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SOMACLONAL VARIANT PLANTS OF WHEAT (*TRITICUM AESTIVUM* L.) WITH INCREASED FLAG LEAF SIZE, HEAD SIZE AND GRAIN NUMBER

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Abstract

Bread wheat (*Triticum aestivum* L. *em* Thell., 2n = 6x = 42) callus cultures of 3 spring wheat cultivars (Glennson, Pavon, PAK-16171) were selected for 90 days on medium containing 0, 10, 15, 20 and 25% (w/v) PEG-4000. Somaclonal variation was examined in the R_1 generation. Comparisons were made among the somaclones and their non-tissue cultured parents. Characteristics examined included days to 50% heading, flag leaf length, width and area; plant height; spike length; number of grains per spike and days to maturity. Significant variation was found in all the characteristics measured. The most remarkable differences were observed in the flag leaf length and area, 3 to 4 times greater in somaclones 71B88R1, 71C88R1 and 71D88R1 and 2 to 3 times greater in somaclones PA88R1 and PB88R1 than the parent lines. The somaclones produced longer spikes and yielded more grains per spike than the parent lines.

Introduction

The designation of the term "somaclonal" variation by Larkin & Scowcroft (1981) to describe genetic variability in plants derived from cell cultures has stimulated the interest in applying tissue culture in crop improvement. The origin of somaclonal variation is not yet clear (Larkin & Scowcroft, 1981) since the expression can differ among plants. Lapitan *et al.*, (1984) found a high degree of chromosome structural changes in wheat by rye hybrids regenerated from tissue culture. In order for variation to be useful in sexually propagated crop breeding programs, the altered traits should be quantitative, have a genetic base, and be passed on to subsequent generations.

Heritable variation is often observed in plants regenerated from tissue, cell, or organ culture (Larkin & Scowcroft, 1981). Wheat has been one of the principal subject for investigating this phenomenon and several laboratories have reported different types of somaclonal variation (Larkin *et al.*, 1984; Ahloowalia & Sherington, 1985; Maddock *et al.*, 1983; Chen *et al.*, 1987).

Davies *et al.*, (1986) have reported an alcohol dehydrogenase (ADH1) mutant of wheat. Recently, Lazar *et al.*, (1988) observed somaclonal variation in regenerated plants of winter wheat which had increased freezing tolerance compared to parent cultivars. Ryan *et al.*, (1987) have reported significant variation in characters such as plant height, grain number per spike, kernel weight, yield, total dry weight and harvest

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index in a population of wheat somaclones. Larkin *et al.*, (1984) documented heritable somaclonal variation from wheat regenerated from cell cultures.

The goal of these experiments is to report on wheat (*Triticum aestivum* L.) somaclones which exhibited increased flag leaf length, area, head size and grain number in the R_1 generation.

Materials and Methods

Callus cultures were initiated from mature embryos of 3 wheat (*Triticum aestivum* L.) genotypes: Pavon, Glennson and PAK-16171. Mature embryos were germinated on callus induction medium of Linsmaier & Skoog's (1965) basal salts, vitamins, 2.0 mg/1 2, 4-dichlorophenoxyacetic acid (2, 4-D) and 2% sucrose. The calli originating from the germinating embryos were subcultured on the L & S fresh medium supplemented with the same hormonal concentrations used for callus induction at 30 day intervals.

After the fifth passage, when sufficient amounts of green, compact yellowish embryogenic calli (Heyser *et al.*, 1985) were produced, 0.5 gms of the E-callus was subjected to PEG-4000 induced water stress of 0, 10, 15, 20 and 25% (w/v). Calli were subcultured on the PEG medium for 90 days with a 30 day subculture interval. The embryogenic calli that survived after 90 days in stress were put on Linsmaier & Skoog's (1965) basal salts and vitamins; the 2, 4-D was replaced by 0.1 mg/1 Indoleacetic acid (IAA) and 0.5 mg/1 benzyladenine (BA) for plant regeneration.

Screening and Selection of Potted plants in Soil: Screening was performed on R_1 plants in the greenhouse in the spring of 1988. Seeds were planted in February in individual pots in a mixture of fine peat: perlite (Sunshine Mix of Fission Western Corporation) supplemented with fertilizers ($CaNO_3$ and NH_4NO_3) and were grown in the greenhouse. Supplemental heating was provided to maintain the greenhouse temperature at 22 $\pm$ 3^{O}C. The experimental design was a randomized complete block with 12 treatments consisting of 3 parents and 9 R_1 populations with 4 replications of 60 plants each. The plants were evaluated for the following characters: (1) Days to 50% heading (number of days from planting to the exertion of the 50% heading; (2) length, width and area of the flag leaf; (3) plant height (main tiller from the soil level to the tip of the awns). After harvesting the following parameters were measured: (1) spike length; (2) number of grains per spike; (3) number of spikelets per spike; (4) number of grains per spikelet; (5) 100- grain weight and (6) days to maturity (visual observations were recorded when all the spikes on a plant turned yellow).

Data on 240 plants for each treatment of the different somaclones and the parent populations were statistically analyzed to determine differences among the populations. Most of the statistical analyses were done using the SAS/STAT statistical procedures employing the General Linear Model. Duncan's Multiple Range Test was applied to separate the means.

Table 1. Variations in characteristics of R_1 plants derived from cv Glennson. The R_0 plants were regenerated from cells selected on medium with 0, 10, 15, and 20% PEG.

Plant Characters	LINES				
	Parent	GA88R1	GB88R1	GC88R1	GD88R1
Days to 50% flowering	73.00 A*	59.00 E	60.00 D	61.00 C	68.00 B
Plant height (cm)	77.67 A	75.00 A	78.25 A	76.50 A	72.00 A
Flag leaf length (cm)	18.07 B	16.85 B	13.88 C	17.63 B	22.08 A
Flag leaf width (cm)	1.30 A	1.25 A	1.15 A	1.38 A	1.33 A
Flag leaf area (cm)	17.66 B	15.78 B	14.36 B	20.24 A	22.02 A
Spike lenght (cm)	10.13 B	10.13 B	9.88 B	11.73 A	15.53 A
No. of grains per spike	48.00 B	55.50 AB	50.00 AB	62.75 A	65.50 A
No. of spikelets per spike	20.00 A	19.00 A	19.25 A	20.75 A	20.25 A
No. of granins per spikelet	2.45 B	3.20 A	2.64 AB	3.03 AB	3.24 A
100 grain weight (g)	3.83 AB	3.85 AB	3.644 B	4.30 A	4.52 A
Days to maturity	111.00 A	105.00 C	106.00 B	106.00 B	105.00 C

* Means in a row followed by the same letter do not differ signi ficantly at 5% level according to Duncan's New Multiple Range Test.

Table 2. Variations in characteristics of R_1 plants derived from cv Pavon. The R_0 plants were regenerated from cells selected on medium with 0, and 10% PEG.

Plant Characters	LINES		
	Parent	PA88R1	PB88R1
Days to 50% flowering	59.00 E	60.00 D	61.00 C
Plant height (cm)	73.00 A	72.25 A	75.00 A
Flag leaf length (cm)	19.10 B	26.78 A	27.75 A
Flag leaf width (cm)	1.27 A	1.43 A	1.35 A
Flag leaf area (cm)	18.29 B	28.71 A	27.99 A
Spike length (cm)	9.33 A	10.55 A	10.15 A
No. of grains per spike	18.33 A	18.25 A	19.25 A
No. of spikelets per spike	18.33 A	18.25 A	19.25 A
No. of grains per spikelet	1.66 C	2.01 B	2.55 A
100-grain weight (g)	4.03 AB	3.64 A	4.22 A
Days to maturity	106.00 A	106.00 A	106.00 A

* Means in a row followed by the same letter do not differ significantly at 5% level according to Duncan's New Multiple Range Test.

Results and Discussion

All the 9 R_1 somaclones differed from the 3 parent lines in most of the characteristics recorded (Tables 1, 2, 3). Plant height did not vary among the somaclones of Glennson and Pavon (Table 1, 2). The differences were greater in the somaclones of PAK-16171 where the parent line averaged 86.7 $\pm$ 5 cm as compared to somaclones 71B88R1, 71C88R1 and 71D88R1 showing an average height of 75.8 $\pm$ 2.4 cm, 72.3 $\pm$ 2.6 cm and 73.0 $\pm$ 3.8 cm, respectively (Table 3).

A feature of potential importance was the increase in length, and area of the flag leaf. Only 3 of the 9 somaclones (GA88R1), GB88R1, GC88R1) had less or similar flag leaf length, width, and area than the parent line (Table 1), while all other somaclones differed significantly from the parent lines (Table 2, 3 and Fig.1). Two of the 9 somaclones (PA88R1, PB88R1) had about twice the flag leaf area of the parent line (Table 2) while 3 of the 9 (71B88R1, 71C88R1, 71D88R1) had about 3 to 4 times more flag leaf area than the parent line (Table 3 and Fig. 1).

Table 3. Variations in characteristics of R_1 plants derived from cv PAK-16171. The R_0 plants were regenerated from cells selected on 10,15, and 20% PEG.

Plant Characters	LINES			
	Parent	71B88R1	71C88R1	71D88R1
Days to 50% flowering	61.00 B	63.00 A	57.00 C	57.00 C
Plant height (cm)	86.67 A	75.75 B	72.25 B	73.00 B
Flag leaf length (cm)	10.30 C	24.75 AB	26.35 A	22.08 B
Flag leaf width (cm)	1.17 B	1.95 A	2.07 A	1.85 A
Flag leaf area (cm)	9.02 D	36.13 B	40.89 A	30.56 C
Spike length (cm)	8.50 B	10.00 A	9.68 A	9.30 A
No. of grains per spike	33.33 B	41.25 A	40.75 A	40.75 A
No. of spike-lets per spike	17.33 A	16.00 A	16.25 A	17.75 A
No. of grains per spikelet	1.94 B	2.59 A	2.53 A	2.46 A
100-grain weight (g)	3.48 B	4.53 A	4.53 A	4.49 A
Days to maturity	116.00 B	117.00 A	111.00 C	111.00 C

* Means in a row followed by the same letter do not differ significantly at 5% level according to Duncan's New Multiple Range Test.

Flag leaf area and flag leaf duration have been shown to have a direct relationship with yield and yield components in wheat. Kyrlasov (1987) observed an increase of >1% in yield/ear and total grain yield with an increase in leaf width by 1% by selecting under optimal conditions. Removal of the flag leaf in six varieties of wheat had a greater effect in reducing yield in the tall and moderately tall (but lower yielding) varieties than in the short higher yielding ones (Nefedov & Py1 Nev, 1984).

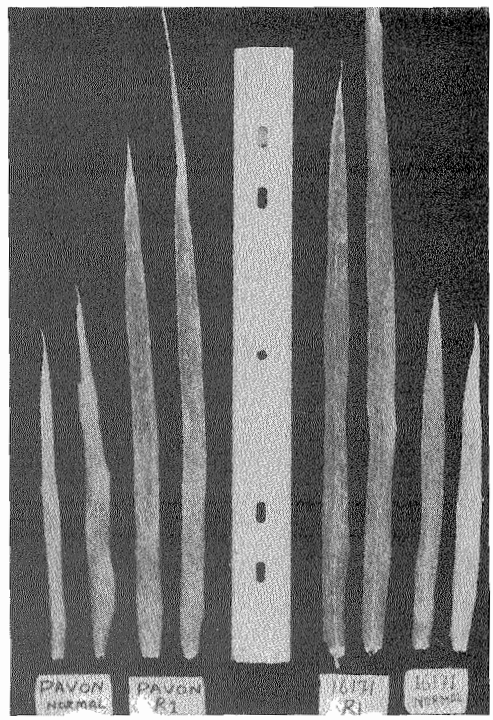

Fig. 1. Flag leaves of regenerants (R) of Pavon and PAK-16171 and their non-tissue cultured parent lines. The term "normal" in the figure refers to the leaves of non-tissue cultured parent lines.

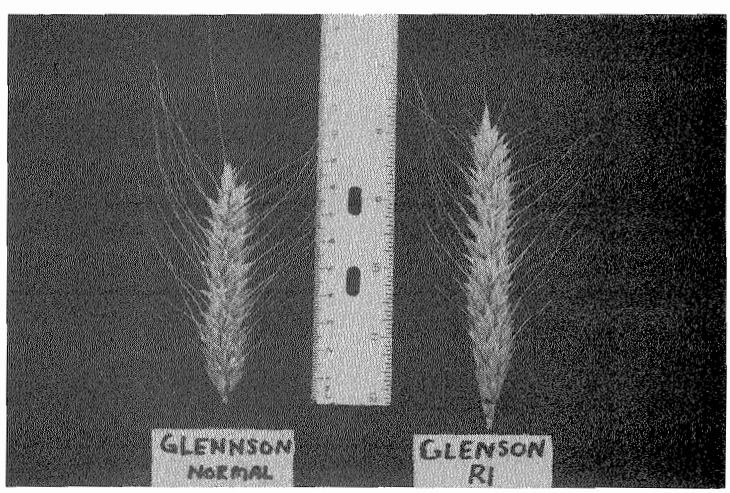

Fig. 2. Mature spikes of regenerants (R) of Glennson and its non-tissue cultured parent line. The term "normal" refers to non-tissue cultured parent.

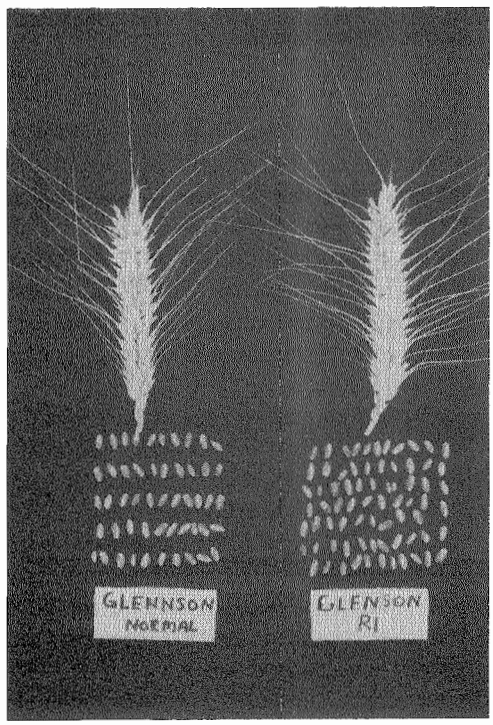

Fig. 3. Spikes and grains per spikes of the regenerants (R_1) and their non-tissue cultured parent lines of Glennson.

Thousand grain weight fell 5 times more in tall than in short winter wheat varieties. Mornhinweg (1985) studied heritability and combining ability of flag leaf area and flag leaf duration and their relationship to grain yield in winter wheat. Proportionally greater increase in main shoot grain yield was observed on the recovering of the flag leaf area in droughted wheat cultivars after mid-season water application (Aggarwal & Sinha, 1987).

Variation in spike length was significantly different from the parent lines in somaclones of Glennson and Pak-16171 (Tables,1, 3, Fig. 1) but did not differ in the somaclones of Pavon (Table 2). The number of grains per spike were also higher in the somaclones with increased flag leaf area. The parental mean was 37.7 $\pm$ 3 grains per spike while in the somaclones the means ranged from 36.0 $\pm$ 3.0 to 65.5 $\pm$ 1.26 (Tables 1, 2, 3, Fig.3). Somaclones GC88R1 and GD88R1 had 62.75 and 65.5 grains per spike, respectively as compared to parent line which produced 48.0 grains per spike (Table 1, Fig.3). Hundred grain weight did not differ from the parent line in the somaclones of Pavon (Table 2) but significant variations were observed from the parent lines in the somaclones of Glennson weighing 3.83 and 4.52 g and PAK-16171 weighing 3.48 and 4.49 g respectively (Table 1,3).

Evaluation for days to maturity (by visual observation when most of the spikes in a plant turned yellow) was recorded. Since variations were present in the days to 50% flowering, variation in days to maturity was apparent. The plants of somaclones of Glennson and PAK-16171 matured 5 to 6 days earlier than the parent lines (Table 1, 3).

These results demonstrate a relatively high frequency of variation in many of the individual characters measured in the R_1 plants with respect to the traits which were evaluated. The somaclones also differed among themselves in different characters.

The variation in the flag leaf size and the associated increase in grain number is the first such observation reported in wheat. Similar yield increases however, have also been reported in somaclones of sorghum (Bhaskaran *et al.*, 1987) and rice (Zongxiu *et al.*, 1983).

The phenotypic variation found among regenerated wheat plants could be due to either genetic changes or to factors such as carry-over of hormonal effects and differences in growth conditions. In our case it might be possible that the phenotypic differences are due to the effects of PEG-induced water stress, PEG *per se* or due to time period in culture since the cultures were maintained for 8 passages, each passage of 30 day duration and then regenerated into whole plants. In view of the increasing evidence for tissue culture-induced variation (Lazar *et al.*, 1988; Larkin & Scowcroft, 1981; Larkin *et al.*, 1984) in the present study it was apparent that the variations seems to be heritable as all the characters observed were among the R_1 plants which had already passed through one stage of meiosis. However, extensive genetic analysis and field evaluation will be necessary to fully evaluate the stability of the traits.

References

Aggarwal, P.K. and S.K. Sinha. 1987. Response of droughted wheat to mid-season water application: recovery in leaf area and its effect on grain yield. *Aust. J. Plant Physiol.,* 14: 227-237.

Ahloowalia, B.S. and J. Sherington. 1985. Transmission of somclonal variation in wheat. *Euphytica*, 34: 525-537.

Bhaskaran, S., R.H. Smith., S. Paliwal and K.F. Schertz. 1987. Somaclonal variation from *Sorghum bicolor* (L.) Moench cell cultures. *Plant Cell Tiss. Org, Cult.,* 9: 189-196.

Chaleff, R.S. 1981. *Genetics of higher plants. Application of cell culture.* Cambridge Univ. Press, New York.

Chen, T.H.H., M.D. Lazar, G.J. Scoles, L.V. Gusta and K.K. Kartha. 1987. Somaclonal variation in a population of winter wheat. *J. Plant Physiol.,* 130: 27-36.

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

Heyser, J.W., M.W. Nabors, C. MacKinnon, T.A. Dykes, K.J. DeMott, D.C. Kautzman and A. Mujeeb Kazi. 1985. Long-term, high frequency plant regeneration and the induction of somatic embryogenesis in callus cultures of wheat (*Triticum aestivum*) *Z. Pflazenzuchtg.,* 94: 218-233.

Kyrlasov, V.G. 1987. Correlated variation in quantitative traits in winter bread wheat. In: *Ispol. ovanie, sovremennykh metdorv seleketsii ernovykh i zernobobomykh kul' tur v Tsentral' non raine Nechernozemnoi zoin,* Moscow, pp. 12-22.

Lapitan, N.L.V., R.G. Sears, and B.S. Gill. 1984. Translocation and other structural changes in wheat by rye hybrids regenerated from tissue culture. *Theor. App. Genet.,* 68: 547-554.

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

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

Lazar, M.D., T.H.H. Chen, L.V. Gusta, and K.K. Kartha. 1988. Somaclonal variation for freezing tolerance in a population of norstar wheat. *Theor. Appl. Genet.,* 75: 480-484.

Linsmaier, F.M. and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue culture. *Physiol. Plant.,* 8: 100-127.

MacKinnon, C. 1985. *Morphogenetic responses of wheat calli derived from developmentally mature embryo explants.* Ph.D. Dissertation, Colorado State University, Fort Collins, CO., USA.

Maddock, S.E., V.A. Lancaster, R. Risiot and J. Franklin. 1983. Plant regeneration from cultured immature embryos and inflorescence of 25 cultivars of wheat (*Triticum aestivum*). *J. Exp. Bot.,* 34: 915-926.

Mornhinweg, D.W. 1985. *Heritability and combining ability of flag leaf area and flag leaf area duration and their relationship to grain yield in winter wheat.* Ph.D. Dissertation. Oklohoma State University, Stillwater, OK, USA.

Nefedov, A.V. and V.V. Pyl Nev. 1984. Role of leaves of particular insertion, heights and of awns in grain filling in different winter wheats. *Nauchno tekhnicheskii Byulleten Vsesoyuznogo Selektsionno-geneticheskogo Instituta,* 2: 11-14.

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

Zong-Xiu, S., Z. Chang-Zhang, Z. Kang-le, Q. Xiu-fang, F. Ya-ping. 1983. Somclonal genetics of rice (*Oryza sativa*). *Theor. Appl. Genet.* 67: 67-73.

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