SCREENING OF HIGH YIELDING WHEAT GENOTYPES FOR CALLUSING ABILITY UNDER NaCl STRESSED CONDITIONS *IN VITRO*

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ABSTRACT

Twenty-eight high yielding advanced lines of bread and durum wheat genotypes were screened for their callusing ability under NaCl stressed conditions in vitro . The screening was performed through culturing wheat root explants on callus-induction medium supplied with 2 mg/l 2,4-D and increasing levels of NaCl. The tolerant genotypes were then identified according to their ability to initiate and maintain callus growth on such medium. The wheat genotypes were also evaluated for their ability to initiate callus under unstressed conditions. Results showed that the bread wheat genotypes expressed better callusing ability than the durum ones, genotypes average percent callusing was 58 % and 22 % for the bread and durum wheat, respectively. The callus of bread wheat genotypes also expressed better tolerance to salinity as reflected by their ability to induce callus on media supplied with increasing NaCl concentrations. Among the 14 bread wheat genotypes screened, genotypes ACSAD 921 and 949 were able to maintain 41 and 51% callus on such a high NaCl level, respectively and therefore, were considered highly tolerant. In particular ACSAD 921 showed good callusing ability under both stressed and unstressed conditions. On the other hand, the bread wheat genotypes Towpe and ACSAD 947 were identified as extremely sensitive to salt where no callus growth could be observed at high NaCl concentrations. The callus of the other bread wheat genotypes showed variation in their tolerance levels ranging from slightly sensitive to moderately tolerant. As for the durum wheat genotypes, genotype ACSAD 1031 had the highest relative salt tolerance being able to maintain 43 % of its callus on medium supplied with 1.5 % NaCl. Others showed moderate degree of tolerance such as genotypes ACSAD 1071, Cham1/brachua and Haurani, while Youssef-1 was slightly tolerant. All other examined durum genotypes were very sensitive and showed no callus growth at 1.5 % NaCl .

Keywords: *in vitro* selection, wheat, salt tolerance, callusing ability

INTRODUCTION

Wheat is one of the most important crops in the world with an average production of more than 340 million tons cultivated on about 220 million hectares (FAO, 1990-1998). The major two wheat types are bread wheat (*Triticum aestivum*) and durum wheat (*Triticum durum*).

Major factors that reduce wheat production are the biotic stresses such as diseases and insects and abiotic stresses such as cold, frost, heat, drought and salinity (Layoun, 1999). Irrigation practices as reported by Shannon in 1997 can lead to an increase in soil salinity, an annual application of 1-1.5 m of irrigation water can contribute to 1-60 tones of salts per hectare. In Lebanon where supplementary irrigation is a common practice, salinity is a potential problem. In addition, it can also be a potential problem in the dry northern part of Bekaa which receives normally less than 300 mm average annual rainfall thus leading to minimum leaching of salts from the top soil.

The best way to fight abiotic stresses such as salinity is to produce new varieties that are tolerant to such stresses. Doing that through classical breeding can be time consuming. *In vitro* selection and screening is a new technique which has been extensively used recently to promote cell tolerance to many biotic and abiotic stresses (Trigiano and Gray, 1999). A pre-requisite to such work is callus formation which is used to select tolerant cells and to regenerate plants. Callus induction from wheat has been extensively reported from different kinds of explants, the most successful ones being reported from immature or mature embryos or from anthers. Only few attempts were done to induce callus from roots, and only recently this was achieved with high frequencies from ten genotypes (Layoun, 1999).

The objective of this study was to screen 28 advanced bread and durum wheat high yielding genotypes for their callusing ability in order to identify better genotypes suitable for regeneration of salt tolerant somaclones.

MATERIALS AND METHODS

Plant material

The twenty-eight wheat genotypes that were used in this study included bread and durum wheat advanced lines and released or pre-released varieties. The seeds were provided by the Lebanese Agriculture Research Institute (LARI) and originated from the International Center for Agricultural Research in the Dry Areas (ICARDA) and from the Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD). The names and pedigrees of the genotypes are described in Table 1.

Seed sterilization and planting

Seeds from each genotype were cleaned, then sterilized by soaking them in 95 % ethanol solution for 5 minutes followed by 20 min. in 2.6 % sodium hypochlorite. The seeds were then rinsed three times in sterile distilled water to remove traces of sodium hypochlorite after which they were planted in glass jars (10 seeds/jar) filled with germination medium, GM (half strength MS salts as described by Murashige and Skoog 1962 , 30 g/l sucrose, 8 g/l agar, pH 5.7) to allow germination and extensive root growth and development at 25 ºC under 16 hours light conditions.

TABLE 1

Names and Pedigrees of Genotypes Screened in the Study

Wheat type	Geno-type	Name / Pedigree
Bread Wheat	1	Towpe
	2	ACSAD 921/Mexipak 65/5/Maya's'/on//II 60.147/3/BB/G1/4/chat's'
	3	ACSAD 943/Mon's'/Aid's'//Deir Alla 4
	4	ACSAD 947/Snb'S'/ACSAD 305
	5	ACSAD 949/Snb'S'/ACSAD 305
	6	Inia/RL4220//7C/3/yr'S'/5//2300//Lr64/8/56/3/Nor67/4//y50 E/6/Tow'S'
	7	4777(2)//FKn/Gb/3/Vee'S'/4/Buc'S'/Rn'S'5/Vee'S'/Tsi
	8	Kasyon/Genaro-81//Tsi/Vee'S'
	9	Kauz'S'/ABE
	10	Tsi/Vee'S'/4/C/82.24/C/68.3/3/cno*2/7C//CC/Tob
	11	Cham-4//Shi # 4414/Crow'S'
	12	MYNA/VUL//TURACO/3/TURACO
	13	BLOYKA
	14	CHIL//Vee'S'/Tsi
Durum Wheat	1	ACSAD 1031
	2	ACSAD 1071
	3	ACSAD 1121 (ACSAD 675 x Syrica 1)
	4	ACSAD 1123 (ACSAD 675 x Syrica 1)
	5	Bicricham
	6	Cham 1/brachua
	7	Cham/Yav 79-7387
	8	Haurani
	9	Haurani 27
	10	Lahn
	11	Outroub 6
	12	Ruff fg//Turk-1
	13	TA2 Uruk 120/62-130 Kobak 2916/Lol 5/3//snipe/9/4 – MG 5730
	14	Youssef-1

Explant preparation and callus culture

Healthy root segments (1 cm in length) were isolated from 2 weeks old seedlings and cultured on callus induction medium, CIM (full strength MS salts, 30 g/l sucrose, 2 mg/l 2,4-D , MS vitamine mixture, 1 mg/l silver nitrate and 8 g/l agar, pH 5.7) supplied with different levels of NaCl (0, 0.25, 0.5, 0.75, 1 and 1.5 %). Thirty explants were cultured from each genotype in each treatment in darkness at 25 ºC for callus induction.

Determination of cellular tolerance

Cellular tolerance of the studied wheat genotypes was determined based on their explants ability to initiate and grow callus *in vitro* under salt stressed conditions. Callusing ability under salt unstressed conditions (expressed in percentage) was determined for both wheat types as well as for each individual genotype. Determination of salt tolerance based on callusing ability has been used in wheat improvement over the last decade (El-Hennawy, 1996; Karadimova and Djambova, 1993).

Experimental design

The experiment made use of a completely randomized design with two factors, namely genotype and salt level and 3 replications for each treatment. The callusing ability was determined by calculating the percentage of responsive explants that were able to initiate and maintain callus formation . Data were collected from calli four weeks after their culture.

RESULTS AND DISCUSSION

Effect of wheat type on callusing ability

There was a clear difference between the two wheat types in terms of callusing potential. The bread wheat genotypes showed better average callusing ability (58 %) as compared to the durum genotypes (22 % only). The callusing frequencies ranged between 23-80 % in bread wheat compared to 7-60 % in durum wheat. Individual performance of all tested genotypes is presented in Figure 1.

Effect of NaCl on callusing ability

NaCl presence in the callus induction medium had a negative effect on the average callusing performance of bread wheat and durum wheat genotypes (Figure 2). In bread wheat, the average callusing frequencies was negatively affected by the increasing levels of NaCl in the culture medium, this value dropped from 58 % in the control to reach 14 % only at 1.5 % NaCl concentration. The same effect but to a weaker extent was also observed for the durum wheat genotype where the average callusing frequency dropped down from 22 % in the control to reach 2 % only at 1.5 % NaCl .

The better ability of bread wheat to tolerate higher concentrations of salt as compared to durum wheat shown by this work was in agreement with the findings of Karadimova and Djambova (1993). The mechanism of this tolerance was earlier illustrated by

Wyn Jones and Gorham (1989) who said that the genes responsible for increasing tolerance to salt were present in the D genome which is present in the bread wheat genome only.

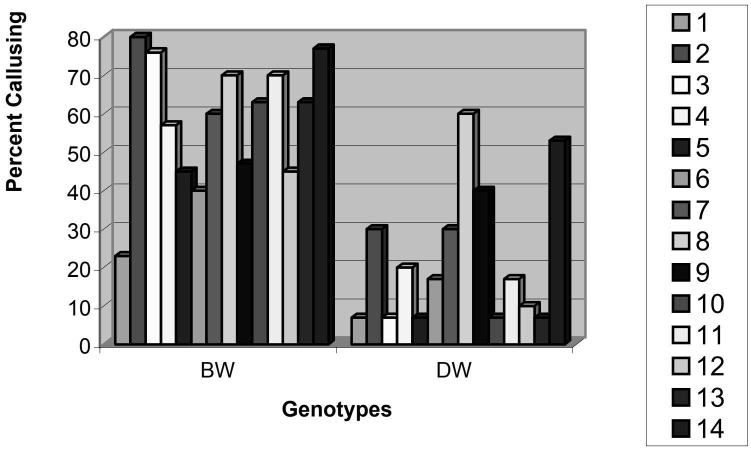

Figure 1. Callusing ability of individual genotypes of bread (BW) and durum wheat (DW) genotypes.

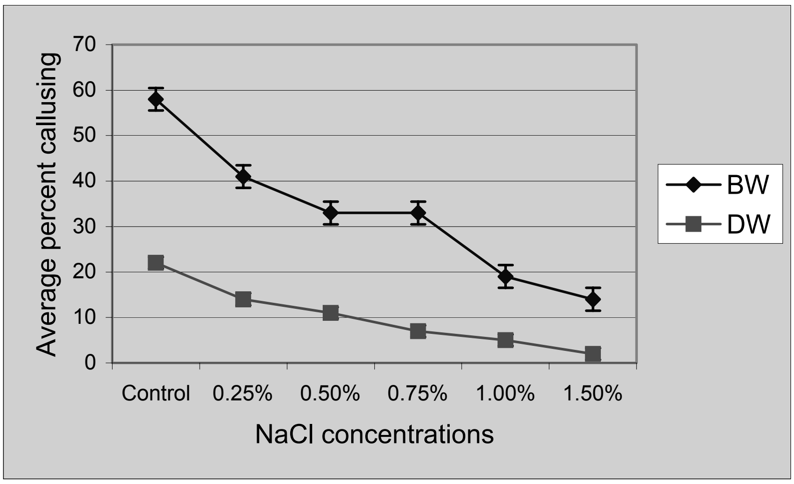

Figure 2. Effect of NaCl on callusing ability of both wheat types, vertical bars are standard error of means.

Effect of genotype on callusing ability

Although low concentrations of NaCl (0.25-0.75 %) in the callus induction medium affected callus formation positively for some genotypes in both bread and durum types, screening concentrations of NaCl above 1.0 % resulted in a reduction in callus growth . Great variation in callusing frequencies under unstressed conditions was seen among individual genotypes. Among the bread wheat, genotypes 2, 3 and 14 were very responsive and produced callus from almost 80 % of the root explants. On the other hand, the durum landrace Haurani (genotype 8) and the improved variety Youssef-1 (genotype 14) gave good callus with frequencies reaching almost 60 %. These genotypes can be recommended for further studies related to wheat cell culture or *in vitro* selection because of their relatively high callus formation ability . The results obtained by this study agrees with previous literature in that callus induction in wheat is genotype-dependent (Al-karaki and Abu-Ein, 1999 ; Arzani and Mirodjagh, 1999; Borojevic *et al*. 1986; Boyadzhiev, 1986; Butenko *et al.*; Dzhos and Kalashnikova,1998; Machii *et al.* 1998; Ozgen *et al.* 1998; Sharma *et. al.* 1982 ; Sidorova *et. al.* 1988). Great variability was also observed in the callusing response of the tested wheat genotypes under salt stressed conditions. The analysis of variance of both wheat types indicated highly significant F values for both factors (genotype and NaCl concentration) as well as for their interaction (Table 2).

TABLE 2
ANOVA Table Using Two-Way Factor Analysis for Bread and Durum Wheat Callusing Potential

Source	Degrees Of Freedom	F-Value Durum Wheat	F-Value Bread Wheat
Genotype	13	12.3683**	10.4439**
NaCl concentration	5	32.3138**	40.1817**
Interaction	65	2.4595**	2.2824**
Error	168	-	-
Total	251	-	-

** Significant at 1 % Probability Level.

Genotype x salt interaction

Based on the highly significant F-values for genotype x NaCl interaction, the treatment means of the interaction component were compared and used to screen the individual genotypes within each wheat type after they were transformed to percentages in relation to callus amount of the controls (Tables 3 and 4).

Under salt stressed conditions, among the tested bread wheat lines, genotypes 2 and 5 were able to maintain 41 and 51% callus on such a high NaCl level, respectively and therefore, were considered highly tolerant (Table 3), such genotypes can be recommended to be used in further studies because of their ability to produce callus in the presence of high salt concentrations. In particular, genotype 2 (ACSAD 921) showed good callusing ability under both stressed and unstressed conditions. On the other hand, the bread wheat genotypes 1 and 4 were identified as extremely sensitive to salt where no callus growth could be observed at high NaCl concentrations. The other bread wheat genotypes showed variation in their tolerance levels ranging from slightly sensitive to moderately tolerant (Table 3).

TABLE 3
Callus Formation of the Bread Wheat Genotypes under Different NaCl Concentrations

NaCl→ Genotypes↓	0.25 %	0.5 %	0.75 %	1.0 %	1.5 %
1	161	74	0	13	0
2	112	46	75	84	41
3	78	74	61	7	17
4	30	5	35	5	0
5	111	117	44	96	51
6	75	43	8	8	33
7	50	72	105	45	22
8	53	71	81	24	39
9	64	106	85	36	28
10	79	52	27	11	32
11	29	10	76	24	14
12	60	67	133	89	22
13	79	40	11	16	21
14	65	48	22	9	9

TABLE 4
Callus Formation of the Durum Wheat Genotypes under Different NaCl Concentrations

NaCl→ Genotypes↓	0.25 %	0.5 %	0.75 %	1.0 %	1.5 %
1	143	143	143	43	43
2	77	23	23	57	23
3	0	0	0	0	0
4	0	0	0	0	0
5	285	143	0	100	0
6	100	76	41	18	17
7	43	33	10	0	0
8	28	17	29	12	17
9	43	33	8	8	0
10	186	143	143	43	0
11	132	118	41	76	0
12	0	70	0	0	0

13	143	100	100	43	0
14	62	38	38	25	6

As for the durum wheat genotypes, genotype 1 (ACSAD 1031) had the highest relative salt tolerance being able to maintain 43 % of its callus on medium supplied with 1.5 % NaCl. Others showed moderate degree of tolerance such as genotypes 2, 6 and 8 (ACSAD 1071, Cham1/brachua and Haurani) while genotype 14 (Youssef-1) was slightly tolerant. All other examined genotypes were very sensitive and showed no callus growth at 1.5 % NaCl (Table 4). Based on the above, genotype 2 (ACSAD 1031) in particular is recommended for *in vitro* screening studies against salinity because of its ability to produce good callus under salt stressed conditions.

CONCLUSION

The results of this study indicate that bread wheat showed a better callusing capacity than durum wheat under unstressed conditions , and that this capacity was genotype dependent. With respect to salt tolerance, bread wheat also showed better tolerance to salinity under *in vitro* stressed conditions than durum wheat as was reflected by the genotypes ability to initiate and maintain callus on NaCl enriched culture media.

Finally, genotypes from both wheat types with good callusing potential and good tolerance to salinity were identified and are therefore, recommended for further research aiming at producing wheat plants tolerant to salinity.

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REFERENCES

Arzani, A. and Mirodjagh, SS. 1999. Response of durum wheat cultivars to immature embryo culture, callus induction and *in vitro* salt stress. *Plant Cell, Tissue and Organ Culture*, 58 (1): 67-72.

Borojević, K., Šesek, S. and Radojević, L. 1986. Responses of different genotypes on development of callus from anther cultures of wheat. In *Nuclear techniques and in vitro culture for plant improvement,* 233-237.

Boyadzhiev, P. 1986. Callus formation and regeneration in anther cultures of bread wheat hybrids. *Rasteniev"dni Nauki,* 23 (2): 10-14.

Butenko, R.G., Dzhardemaliev, ZH. K. and Gavrilova, N.F. 1986. Callus formation ability in explants of different organs of winter wheat cultivars. *Fiziologiya Rasteniĭ,* 33 (2): 350-355.

Dzhos, L. and Kalashnikova, E.A. 1998. Influence of genotype and spring wheat embryo culture conditions on the process of callus formation and morphogenesis. *Izvestiya Timiryazevskoĭ Sel'skokhozyaĭstvennoĭ Akademii,* N° 3, 94-99.

Food and Agricultural Organization. Production yearbooks, 1990-1998.

El-Hennawy, M.A. 1996. Salt tolerance in wheat using tissue culture techniques. *Annals Agric. Sci.,* 41(2): 785-803.

Karadimova, M. and Djambova, G. 1993. Increased NaCl-tolerance in wheat (*Triticum aestivum* L. and *T. durum* DESF.) through *in vitro* selection. *In vitro Cell. Dev. Biol.,* 29: 180-182.

Al-Karaki, G. and Abu-Ein, A. 1999. Callus induction and plant regeneration in wheat (*Triticum durum* L.) cultivars. *Crop research (Hisar),* 17 (1): 80-84.

Layoun, R. 1999. *in vitro* selection and screening of wheat genotypes for tolerance to high boron concentrations. Thesis. 72 pp. Faculty of Agricultural Sciences, the Lebanese University, Beirut, Lebanon.

Machii, H., Mizuno,H., Hirabayashi, T., Li,H., Hagio,T. 1998. Screening wheat genotypes for high callus induction and regeneration capability from anther and immature embryo cultures. *Plant Cell, Tissue and Organ Culture,* 53 (1): 67-74.

Murashige , T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*, 15:473-497.

Özgen, M., Türet, M., Altiinok, S. and Sancak, C. 1998. Efficient callus induction and plant regeneration from mature embryo culture of winter wheat (*Triticum aestivum* L.) genotypes. *Plant Cell Reports,* 18 (3/4): 331-335.

Shannon, M.C. 1997. Adaptaion of plants to salinity. *Adv. Agron.,* 60:75-120.

Sharma, G.C., Wang, W.C. and Sapra, V.T. 1982. Effect of genotype, media and temperature pretreatment on callus initiation in triticale, wheat, and rye anther cultures. *Cereal Research Communications*, 10 (3/4): 143-150.

Sidorova, N.V., Morgun, V.V. and Loyvihenko, V.F. 1988. Peculiarities of callus and morphogenesis in immature embryo culture of different winter wheat genotypes. *Fiziologiya i Biokhimiya Kul'turnykh Rasteniĭ,* 20 (4): 349-353.

Trgiano, R.N. and Gray, D.J. 1999. *Plant Tissue Culture Concepts and Laboratory Exercises,* Second edition. CRC press, 454 pp.

Wyn Jones, R.G. and Gorham, J.1989. *Drought resistance in cereals*. Chapter 7. p. 96. Baker edition. C.A.B. International, UK.