 12 h photoperiod illuminated with florescent light to produced 2500 lux.

Regeneration of plantlets: Surviving calli under salt stress were shifted to regeneration medium for the development of plantlets. MS basal medium without 2, 4-D was used for the regeneration of plants under same salinity levels after following Hussain *et al*. (2001). These developed plantlets were transferred on rooting medium salinized with the same salinity levels (MS +1 mg L^{-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 & Bhakhar-2002) developed roots and turned in to complete plants. The plantls developed by this method were shifted to the pots filled with sand and gravel under the same salinity levels under controlled conditions up to maturity to produced R_0 seeds. R_0 seeds of 10 wheat somaclones i.e. Ufaq-2002, Parwaz-94, LU-26S, Punjab-76, Pasban-90, Inqulab-91, Uqab-2000, Chenab-70, AS-2000, and Bhakhar-2002 were germinated and grown in plastic buckets containing vermiculite and gravel (1:1 by volume) in a wire house to avoid the birds. Four levels of salinity, non-saline and saline (50, 100 & 150 mM NaCl) were used.

The control and saline buckets were filled and drained daily. Full strength Hoagland nutrient solution (Hoagland & Arnon, 1950) was used as the nutritional source. The buckets were recycled with the same solution throughout the week. Calculated amount of NaCl was mixed to the nutrient solution to develop the desired salinity levels.

Ten seeds of each somaclones were grown in each treatment and were divided into five replication of each plant. Plants were harvested five weeks after sowing. The youngest expanded leaves were preserved in the tubes at freezing temperature for the extraction of sap. Na^+, K^+ and K^+:Na^+ ratio were determined from the leaf sap.

Plant Analysis

Plant sample collection: After uprooting the plants, soil was removed carefully from the roots. Roots and shoots were separated and the later were dried with tissue paper. Root and shoot fresh weights were recorded immediately. The youngest fully expanded leaf was detached, washed with distilled water, blotted and stored in 1.5 cm^3 polypropylene microcentrifuge tubes at freezing temperature. The leaf was used later for tissue sap extraction. Fresh weight of shoot was again recorded after removing the leaf and the sample was preserved in the paper bag. To obtain dry weight, the plant shoot was dried at 70°C till the weight was constant. Calculations were made to get the dry weight of shoot including the leaf separated for tissue sap extraction.

Extraction of tissue sap: For the extraction of sap the plant material was frozen in 1.5 cm^3 micro centrifuge tubes, thawed and crushed by using a metal rod with a tapered end. Small holes were made in the cap and in the base of the tube and the sap were centrifuged in a second centrifuged tube at about 6000 × *g*. Another centrifugation of second tube was done at 9000 × *g* for 3 min and the sap was diluted for the estimation for inorganic ions following Gorham *et al*. (1984).

Determination of Na^+ and K^+: Tissue sap was diluted as required by adding distilled water and sodium and potassium estimation was done by using Flame photometer with the help of standard solutions of NaCl and KCl prepared from reagent grade salts (Richards, 1954).

Statistical analysis: The experiment was conducted in a completely randomized design (CRD) in factorial arrangement (Steel & Torrie, 1980) with five replications in each treatment using MSTATC computer software. The least significant differences (LSD) test to assess the significant differences between the mean values was calculated.

RESULTS

Regeneration studies: This experiment was aimed at production of callus derived plants from varieties previously screened for callogenesis under salt stress. The response of genotypes to plantlet development under 4 levels of NaCl is presented in Table I. Range of plantlets developed varies from 1 to 48 plants among all the varieties. Maximum shoots (48) were developed by LU-26S followed by Pasban-90 (40), Ufaq-2002 (31), Inqulab-91 (29) and Uqab-2000

(26). Minimum shoot developed in SA-42 (1). Total number of plants ranged from 1 to 48. Shoot development was suppressed with increase in salinity that indicated deleterious effect on morphogenetic process in wheat. Maximum number of developed shoots (154) was observed where no salt was applied *i.e.,* 0 mM NaCl followed by 83 and 60 at salinity levels 50 and 100 mM NaCl, while minimum number of plants (27) was produced at the higher salinity level of 150 mM NaCl.

At control level LU-26S was at the top with maximum plant (21), while SA-42, Kohinoor-83 and V-04189 were at the bottom with one plant each. At 50 mM NaCl level varieties LU-26S was also at the top at par with Pasban-90 having 10 plants each. Minimum response to regeneration was exhibited by Pothowar at par with Kohinoor-83, Chakwal-86, Punjab-96, V-03079 and V-04189 having 1 plant each. At 100 mM NaCl LU-26-S again showed best performance, 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 10 varieties SA-42, Pothawar, Barani-83, Kohinoor-83, Faisalabad-85, Chakwal-86, Punjab-96, Iqbal-2000, V-03179 and V-04189 did not produced any plant; whilst LU-26S was also on the top at this level of salinity.

Ionic Analysis

Na^+ concentration in the leaf sap: The data relating Na^+ concentration in the sap of fully expanded leaf on different wheat somaclones is presented in Table II. On overall basis, youngest expanded leaf had much lower Na^+ concentration in controlled treatment than the salinity applications. Comparison of treatment means indicated non-significant differences in Na^+ concentration under controlled conditions, but the addition of salt significantly increased Na^+ concentration under these conditions. Maximum Na^+ (52.08 mM) was recorded at higher salinity level *i.e.,* 150 mM NaCl followed by 20.81 mol m^{-3} and 7.52 mol m^{-3} at the level of 100 and 150 mM NaCl. The minimum Na^+ concentration (2.26 mM) was observed in control where no salt was applied.

Data revealed significant differences in Na^+ concentration within genotypes. Somaclone AS- 2000 proved more salt sensitive with the value of 27.87 mM Na^+ concentration in the leaf followed by Parwaz-94 and LU-26S with the value of 23.91 mM and 23.65 mM respectively, less affected somaclones were from variety Uqab-2000 with the mean values of 20.20 mM Na^+. Interaction between the salinity and somaclones was also found statistically significant. There was positive correlation between genotypes and salinity levels as the salinity increased Na^+ concentration also increased. Maximum Na^+ concentration (70.04 mM) was found for wheat somaclone AS- 2000 at 150 mM NaCl level while minimum for LU-26S at controlled level.

K^+ concentration in younger fully expanded leaf: The effect of somaclones and salinity levels on K^+ concentration in wheat crop has been shown in Table III. Analysis of variance indicated the somaclones, salinity and there interaction decreased the K^+ concentration in wheat plants significantly. K^+ concentration decreased with increasing soil salinity. K^+ decreased from 231.6 mM at controlled salt level to 133.7 mM at highest salinity level of 150 mM NaCl. The observed decreased in K^+ concentration with increasing salinity may be due to competition among sodium and potassium because sodium antagonizes potassium uptake due to which sodium partially can substitute potassium in certain metabolic reactions at moderate salinity level.

Maximum K^+ concentration (204 mM) was found in Punjab-76 and minimum (183.9 mM) in the somaclone of Bhakhar-2002. The other genotypes responded in between these two ranges. The resultant reduction in potassium may be the result of growth dilution effect. Interaction between genotypes and salinity was also observed statistically significant. As salinity increase, K^+ concentration decreased in a consistent pattern. Maximum K^+ (238.0 mM) was found in Parwaz-94 at controlled salinity level and minimum

Table I: Regeneration Potential of 20 wheat varieties under salt stress (No. of plants)

Variety	NaCl (mM)			
	0	**50**	**100**	**150**
Ufaq-2002	15	10	4	2
SA-42	1	0	0	0
Parwaz-94	7	6	5	2
LU-26S	21	10	10	7
Pothowar	4	1	1	0
Punjab-76	9	3	2	1
Barani-83	2	0	0	0
Kohinoor-83	1	1	0	0
Faisalabad-85	4	2	0	0
Chakwal-86	7	1	0	0
Pasban-90	17	10	8	5
Inqulab-91	12	8	6	3
Punjab-96	4	1	1	0
Uqab-2000	13	7	5	1
Chenab-70	6	4	2	1
Iqbal-2000	6	2	2	0
AS-2000	12	9	7	3
Bhakhar-2000	10	6	6	2
V-03079	2	1	0	0
V-04189	1	1	1	0
Total	**154**	**83**	**60**	**27**

Table II: Effect of Salinity on Na^+ concentration (mol m^{-3}) of 10 wheat varieties

Variety	NaCl (mM)			
	0	**50**	**100**	**150**
Ufaq-2002	1.64z	8.02u	19.88n	46.08g
Parwaz-94	2.22yz	10.06qr	27.66j	55.68c
LU-26S	1.44z	8.56st	24.76l	59.82b
Punjab-76	2.20yz	5.14v	14.88o	44.8h
Pasban-90	2.30y	5.18v	14.34p	46.66f
Inqulab-91	1.84z	3.44wx	10.35q	39.70i
Uqab-2000	2.40y	7.82u	22.32m	48.68e
Chenab-70	2.96x	9.06s	25.94k	55.60d
AS-2000	3.72w	9.82r	27.86j	70.04ac
Bhakhar-2002	1.82z	8.10tu	20.08n	55.88

Mean sharing the same letter do not differ significantly at P = 5%

Table III: Effect of salinity on K^+ concentration (mol m^{-3}) in leaf sap of 10 wheat varieties at different salt levels

Variety	NaCl (mM)			
	0	50	100	150
Ufaq-2002	237.2a	228.6d	208.2l	115.4v
Parwaz-94	238.6a	217.8i	202.0m	142.6q
LU-26S	224.0fg	223.4g	211.4k	119.0t
Punjab-76	235.6b	228.4d	214.40j	137.6r
Pasban-90	233.2c	210.4i	199.2m	137.0s
Inqulab-91	229.4d	215.4k	201.6n	141.6rs
Uqab-2000	226.0e	225.6j	210.2m	146.8q
Chenab-70	232.4c	220.2ef	194.8k	144.2p
AS-2000	237.8a	201.18h	195.4o	117.4p
Bhakhar-2002	222.4h	218.6m	203.0o	135.4ub

Table IV: Effect of Salinity on K^+/Na^+ of wheat somaclonal

Variety	NaCl (mM)			
	0	50	100	150
Ufaq-2002	151.618b	28.5l	10.47r	2.5u
Punjab-94	82.778e	21.6o84	7.302t	2.55u4
LU-26S	156.038a	26.13mn6	8.542st	1.986u
Punjab-76	107.344e	43.21k	14.412q	3.06u
Pasban-90	99.97f	42.214k	14.308q	2.898u
Inqulab-91	124.9c	62.194j	19.696p	3.486u
Uqab-2000	94.924g	27.558lm	9.024rs	2.93u
Chenab-70	78.622h	24.872n	8.098st	2.636u
AS-2000	63.012i	22.438o	6.99t	2.078u
Bhakhar-2002	122.38d	24.894n	9.63rs	2.096u

Mean sharing the same letter do not differ significantly at P = 5%

(115.4 mM) for Ufaq-2002 at higher salinity level that is 150 mM NaCl.

K^+:Na^+ ratio: The K^+:Na^+ ratio assessed from leaves of wheat somaclones was quite high under saline conditions. It decreased drastically as the salinity level enhanced. Comparison of treatments means indicated a significantly decreased K^+:Na^+ ratio with increase in salinity. Maximum K: Na ratio 110.7 was in control (0mM NaCl) while lowest (2.623) K^+:Na^+ ratio was observed at salinity level 150mM NaCl and 100mM NaCl level, it was 32.21 and 10.85, respectively.

Comparison of means among the genotypes was also significant. Genotype inqlab-91 had the higher K^+: Na^+ ratio in comparison with other genotypes. It was followed by Ufaq-2002 and LU-26S were proved to have almost the same K^+:Na^+ ratio while AS-2000 had the lowest K^+:Na^+ ratio (23.92). Interaction between salinity and genotypes was also found significant .Ufaq-2002 had maximum K^+:Na^+ ratio under control (having no salt) condition. Inqulab-91 showed maximum K^+:Na^+ ratio at all salt levels with the value of 62.19, 19.70 and 3.49, respectively.

DISCUSSION

In this investigation, the effect of salinity was quite dominant on the development of plant, which decreased as the salinity level increased. Maximum shoots (48) were developed by LU-26S followed by Pasban-90(40) and Ufaq-2002 with 31 plants. Minimum shoot development was observed in SA-42 (1). Accordingly, Ozgen *et al*. (1996) and Poustini and Salmasi (1997) reported that plant regeneration is apparently influenced by culture medium and found significant reduction in total dry mass. The potential of superior genotypes for salt tolerance together with their high embryogenic callus induction led us to recommend these varieties as a good model to observe physiological mechanism involved in *in vitro* selection for salt tolerance in wheat.

As for mineral contents, salinity enhanced the Na^+ and reduced the K^+ contents gradually. However, difference in the accumulation of Na^+ and K^+ concentrations in their leaves at all the salinity levels indicated their genetic behavior (Ashraf & Naz, 1994). This indicated the suppressive effect of Na^+ on K^+, as the former outcompetes the latter during transport at membrane level (Taiz & Zeiger, 2010), although there is proof that cultivar with high K^+ can better tolerate Na^+ (Caterina & Guiliani, 2007). In our study, maximum K^+ concentration was found in somaclones of Punjab-76. The relative shoot dry yield produced at high salinity has been considered as the standard (most rational) critical of relative salt tolerance of sunflower genotypes (Wahid *et al.*, 1999) while the result due to other growth parameters were evaluated against it. A consistent pattern among the growth parameters and salt tolerance of genotypes tested. Thus, the rating of tolerance to salinity based on growth parameters cannot be considered worthwhile. In assessing the plant to salinity tolerance, both the K^+ and Na^+ concentration at higher salinity level has been used by different workers (Poustani & Ciocemardeh, 2001). It is found that considering the K^+:Na^+ value is necessary when comparing genotypes that are differed widely in growth habit or, are grown under various environment conditions (Javed, 2002). Moreover, research is required to develop such correlation among genotypes to exploit their potentials in salt tolerance.

In present study an addition of salt to the rooting medium caused higher accumulation of sodium ions in leaf and roots, which is commonly seen in salt stress studies (Ashraf & Naz, 1994). However, increase in accumulation of Na^+ ions in leaves under saline environments was noted in all somaclones. This indicated similar toxic effect of increase accumulation of Na^+ under saline stress; plant growth is stunted through osmotic effects as well as specific ion toxicity and nutritional disturbance. It is clear that only accumulation of Na^+ may have a role in increasing the osmotic potential of wheat somaclone. We found negative correlations between Na^+ contents and yield components of plants, which corroborate the findings of Salam *et al*. (1999). The somsclones i.e. Inqulab-91, Ufaq-2002, LU-26S, Punjab-76 and Pasban-90 having more K^+ concentration and K^+:Na^+ can be grown successfully under saline soils and give considerable yield.

In conclusion, NaCl salinity increased Na^+ concentration and decreases K^+ concentration. However,

somaclones developed having salt tolerance at different salinity levels can be sown in marginally saline lands.

REFERENCES

Anonymous, 2008-2009. *Agricultural Statistics of Pakistan*. Ministry for Food, Agriculture and Livestock, Islamabad

Ashraf, M. and F. Naz, 1994. Response of some arid zone grasses to potassium deficiency. *Acta Physiol. Plant,* 16: 769–801

Aslam, M., R.H. Qureshi and N. Ahmad, 1993. A rapid screening technique for salt tolerance in rice. *Plant Soil*, 150: 99–107

Caterina, R. and M.M. Giuliani, 2007. Influence of salt stress on seed yield and oil quality of two sunflower hybrids. *Ann. Appl. Biol*., 151: 145–154

Duncan, D.R., M.E. Williams, B.E. Zehr and J.M. Widholm, 1985. then production of callus capable of plant regeneration from immature embryos of numerous *Zea mays* genotypes. *Planta,* 165: 322–332

Flowers, T.J., M.A. Hajibagheri and A.R. Yeo, 1991. Ion accumulation in the cell wall of rice plants grown under saline conditions: evidence for the oertli hypothesis. *Plant Cell Environ.*, 14: 319–325

Ghannadha, M.R., M. Omidi, R.A. Shshi and K. Poustini, 2005. A study of salt tolerance in genotypes of bread wheat using tissue culture and germination test. *Iranian J. Agric. Sci.,* 36: 75–85

Gorham, J. and R.G. Wyn Jones, 1993. Utilization of Triticeae for improving salt tolerance in wheat. *In:* Leith, H. and A.A. Massoum (eds.), *Towards the Rational use of High Salinity Tolerants Plants,* pp: 27–33. Kluwcr Acad. Publication, The Netherlands

Gorham, J., E. Mc Donnell and R.G. Wyn Jones, 1984. Salt tolerance in the Triticeae: I. Leymus sabulosus. *J. Exp. Bot*., 35: 1200–1209

Green, C.E., 1977. Prospects for crop improvement in the field of cell culture. *Hort. Sci.,* 12: 131–134

Hoagland, D.R. and O.J. Arnon, 1950. The water-culture method for growing plants without soil. *California Agric. Exp. Stn. Circ.*, No. 347

Hussain, M., G.S. Khan, M.S. Shaheen and A. Ahmad, 2001. Somaclonal variation in regenerated plants of ten wheat genotypes. *J. Agric. Res*., 39: 1–7

Javed, F., 2002. *In vitro* salt tolerance in wheat. II.Organic solute accumulation in callus. *Int. J. Agric. Biol*., 4: 462–464

Khan, M.A. and J. Ahmad, 2011. In vitro wheat haploid embryo production by wheat x maize cross system under different environmental conditions. *Pakistan J. Agric. Sci.,* 48: 39–43

La Rue, C.D., 1949. Cultures of the endosperm of maize. *American J. Bot.*, 34: 585–586

Larkin, P.J. and W.R. Scowcroft, 1981. Somaclonal variation: A novel source of variability from cell cultures for plant improvement. *Theor. Appl. Genet*., 60: 197–214

Luhrs, R. and H. Lorz, 1987. Initiation of morphenogenic cell suspension and protoplast culture of barley. *Planta,* 175: 71–85

Munns, R., D.P. Schachtman and A.G. Condon, 1995. The significance of a two-phase growth response to salinity in wheat and barley. *Australian J. Plant Physiol.,* 22: 561–569

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*, 15: 473–479

Ozgen, M., M. Turet, S. Ozean and C. Saneak, 1996. Callus induction and plant regeneration from immature and mature embryos of winter durum wheat genotypes. *Plant Breed.,* 115: 455–458

Patade, V.Y., P. Suprasanna and V.A. Bapat, 2008. Effect of salt stress in relation to osmotic adjustment on sugarcane (*Saccharum officinarum* L.) callus cultures. *Plant Growth Regul.,* 55: 169–173

Poustani, K. and A. Ciocemardeh, 2001. K^+/Na^+ ratio and ion selectivity in response to salt stress in wheat. *Iranian J. Agric. Sci*., 32: 525–532

Poustini, K. and S.Z. Salmasi, 1997. Effect of salinity on dry matter production and remobilization in two wheat cultivars. *Iranian J. Agric. Sci*., 28: 11–17

Redway, F.A., V. Vasil, D. Lu and I.K. Vasil, 1990. Identification of callus types for long-term maintenance and regeneration from commercial cultivars of wheat (*Ttriticum aestivum* L.). *Theor. Appl. Genet.,* 79: 609–617

Richards, L.A., 1954. *Diagonosis and Improvement of Saline and Alkali Soils.* U.S.D.A. Hand book No. 60, Washington D.C. USA

Salam, A., P.A. Hollington, J. Gorham, R.J. Wyn Jones and C. Gliddon. 1999. Physiological genetics of salt tolerance in wheat (*Triticum aestivum* L.): performance of wheat varieties\ inbred lines and reciprocal hybrids under saline conditions. *J. Agron. Crop Sci.,* 183: 145–156

Sibi, M.L. and M. Fakiri, 2000. Androgenese et gynogenese, sources de vitrovariation et de tolerance a la salinite chez l'orge *Hordeum vulgare*. *Secheresse,* 11: 125–132

Steel, R.G.D. and J.H. Torrie, 1980. *Principles and Procedures of Statistics: A Biological Approach*, 2nd edition. McGraw Hill Inc., New York, USA

Taiz, L. and E. Zeiger, 2010. *Plant Physiology*, 5th edition. Sinauer Associates Inc. Publishers, Sunderland, Massachusetts, USA

Vasil, I.K., 1987. Biotechnology and food security for the 21st century: real world perspective. *Nat. Biotechnol.*, 16: 399–400

Vasil, V., F. Redway and I.K. Vasil, 1990. Regeneration of plants from embryogenic suspension culture protoplasts of wheat (Triticum aestivum L.). *Biotechnology*, 8: 429–434

Wahid, A., I. Masood, I.l.H. Javed and E. Rasul, 1999. Phenotypic flexibility as marker of sodium chloride tolerance in sunflower genotypes. *Environ. Exp. Bot.*, 42: 85–94

Yamada, Y. and D.T. Tang, 1986. Plant regeneration from protoplast derived callus of rice (*Oryza sativa* L.). *Plant Cell Rep.*, 5: 85–88

(Received 31 December 2011; Accepted 28 May)