

Genetics of regeneration of wheat (*Triticum aestivum* L.) plants

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ABSTRACT

Five genotypes of hexaploid wheat and their F1 hybrids were grown in tissue culture after three different periods of callus maintenance, and were evaluated for plant regeneration capability. Evaluation of the number of regenerated plants per callus showed that this trait is mainly determined by additive gene effects, with high heterotic potential. Plant regeneration capability is relatively easy to select for, especially when this regeneration is obtained after a short period of callus maintenance. Longer periods of callus maintenance led to a considerable reduction in plant regeneration capability in wheat.

INTRODUCTION

There is increasing evidence that genetic factors are the main contributors to the *in vitro* response of cereal tissues in culture (Hartmann *et al.*, 1989). Genotypic differences in callus induction and plant regeneration capability have been demonstrated in several cereals, such as corn (Green *et al.*, 1974; Green and Phillips, 1975), oats (Cummings *et al.*, 1976; Rines and McCoy, 1981), barley (Hanzel *et al.*, 1985; Bregitzer, 1982), triticale (Sharma *et al.*, 1980), and wheat (Sears and Deckard, 1982; Ahloowalia, 1982; Milach *et al.*, 1991a).

The genetic control of plant regeneration has been investigated in various species. In corn, the formation of somatic embryos and plant regeneration are under the control of nuclear genes, exhibiting dominant gene action (Hodges *et al.*, 1986). Tomes and Smith (1985), also working with corn, demonstrated that, although heterosis had a positive effect on regeneration, additive effects prevailed. In rice, the inheritance of callus induction and plant regeneration

is transmitted by blocks of genes with predominantly additive effects, with the occurrence of partial dominance and with no indication of interallelic interactions (Miah *et al.*, 1985; Quimio and Zapata, 1990).

In wheat, De Buyser *et al.* (1992) detected the involvement of few genes in the control of plant regeneration, with an expressive maternal effect. A high genetic control (h^2 between 0.6 and 0.7) of callus formation and plant regeneration and important additive effects were observed by Lazar *et al.* (1984), who also detected the involvement of cytoplasmatic genes.

In a study of Brazilian wheat genotypes, Milach *et al.* (1991b) concluded that plant regeneration was controlled by additive and dominance effects, with reciprocal effects having no importance, demonstrating that this is a selectable trait in plant breeding programs. Lange *et al.* (1995) studied plant regeneration, embryogenesis, early germination and organogenesis of Brazilian wheat genotypes and concluded that the additive and dominance effects were significant for all four traits. The dominance effects, especially for plant regeneration, were elevated, and epistatic effects were also expressive.

MATERIAL AND METHODS

The first phase of the present study involved a field assay at the Estação Experimental Agronômica da Universidade Federal do Rio Grande do Sul (UFRGS), Eldorado do Sul, RS. Five genotypes with different plant regeneration capabilities were sown: Batuíra (BAT) - good regeneration; BH1146 (BH) - low regeneration; BR23 (BR) - good regeneration; Cajeme 71 (CAJ) - low regeneration; and IAC5 (MAR) - good regeneration. All possible crosses, excluding reciprocals and including parents, were performed between these genotypes.

Around 100-150 immature embryos were collected from each of these crosses and from parental lines for tissue culture, on average 12 days after hand pollination, and placed in appropriate culture medium for callus formation, i.e., MS medium (Murashige and Skoog, 1962) with 2.0 mg/l 2,4D (2,4-dichlorophenoxyacetic acid), 3% sucrose and 0.8% carrageenin added. They were maintained at 25°C in the dark for three weeks. At the end of this period, the calli that formed were transferred to an appropriate medium for callus growth (MS containing 0.5 mg/l 2,4D) and maintained there at 25°C in the dark for an additional period of three weeks. After this time, one third of each callus was placed again in callus growth medium, and the remaining two thirds of the calli were fragmented and placed in plant-regeneration medium (MS medium with no 2,4D added) at 25°C and under constant light.

The callus piece left in the callus-growth medium was maintained under the same conditions as described above. The process was repeated during two more three-week periods (second and third regeneration period), and on the occasion of the third replating the calli were not divided but simply fragmented and placed in regeneration medium, where they were maintained at 25°C under constant light. As the formation of complete plants of a size considered to be adequate (five to ten centimeters aerial part, and developed roots) was observed, the plants were removed from the container and washed in running water to remove residual culture medium and to separate the callus residues from the plant roots. Regeneration was evaluated during this phase and complete plants ready to be transferred to vermiculite pots were considered to be regenerant. The calli that did not regenerate any plant after two months in regeneration medium were discarded.

Plant regeneration was evaluated at three different times (after one, two and three passages through the callus-growth medium). The genetic analysis of general and specific combining abilities (GCA and SCA) was done based on Griffing (1956),

method 2, model I. The variance analysis for a complete randomized design was done using a general linear model (GLM), because of the unequal number of replications, and the means comparison (Duncan test) was made by SAS software.

The heterosis estimates were made based on the difference between the F1 mean and half of the parental mean, according to the expression (Fehr, 1987):

$$\text{Heterosis (\%)} = \frac{100 \times (\text{F1 mean} - \text{mean of parents})}{\text{Mean of parents}}$$

RESULTS AND DISCUSSION

During the first regeneration period, the mean number of regenerated plants per callus indicated that Batuíra (BAT), BR23 (BR) and IAC5 (MAR) were lines with best and similar performance, with Cajeme (CAJ) and BH1146 (BH) showing the lowest regeneration capability. The highest mean obtained for the F1's was that of the CAJ x BAT cross, followed by CAJ x BR (Table I).

The differences found in plant regeneration capability were predicted in several previous studies carried out on this species, including the Brazilian genotypes studied here (Sears and Deckard, 1982; Ahloowalia, 1982; Miláč *et al.*, 1991b; Lange *et al.*, 1995), demonstrating differences in potential for use in tissue culture.

During the longer regeneration periods (the second and third), the mean number of regenerated plants per callus was very low, preventing any type of genetic analysis, as shown by the parental behavior (Figure 1).

Starting from the second period, many calli became necrotic or simply stopped developing, becoming oxidized in some cases (visual observations),

Table I - Mean number of regenerated plants per callus obtained for the five parental lines and their F1's during the first period of plant regeneration, Porto Alegre, UFRGS, 1992/1993.

Genotypes	Means*				
	CAJ	BAT	BH	MAR	BR
CAJ	0.55 ^e	3.77 ^a	0.18 ^{ef}	2.35 ^c	3.61 ^a
BAT		1.80 ^d	1.50 ^d	2.76 ^b	2.26 ^c
BH			0.10 ^f	1.38 ^d	1.72 ^d
MAR				1.62 ^d	2.55 ^{bc}
BR					1.76 ^d

*Means followed by the same letter did not differ significantly at the 5% level.
Y = 1.89.

with very low plant regeneration rates, that considerably impaired the reliability of the results. Sears and Deckard (1982) found considerable variation among genotypes, both in terms of production of regenerable calli and the period of time during which the calli maintain their regeneration capability. There was no evidence of a correlation between the percentage of regenerating calli and the time during which these calli continue to regenerate plants.

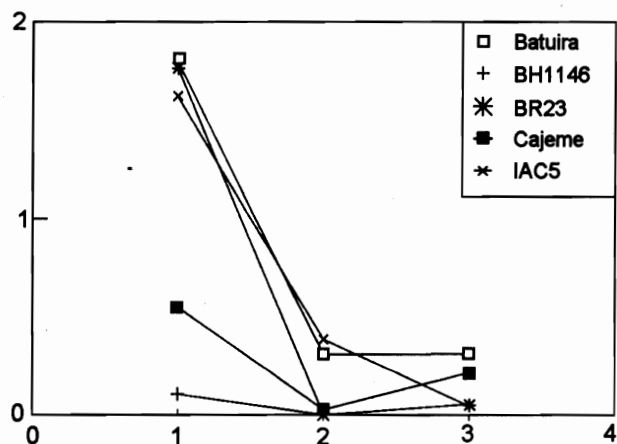


Figure 1 - Mean number of regenerated plants per callus for the five wheat parental lines during the three plant regeneration periods. Porto Alegre, UFRGS, 1992/1993.

Table II - Variance analysis for general combining ability (GCA) and specific combining ability (SCA) for the number of regenerated plants per callus during the first period of plant regeneration. Porto Alegre, UFRGS, 1992/1993.

Sources of variation (CV)	Degrees of freedom (d.f.)	Mean squares (MS)	F
Treatments	14	60.23	55.36
GCA	4	98.10*	90.16
SCA	10	45.08*	41.44
Residue	733	1.09	

*Significant MS values at the 5% level (F test).

Table III - General combining ability (GCA) and specific combining ability (SCA) for the number of regenerated plants per callus for the five parental lines and their F1's during the first period of plant regeneration. Porto Alegre, UFRGS, 1992/1993.

Genotypes	SCA					GCA
	CAJ	BAT	BH	MAR	BR	
CAJ	-1.27	1.54	-0.78	0.35	1.41	-0.02
BAT		-0.84	0.13	0.35	-0.35	0.39
BH			0.01	0.24	0.39	-0.88
MAR				-0.56	0.17	0.16
BR					-0.81	0.36

The effects of both general (GCA) and specific (SCA) combining abilities for the number of plants regenerated per callus, calculated for the first period of plant regeneration, were significant at the 5% level of probability. The data demonstrate that GCA mean squares were much higher than the SCA mean square, showing the importance of additive effects in the genetic variance (Tables II and III). These differences between GCA and SCA demonstrated that selection for plant regeneration capability is viable, especially when carried out after a short time of callus maintenance. The predominance of additive effects, although with significant nonadditive effects, was observed by Lazar *et al.* (1984) and by Milach *et al.* (1991b) with Brazilian genotypes. High values were detected by Lange *et al.* (1995) for dominance and epistasis effects, although additive effects were also expressed.

BAT and BR had the highest GCA values (Table III), and the SCA values from the F1 hybrids with their participation can explain the mean values for these F1s (Table I), all quite high and most of them higher than the overall mean (except for BH x BR and BAT x BH, which were much higher than the BH mean). Thus, the deviations observed in some F1's, in addition to demonstrating a large additive effect, also showed nonadditive effects. Similar behavior was observed in genotype MAR with F1's which always had a greater response than the parents. This was further supported by the predominance of additivity, with considerable nonadditive effects (Table I). BH was the genotype with the most negative GCA value, together with very low (close to zero) SCA values for its F1's, as demonstrated by the mean values obtained in crosses (Table III), which were always lower than those of the other parent (with very small additivity deviations). In turn, CAJ presented GCA values closest to zero and the highest SCA values in its combinations. The highest SCA effects were obtained with crosses CAJ x BAT and CAJ x BR, which demonstrated deviations considerably above the expected values, considering only additivity, and which presented the highest mean values (Table I).

The GCA of the BR parent (Table III) had a negative general combining ability, while the non-additive effects were quite elevated for regenerating capability, different from Lange *et al.* (1995). However, the only genotypes used in both studies were BR and MAR. Furthermore, the form of evaluating regeneration in the study by Lange *et al.* (1995) was the relationship between number of calli that regenerate plants and total number of calli of the genotype, while the present study evaluated the mean number of plants regenerated per callus, which may not represent the same genetic control.

Table IV - Heterosis (%) for the number of regenerated plants per callus for the ten F1's resulting from semidialele crosses between the five parental lines during the first regeneration period. Porto Alegre, UFRGS, 1992/1993.

Genotypes.	Heterosis (%)				
	CAJ	BAT	BH	MAR	BR
CAJ		220.85	-43.75	116.59	212.55
BAT			57.89	61.40	26.97
BH				60.46	84.95
MAR					50.89
BR					

Calculated heterosis (Table IV) showed that CAJ can be an efficient genotype as a parent to obtain hybrids with high scores of plant regeneration per callus. Except for the CAJ x BH cross, all others presented hybrid vigor.

Heterosis was observed for regeneration capability. BR and BAT were the genotypes yielding the highest GCA potentials and high capability in the combination with the CAJ genotype. As shown in Tables I, III and IV, the mean values obtained for the CAJ x BAT and CAJ x BR crosses revealed a mean number of regenerated plants per callus considerably exceeding the values expected from the parental means, and the highest heterosis values among all hybrids tested. Furthermore, GCA and SCA data also showed that higher SCA effects coincide with the highest means, raising the hypothesis of genes in the CAJ genotype that, in combination with genotypes such as BAT and BR, express a high regeneration capability that is not expressed in the constitution of the CAJ genotype, because CAJ was the second worst genotype among the parental lines in number of regenerated plants per callus.

ACKNOWLEDGMENTS

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - RHA/E), and with the financial support of the Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS).

RESUMO

Cinco genótipos de trigo hexaplóide e seus híbridos F1's foram submetidos à cultura de embriões imaturos, com três períodos de manutenção de calos, e avaliados quanto à capacidade de regeneração de plantas. As avaliações do número de plantas regeneradas por calo mostraram ser este

caráter controlado principalmente por efeitos aditivos, com alto potencial de heterose. Regeneração de plantas é um caráter de seleção relativamente fácil, principalmente quando obtida após um curto período de manutenção de calos. Períodos mais prolongados de cultura apresentaram uma redução expressiva na capacidade de regeneração de plantas de trigo.

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(Received August 15, 1994)