Agronomic performance and quality characteristics of tissue culture-derived lines of spring wheat (*Triticum aestivum* L.) cultivar Pavon

R. L. Villareal*, A. Mujeeb-Kazi and R. J. Peña
International Maize and Wheat Improvement Center (CIMMYT), Lisboa 27, Apartado Postal 6-641, Deleg. Cuauhtemoc, 06600 Mexico, D.F., Mexico

*** Corresponding author**

Summary
The potential of somaclonal variation to contribute genetic variation for wheat improvement has been widely recognized. Thirty selected lines derived from tissue culture of spring bread wheat (*Triticum aestivum* L.) cultivar Pavon were grown in replicated trials at CAEVY, Sonora, northwest Mexico, during two production seasons to assess their agronomic performance and quality traits. Field evaluation indicated that the tissue culture-derived lines (TCDL) possessed more grains/m^2, spikes/m^2, grains/spike, earlier flowering date, longer grain-filling period and shorter height than the original breeder line Pavon. Laboratory results revealed that 20% of the TCDLs had improved flour protein. In contrast, 1000-grain weight and test weight of the regenerants were inferior to the Pavon parent. No significant difference was observed between the mean grain yield of TCDLs and the breeder line. In conclusion, it appears that the variations observed in the study could have direct implications in a wheat germplasm enhancement program.
Key words: Somaclonal variation - *Triticum aestivum* - agronomic performance - quality - yield components

Somaclonal variation (variation among tissue culture-derived lines, TCDL) has been widely recognized as a novel diversity source that could give new genotypes with superior agronomic performance and quality (Ahloowahlia and Sherington 1985, Hanson et al. 1994, Larkin et al. 1984, Maddock et al. 1985, Ryan et al. 1987, Scowcroft et al. 1987). The resulting genetic changes that occur in plant tissue culture are transmitted to regenerant plants and their progeny (Larkin and Scowcroft 1981, Shepard 1981, Scowcroft and Larkin 1983). Some of the genetic events responsible for the variations are changes in ploidy level, aneuploidy, chromosome breakage, rearrangements, gene amplification or deamplification, single gene and multigene mutations, and single base pair substitutions (Ahloowahlia 1982, Davies et al. 1986, Dutta Gupta and Ahmed 1986, Evans and Sharp 1983, Fedak et al. 1987, Karp and Maddock 1984, Larkin et al. 1989, Lee and Phillips 1988).

Somaclonal variation in wheat (*Triticum aestivum* L.) is widely documented. Yield components such as grains/m^2, grains/spike, grain weight, and spike length have been found to be variable in regenerant lines (Hanson et al. 1994, Mohmand and Nabors 1990, Ryan et al. 1987, Villareal et al. 1993). Most grain yield results indicate TCDLs to be inferior to their parental germplasm (Borja et al. 1994, Ryan et al. 1987, Villareal et al. 1993). The study conducted by Villareal et al. (1993) using three advanced spring bread wheat lines found height reduction of the somaclones to be the most consistent effect

across the breeder lines studied. Ryan et al. (1987) had earlier identified somaclonal variants that were both taller and shorter than the original parents. Lines with improved harvest index were also found. Regenerant lines with variable flowering days, grain-filling period and physiological maturity were obtained as well (Hanson et al. 1994; Villareal, et al. 1993).

On wheat quality traits, Ryan et al. (1987) found that most of the regenerant lines derived from the cultivar Millewa had higher protein content than the parental material. Inferior variants were detected for flour yield and mixograph values only. Somaclones possessing harder and softer kernels than Millewa were also found. Hanson et al. (1994) obtained similar results on increased flour protein of the regenerant lines; in contrast, SDS sedimentation volumes, indicative of gluten strength, were higher than those of the breeder line. Somaclonal variants also possessed greater test weight.

Limited field studies have been performed to rigorously evaluate the extent of variability in agronomic traits exhibited in lines regenerated from tissue culture. Although it was generally shown that a wide variation was found among the somaclonal lines, very few lines were identified that significantly outperformed their parents in important agronomic characters. The objective of this study was to assess the agronomic performance and quality traits of selected advanced (R9) tissue culture derivatives possessing good agronomic characteristics. Unlike other evaluation studies on somaclonal variants, the intent of this study was to measure not only the variation due to tissue culture among the regenerated lines, but more importantly, to evaluate the yield potential of the TCDLs using field plots.

Materials and Methods

Plant materials: Somaclonal lines of the spring wheat cultivar Pavon were developed via somatic embryo culture by R. Waskom and his group at the Colorado State University, Fort Collins, Colorado, U.S.A. Pavon was bred by the CIMMYT bread wheat program in Mexico and originated from the cross Vicam//Ciano/7C/3/Kalyan//Bluebird. Tissue culture was used to select and regenerate somaclones following previously published methodologies in wheat (MacKinnon et al. 1987, Timm et al. 1991). *In vitro* selection for salt tolerance in callus cultures was accomplished following the procedure of Nabors et al. (1980). Plants regenerated directly from culture has been termed the R1 generation, and the selfed progeny and subsequent generations R2, R3, and so on. In 1988, CIMMYT received more than 800 R2 regenerated plants from Colorado. Since then, the materials were selected for good agronomic attributes and advanced to the R9 generation under no-salt stress field conditions in CIMMYT, Mexico. Each original regenerant selected was considered a distinct line. The spikes were self-pollinated, seed bulked to increase homozygosity and be utilized for replicated yield tests. Of the 139 R9 plants left, the best 30 TCDLs were finally chosen based on uniformity of the plot using plant height, maturity, yield potential, good agronomic characteristics, with field resistance to leaf rust (*Puccinia recondita* Rob. et Desm. f. sp. *tritici*) and stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Erikss. et Henn.) as selection criteria. The selected TCDLs were from the following tissue culture treatments: TCDLs 1 to 10 were derived from Pavon no stress treatment (Pvn NS), TCDLs 11 to 26 were from Pavon 3g/l NaCl stress (Pvn 3G), TCDLs 27 to 28 were from Pavon 6g/l NaCl stress (Pvn 6G) and TCDLs 29 to 30 were from

"stepwise" NaCl stress (Pvn SS). The Colorado reference treatment numbers for each genotype are indicated in Table 1.

Evaluation. Test genotypes were grown in two field trials at the Mexican National Institute of Forestry, Agriculture and Livestock, Campo Agricola Experimental Valle del Yaqui (CAEVY) research station (27°25'N, 109° 55'W; elevation 40 m above sea level) during two wheat production seasons. Each trial was grown as a randomized complete block design with three replications. Plots consisted of eight rows, each 5 m long and 20 cm apart, were machine-drilled at a seed rate of 120 kg/ha. In each year the trials were seeded in late November, considered to be the optimum seeding date for bread wheat in the State of Sonora, Mexico; they reached physiological maturity during April.

The trials received high levels of agronomic inputs and management regarding fertilizer (200 kg N/ha and 40 kg P/ha), irrigation (as required until the latest maturing genotypes reached physiological maturity), weed control (selective herbicide) and Folicur for disease control (leaf and stem rust), while an insect control program was not required at any time during the season. When mature, the crop from the central 3.75m^2 of each plot was machine harvested. The outer row and a 0.5m^2 border at each end of the plot were excluded from the samples. The procedure used in determining grain yield, yield components, and other agronomic characteristics was essentially similar to that described by Villareal et al. (1995).

Test weight was recorded using an electronic hectoliter balance for each entry. Flour protein (N x 5.7) was determined using the Kjeldahl procedure 46-14 of the American Association of Cereal Chemists (AACC 1983). SDS-sedimentation volume was determined on 1-g flour samples as described previously by Peña et al. (1990). Alveograph characteristics were obtained with the Chopin Alveograph (Tripette and Renaud, Paris, France) using 60-g flour samples and variable absorption (55-60%) to maintain uniform dough handling consistency as judged by the operator. The deformation energy (W), an indicator of gluten strength, and the overpressure (P) to swelling index (G) ratio (P/G), an indicator of tenacity/extensibility, were calculated from the alveograms. Mixograph mixing time was determined using AACC method 54-40 (AACC 1983). The breadmaking procedure used was the AACC straight-dough baking test 10-10 using the 100-g flour formula. Bread loaf volume was determined by rapeseed volume displacement. Most characters were measured on two replications in both experiments.

Data analysis. All variables measured for each genotype were subjected to analysis of variance (ANOVA). Treatment means were compared by Least Significant Difference (LSD), and contrasts were carried out using the general linear model (GLM) procedure of the Statistical Analysis System (SAS Institute 1985).

Results

Comparison of the 30 somaclonal lines and the parental breeder line (Table 1) showed significant variation for days to flowering ($P<0.001$), physiological maturity ($P<0.05$), grain-filling period ($P<0.01$), and plant height ($P<0.001$). More than 63% of TCDLs have comparable flowering dates with Pavon while 11 lines flowered 2 to 3 days earlier. The mean flowering of the TCDLs was 81.8 days, compared to Pavon's 83 days. TCDLs 21 and 23 needed a longer grain-filling period (46 days) than the parental cultivar (43 days). On physiological maturity, most of the TCDLs had comparable maturity with the breeder

Table 1. Agronomic traits, grain yield and yield components and quality traits of 30 tissue culture-derived Pavon lines as compared to the original Pavon breeder's line, combined over two years of yield trials at CAEVY, Sonora, Mexico.

TCDL no.	Genotype	FL d	PM d	GF d	PH cm	SL cm	GY t/ha	BIO t/ha	HI %	SPM no.	GPM no.	GPS no.	TGW g	TW kg/hl	PN %	SDS ml	ALV W	ALV P/G	MMT min	LV ml
1	Pvn NS(4000103)	82	127	45	99	11.0	5.7	15.9	36.8	447	15600	38	36.9	78.9	10.2	17.5	325	4.3	2.6	861
2	Pvn NS(4000113)	81	126	45	98	10.7	5.9	15.7	37.8	440	15890	39	37.2	79.1	10.0	16.2	337	5.4	2.6	884
3	Pvn NS(4000116)	82	126	44	98	11.3	5.6	15.1	37.6	426	14768	37	38.0	79.2	10.2	18.0	318	5.0	2.6	869
4	Pvn NS(4000121)	82	126	44	98	11.6	5.9	16.6	36.2	470	15809	36	37.5	78.8	10.0	17.0	279	5.1	2.6	861
5	Pvn NS(4000123)	83	127	44	95	11.1	5.6	15.2	36.2	451	14059	34	39.6	79.1	10.9	18.2	330	4.3	2.5	886
6	Pvn NS(4000128)	82	125	43	97	11.1	5.8	14.8	38.3	426	15208	36	37.7	79.2	10.2	16.2	307	5.3	2.6	847
7	Pvn NS(4000129)	83	127	44	99	11.3	5.7	16.0	35.4	434	16423	40	35.9	78.9	10.4	16.5	335	4.3	2.5	837
8	Pvn NS(4000137)	82	125	43	98	11.3	5.7	15.9	36.2	466	15112	33	37.7	79.0	10.1	15.2	313	5.6	2.7	831
9	Pvn NS(4000138)	82	125	43	97	11.2	5.7	15.7	36.8	459	15747	35	36.5	78.7	9.8	16.2	327	5.1	2.7	814
10	Pvn NS(4000143)	81	126	45	95	10.8	5.7	16.2	36.3	474	15113	34	38.2	79.1	9.9	16.2	300	5.8	2.7	875
11	Pvn 3G(4030105)	82	126	44	98	11.2	5.7	15.7	36.6	427	15122	36	37.9	78.7	10.0	15.0	318	5.0	2.6	848
12	Pvn 3G(4030107)	82	126	44	97	10.7	5.7	15.1	38.0	419	15309	39	37.1	78.5	10.6	15.2	332	5.0	2.6	842
13	Pvn 3G(4030114)	82	125	43	100	10.9	5.9	16.1	36.9	462	15708	34	38.0	79.5	10.0	15.7	313	3.9	2.7	877
14	Pvn 3G(4030153)	81	124	43	97	11.5	5.4	14.7	37.2	434	14556	36	37.3	78.1	10.8	16.7	300	4.1	2.4	865
15	Pvn 3G(4030162)	83	128	45	100	11.2	5.6	14.8	37.2	418	14479	36	38.8	79.3	10.6	15.5	313	4.8	2.5	826
16	Pvn 3G(4030163)	80	125	45	98	11.2	6.0	16.1	37.8	459	15640	35	38.7	78.8	11.0	17.5	347	4.2	3.1	912
17	Pvn 3G(4030173)	82	127	45	99	11.5	5.6	15.6	37.0	457	14825	36	38.8	78.5	10.4	15.7	226	4.6	2.6	862
18	Pvn 3G(4030187)	81	126	44	100	10.8	5.5	15.0	37.1	425	13831	34	40.0	79.1	10.6	16.0	302	4.4	2.5	840
19	Pvn 3G(4030193)	81	125	44	98	11.5	5.8	15.5	38.0	427	14910	36	39.3	79.8	10.3	16.5	288	5.9	2.5	873
20	Pvn 3G(4030209)	82	125	43	98	10.6	6.1	16.2	37.2	484	15369	34	39.8	79.6	10.4	15.0	299	4.5	2.6	842
21	Pvn 3G(4030211)	82	128	46	99	11.4	5.9	15.1	38.7	418	14826	37	39.1	80.0	10.7	16.2	235	4.5	2.5	864
22	Pvn 3G(4030298)	81	125	44	98	11.1	5.5	15.2	36.7	440	14631	35	37.7	79.3	9.9	16.0	280	5.2	2.7	861
23	Pvn 3G(4030302)	81	127	46	97	11.2	5.8	15.0	39.3	410	15145	41	38.4	79.4	10.0	15.5	322	5.0	2.6	834
24	Pvn 3G(4030306)	83	126	43	100	11.5	5.5	15.3	36.4	405	14205	37	39.2	78.7	9.8	15.5	289	3.7	2.6	862
25	Pvn 3G(4030318)	81	126	45	99	11.7	5.7	15.4	37.3	422	14511	36	39.2	79.4	10.6	16.5	308	4.4	2.5	885
26	Pvn 3G(4030326)	83	127	44	98	11.4	5.7	16.1	35.6	468	15339	34	37.2	78.3	10.8	16.7	292	4.2	2.8	895
27	Pvn 6G(4060104)	81	125	44	97	11.0	5.8	15.9	37.1	456	14953	34	39.0	79.6	10.2	15.7	280	4.4	2.6	817
28	Pvn 6G(4060110)	83	126	43	98	11.5	5.5	15.7	36.4	439	14090	35	39.5	79.7	10.3	14.7	286	4.8	2.5	863
29	Pvn SS(4290104)	82	126	44	100	11.0	5.8	16.3	36.2	436	15304	39	38.0	78.8	10.8	16.2	330	4.4	2.6	885
30	Pvn SS(4290109)	81	126	45	98	10.8	5.7	15.5	35.4	451	14430	34	39.4	79.5	10.5	15.2	279	5.4	2.6	857
OBL	Pvn breeder's line	83	126	43	100	11.3	5.7	15.4	37.4	426	14230	35	40.3	80.0	9.6	17.5	269	5.1	2.7	853
	LSD (0.05)	1	1	2	2	0.8	0.4	1.2	2.4	57	1251	4	1.9	0.8	1.1	2.6	88	1.3	0.3	67
	CV (%)	1	1.4	3.9	2	6.1	6.4	6.4	5.6	11	7.3	10	4.3	1	5.2	8.1	14	14.0	5.9	4

Abbreviations: Number in parenthesis indicates treatment number; OBL, original breeder's line, FL, days to anthesis; PM, days to physiological maturity; GF, grain-filling period; PH; plant height ; SL, spike length ; GY, grain yield; BIO, biomass at maturity; HI, harvest index; SPM, spikes/m^2; GPM, grains/m^2; GPS, grains/spike; TGW, 1000-grain weight; TW, test weight; PN, flour protein; SDS, sodium dodecyl sulphate sedimentation; ALV, alveogram W and P/G; MMT, mixograph mixing time; and LV, bread loaf volume.

line except for TCDLs 14 (2 days earlier) and 15 and 21 (2 days later). Overall mean maturity for all TCDLs and Pavon was 126 days. Mean maturity range among the TCDLs was 124 - 128 days. Eight somaclones were 3 - 5 cm shorter than the original Pavon line. TCDLs 5 and 10 were recorded as the shortest (95 cm) among the somaclones. Spike length difference among the test entries was not significant ($P>0.05$). The mean overall spike length of the TCDLs (11.2 cm) and Pavon (11.3 cm) was comparable. Coefficients of variation for all characters were satisfactory and ranged from 1.0 to 6.1%, which is indicative of a well executed field experiment.

The ANOVA failed to detect significant differences between TCDLs and Pavon for grain yield, above-ground biomass at maturity and harvest index. Significant variation, however, was found for spikes/m^2 ($P<0.05$), grains/m^2 ($P<0.001$), grains/spike ($P<0.01$) and 1000-grain weight ($P<0.001$). One regenerant line (TCDL 20) produced 13.6% more spikes/m^2, and 7 lines (TCDLs 1,2,4,7,9,13 and 16) had a 9.6% to 15.4% increase in grains/m^2 as compared to the breeder line. The overall mean grains/m^2 of the TCDLs was 15,030 compared to Pavon's 14,230. The range of mean grains/m^2 among the TCDLs was 13,831 (TCDL 18) to 16,423 (TCDL 5). Five somaclonal variants (TCDL 2,7,12,23 and 29) had 4 - 6 more grains/spike than the original breeder line. The TCDLs' overall mean grains/spike was 36 while that of Pavon was 35. On 1000-grain weight, 53.3% of the TCDLs produced lighter grains than the original breeder line; the rest of the lines were comparable to Pavon (40.3 g). Very good coefficients of variation (4.3 to 11.0%) were calculated for all of the above traits.

Grain volume weight varied significantly ($P<0.01$) among the entries, from 78.1 kg/hl (TCDL 14) to 80.0 kg/hl (TCDL 21 and Pavon). Seventeen TCDLs had test weights inferior to that of Pavon. Average test weight of the TCDLs was 79.1 kg/hl. A significant variation ($P<0.05$) was also detected for flour protein content in the genotypes examined. Twenty percent of the somaclones had increased flour protein than Pavon. Average flour protein content of the TCDLs was 10.3%, while Pavon had 9.6%. Flour protein ranged from 9.8% (TCDL 9 and 24) to 11.0% (TCDL 16) among the TCDLs. No large variation was observed for the gluten strength-related parameters: SDS sedimentation, Alveograph strength value-W and Mixograph mixing time. Exceptions, however, were detected on TCDL 28, which showed lower sedimentation volume (14.7 ml), TCDL 24 with a smaller P/G ratio (3.7) and TCDL 16, with a longer mixing time (3.1 min) than the original cultivar Pavon. Alveograph strength values of all the test materials were similar. The average mean of the 30 somaclones for SDS sedimentation was 16.1 ml, W value was 304, P/G ratio was 4.7 and dough mixing time was 2.6 minutes. Bread loaf volume data showed no significant differences ($P>0.05$) among all of the entries. The mean loaf volume of the TCDLs was 859 ml and that of Pavon was 853 ml; all were considered "large" loaf volumes (>800 ml).

Discussion

The results of this research indicated that significant somaclonal variation for agronomic and quality characteristics can be obtained from genotypes originating from *in vitro* calli and further selected for yield potential under optimum management field conditions. Differences in agronomic variability between the TCDLs and the original breeder line (Pavon) were most pronounced for grains/m^2, grains/spike, spikes/m^2, plant height, days

to flowering and grain-filling period. These results agree with previous findings of Villareal et al. (1993) where a number of regenerated lines from three spring bread wheat advanced lines possessed more grains/m^2, spikes/m^2, grains/spike, earlier flowering days, longer grain-filling period and reduced plant height. The study however, involved a limited number of regenerant genotypes (maximum of four) from each test line. The results of Borja et al. (1994), Mohmand and Nabors (1990), Ryan et al. (1987) partly agree with the current findings wherein the somaclonal lines had increased spikes/m^2 and grains/spike. Moreover, for plant height Ryan et al. (1987) and Larkin et al. (1984) identified both tall and short somaclones. The frequency of tall variants generated, however, was higher than short ones. During the two years of field testing, none of the shorter Pavon TCDLs lodged (data not shown); hence, they can be considered an improvement over the original breeder line that lodged during both testing years. Somaclonal variation on 1000-grain weight was also significant but inferior to that of Pavon. Hanson et al. (1994) reported similar results using tissue culture regenerants for spring wheat cultivar HY320. However, some studies proved otherwise, and TCDLs with larger and presumably heavier grains were generated (Mohmand and Nabors 1990, Ryan et al. 1987).

As for quality characteristics, flour protein was higher for 20% of the TCDLs as compared to the original material. This was consistent with the findings of Hanson et al. (1994) using somaclones of cultivar HY320 and Ryan et al. (1987) on Millewa regenerant lines. Flour protein concentration is very important because it affects most quality properties (Finney et al. 1987). Test weight of more than 50% of the somaclones was inferior to Pavon which is indicative of less plump and/or unfilled grains. In addition, the lighter 1000-grain weight of the somaclones might have led to their increased protein concentration, since smaller grains result mostly from decreased starch deposition with similar protein, which increases protein concentration (Simmons 1987).

The yield potential of all selected TCDLs was comparable to that of parental cultivar Pavon. Obviously, test weight and 1000-grain weight did not affect grain yield significantly; otherwise, regenerants with inferior yield would have resulted. This may have been neutralized also by the increase in yield components such as grains/m^2, spikes/m^2 and grains/spike. Since selection of the TCDLs was biased towards Pavon-looking plants, there might be certain regenerant lines that are higher yielding but were excluded from the study. Assessment of somaclonal variation per se could also be complicated by the fact that lines entered in the experiment were not randomly selected and do not represent the entire variation extremes of the original tissue-cultured materials. Hence, conclusions were only based on the results obtained from the 30 Pavon TCDLs studied. Based on our current findings, it appears that the application of the tissue culture technique to adapted cultivars in conjunction with appropriate screening and evaluation schemes may provide useful variation to wheat breeders. Early generation (R3 or R4) field tests are recommended to facilitate identification of somaclones with improved yield potential. Discarding inferior regenerants at an earlier stage may hence better serve the conventional breeding program.

Acknowledgements
The authors gratefully acknowledge the contribution of CIMMYT's bread wheat and wide crosses program field staff especially to Mr. Efren del Toro for the field experiments. They also thank Ms. Alma McNab for editing the manuscript and Ms. Martha Larios for her secretarial help.

References

AACC. 1983. Approved methods of the American Association of Cereal Chemists. AACC, St. Paul, MN.

Ahloowahlia, B. S.. 1982. Plant regeneration from callus culture in wheat. Crop Sci. 22: 405-410.

Ahloowahlia, B. S., and J. Sherington. 1985. Transmission of somaclonal variation in wheat. Euphytica. 34: 525-537.

Borja, J., O. S. Abdalla, A. Mujeeb-Kazi, N. Ter-Kuile,and E. Autrique. 1994. Agronomic evaluation of somaclonal variants from durum wheat cultivars. Agron. Abstr., American Society of Agronomy, Madison, Wisconsin, p.

Davies, P. A., M. A. Pallota, S. A. Ryan, W. R. Scowcroft, and P. J. Larkin. 1986. Somaclonal variation in wheat: genetic and cytogenetic characterization of alcohol dehydrogenase mutants. Theor. Appl. Genet. 72: 644-653.

Dutta Gupta, S., and R. Ahmed. 1986. Structural alterations of chromosomes during callus culture of *Triticum aestivum* L. Cereal Res. Commun. 14: 33-41.

Evans, D. A., and W. R. Sharp. 1983. Single gene mutations in tomato plants regenerated from tissue culture. Science 221: 949-951.

Fedak, G., K. C. Armstrong, and R. J. Handyside. 1987. Chromosome irregularities in wheat and triticale plants regenerated from leaf base callus. Plant Breed. 99: 151-154.

Hanson, K., P. Hucl, and R. J. Baker. 1994. Comparative field performance of tissue culture-derived lines and breeder lines of HY320 spring wheat. Plant Breed. 112: 183-191.

Karp, A., and S. E. Maddock. 1984. Chromosome variation in wheat plants regenerated from culture immature embryos. Theor. Appl. Genet. 67: 249-255.

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.

Larkin, P. J., P. M. Banks, R. Bhati, R. I. S. Brettell, P. A. Davies, S. A. Ryan, W. R. Scowcroft, W. R. Spindler, and G. J. Tanner. 1989. From somatic variation to variant plants: mechanisms and applications. Genome 31: 705-711.

Larkin, P. J., S. A. Ryan, R. I. S. Brettell, and W. R. Scowcroft. 1984. Heritable somaclonal variation in wheat. Theor. Appl. Genet. 67: 443-455.

Lee, M., and R. L. Phillips. 1988. The chromosomal basis for somaclonal variation. In: W. R. Briggs (ed.). Annu. Rev. Plant Physiol. Plant. Mol. Biol. 39: 413-437.

MacKinnon, C., G. Gunderson, and M. W. Nabors. 1987. High efficiency plant regeneration by somatic embryogenesis from callus of mature embryo explants of bread wheat (*Triticum aestivum*) and grain sorghum (*Sorghum bicolor*). In Vitro Cell. & Dev. Biol. 23: 443-448.

Maddock, S. E., R. Rissiott, S. Parmar, M. G. K. Jones, and P. R. Shewry. 1985. Somaclonal variation in the gliadin patterns of grains of regenerated wheat plants. J. Exp. Bot. 36: 1976-1984.

Mohmand, A. S., and M. W. Nabors. 1990. Somaclonal variant plants of wheat derived from mature embryo explants of three genotypes. Plant Cell Rep. 558-560.

Nabors, M. W., S. E. Gibbs, C. S. Bernstein, and M. E. Meis. 1980. NaCl-tolerant tobacco plants from culture cells. Z. Pflanzenphysiol. Bd. 97: 13-17.

Peña, R. J., A. Amaya, S. Rajaram, and A. Mujeeb-Kazi. 1990. Variations in quality characteristics associated with some spring 1B/1R translocation wheats. J. Cereal Sci. 12: 105-112.

Ryan, S. A., P. J. Larkin, and F. W. Ellison. 1987. Somaclonal variation in some agronomic and quality characters in wheat. Theor. Appl. Genet. 74: 77-82.

SAS Institute. 1985. SAS/STAT: Guide for personal computers, version 6 ed. SAS Inst. Inc., Cary, N.C.

Scowcroft, W. R., and P. J. Larkin. 1983. Somaclonal variation, cell selection and genotype improvement. In: C. W. Robinson, and H. J. Howell (eds.) Comprehensive Biotechnology Vol. 3. Pergamon Press, Oxford.

Scowcroft, W. R., R. I. S. Brettel, S. A. Ryan, P. A. Davies, and M. A. Pallotta. 1987. Somaclonal variation and genomic flux. In: C. E. Green, D. A. Somers, W. P. Hackett, and D. D. Biesboer (eds.). Plant Tissue and Cell Culture, p. 275-286. Alan R. Liss, New York.

Shepard, J. F.. 1981. Protoplasts as source of disease resistance in plants. Ann. Rev. Phytopathol. 19: 145-166.

Timm, D. A., R. M. Waskom, D. R. Miller, and M. W. Nabors. 1991. Greenhouse evaluation of regenerated spring wheat for enhanced tolerance. Cereal Res. Commun. 19: 451-457.

Villareal. R. L., E. Del Toro, A. Mujeeb-Kazi and S. Rajaram. 1995. The 1BL/1RS chromosome translocation effect on yield characteristics in a *Triticum aestivum* L. cross. Plant Breed. 114: 497-500.

Villareal, R. L., N.Ter-Kuile, E. Del Toro, S. Rajaram, and A. Mujeeb-Kazi. 1993. Agronomic evaluation of somaclonal variants from three bread wheat (*Triticum aestivum* L.) cultivars. Agron. Abstr., American Society of Agronomy, Madison, Wisconsin. p.196.

Received 19 August, 1998, accepted 5 October, 1998