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Callus induction and plant regeneration by Brazilian triticale and wheat genotypes

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ABSTRACT

In order to determine the *in vitro* behavior of Brazilian triticale, 16 triticale genotypes, and three wheat genotypes used as checks, were sown in June 1994. The explants used were immature embryos. In addition to the genotype tests, two culture media for callus induction were also evaluated, i.e., MS (Murashige and Skoog, *Physiol. Plant. 15*: 473-497, 1962) medium containing 2.0 mg 2,4D/l, and MS medium containing 4.0 mg 2,4D/l. The plant regeneration protocol used was the one employed at the Laboratório de Cultura de Tecidos, Departamento de Plantas de Lavoura, Universidade Federal do Rio Grande do Sul, for wheat. Differences in plant regeneration were observed both among triticale and wheat genotypes, with triticale usually showing better regeneration than wheat. No differences were observed between the callus induction media.

INTRODUCTION

Triticale is an artificial hybrid of wheat and rye obtained by Wilhelm Rimpau a little more than a century ago (Carvalho *et al.*, 1977; Rimpau, 1891, cited by Baier, 1991) which has been cultivated for approximately 30 years. This hybrid offers good perspectives, especially in marginal growing regions of the parental species, because of its greater tolerance to adverse environmental conditions such as drought and soil aluminum toxicity, and greater resistance to some pests and diseases, although its grain quality is inferior to that of wheat. In southern Brazil, Mairesse (1976) tested eight lines of hexaploid triticale sown on four different dates and observed that the hybrid had higher adaptability than wheat or rye.

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In addition to its direct utilization for culture, triticale has proven to be valuable for wheat improvement as a means of introgression of rye genes. Data reported by Gustafson *et al.* (1985) have confirmed the value of this technique for total or partial substitution of chromosomes of the D genome of wheat for the R genome (rye).

In vitro culture of plant cells and tissue has attracted considerable interest over the last few years because it provides the means to study the physiological and genetic processes of plants, in addition to offering the possibility of increasing genetic variability for use in breeding programs (Hanzel *et al.*, 1985). According to Hartmann *et al.* (1989), genetic factors are major contributors to the *in vitro* response of cereal tissues in culture. Genotypic differences in the ability to initiate a callus and to regenerate a plant have been demonstrated in different cereals, such as maize (Green *et al.*, 1974; Green and Phillips, 1975), oats (Cummings *et al.*, 1976; Rines and McCoy, 1981; Bered, 1994), barley

(Hanzel *et al.*, 1985; Bregitzer, 1992), triticale (Sharma *et al.*, 1980), and wheat (Sears and Deckard, 1982; Ahloowalia, 1982; Milach *et al.*, 1991a,b; Lange, 1991; Dornelles, 1994).

MATERIAL AND METHODS

The study was conducted in the experimental fields of the Estação Experimental Agronômica of the Universidade Federal do Rio Grande do Sul (UFRGS), municipality of Eldorado do Sul, and in the Laboratório de Cultura de Tecidos, Departamento de Plantas de Lavoura, UFRGS, Porto Alegre. Sixteen triticale genotypes and 3 wheat genotypes obtained from the 1994 South Brazilian Trial of Triticale Lines were sown, to be later used as sources of explants (immature embryos) for tissue culture. The culture techniques and phytosanitary treatments were performed according to the recommendations for research on triticale production in Rio Grande do Sul (Baier *et al.*, 1994).

Approximately 12 days after anthesis, triticale heads were picked in the field and carried to the Laboratório de Cultura de Tecidos. The karyopses were removed and disinfected by successive immersions into 70% alcohol for 1 min and into two sodium hypochlorite solutions (1.0% and 0.5% active principle) for 15 min each, and washed three times with sterile distilled water, in a laminar flow hood. After disinfection, the immature embryos were removed with the aid of a forceps and surgical knife and placed in two different culture media for callus induction, i.e., Murashige and Skoog (1962) medium at concentrations of 2.0 mg 2,4D/l (MS2) and 4.0 mg 2,4D/l (MS4). Immature embryos were placed in Petri dishes (10 per dish) measuring 7 cm in diameter, with the scutellum side up. The dishes were placed in a growth chamber for three weeks under conditions of total darkness and 25 ± 1°C temperature. At the end of this time, induced calli were counted and transferred to culture medium for callus growth (MS medium containing 0.5 mg 2,4 D/l) on Petri dishes measuring 10 cm in diameter, 10 calli per dish. The calli were then returned to the growth chamber under the same conditions as used for callus induction and left there for three weeks, after which they were placed in plant regeneration medium (MS with no 2,4D added) in individual flasks under constant light, at 25°C.

As the regenerated plants reached a size considered to be adequate (5 to 10 cm) and good rooting, they were removed from the culture medium and counted. Four determinations were made to evaluate the differences among triticale and wheat genotypes and between callus-induction media: 1) number of calli formed per plate, 2) percentage of formed calli, 3) number of regenerated plants per callus, and 4) percentage of calli that regenerated a plant in relation to the number of immature embryos placed in callus induction medium.

The data were analyzed by weighted analysis of variance (GLM) and the Duncan test (5%) was used to compare the means.

RESULTS AND DISCUSSION

No significant interactions were observed between the wheat and triticale genotypes and the culture media used for callus induction (data not shown).

In the evaluation of differences between genotypes within each species, analysis of variance (Table I) indicated significant differences only for plant regeneration traits. In triticale some genotypes were clearly superior in terms of number of plants regenerated per callus and percent calli that regenerated plants (Table II). Five of these (IAPAR 54, EMBRAPA 18, IAPAR 23, EMBRAPA 17, and PFT 107) demonstrated this superiority for both traits, all of them presenting a mean number of more than 2.0 regenerated plants per callus and approximately 70% regenerating calli. The PFT 8922 genotype produced more than 80% regenerating calli.

Because of the regeneration methodology employed (i.e., without sectioning the calli to be transferred to the regeneration medium), the number of plants/callus was affected. In many cases plants that might have developed did not, because of space limitations in the flask and insufficient culture medium.

Table I - Analysis of variance for triticale and wheat genotypes submitted to one cycle of tissue culture. Porto Alegre, 1994/1995.

Species/ variable	Degrees of freedom	Mean squares	F	
Triticale				
No. of calli formed	15	3.22	1.40	NS
% Calli formed	15	231.01	1.59	NS
Plants/callus	15	1.55	2.61	*
% Regenerating calli	15	987.12	2.52	*
Wheat				
No. of calli formed	2	0.65	0.45	NS
% Calli formed	2	120.80	1.05	NS
Plants/callus	2	10.74	40.07	*
% Regenerating calli	2	9300.40	18.91	*

*Significant F value (1%); NS, nonsignificant.

Table II - Number of calli formed, percent of calli formed, number of regenereted plants, and percent of regenerating calli in wheat and triticale genotypes submitted to one cycle of tissue culture. Porto Alegre, 1994/1995.

Genotype	No. of calli formed	% Calli formed	No. of plants regenerated	% Regenerating calli
Triticale				
BR 1	8.86	88.57	1.48 C	62.01 ABCD
BR 4	9.25	93.89	1.75 BC	62.90 ABCD
CEP 18	9.25	92.50	1.76 BC	59.70 ABCD
CEP 22	8.69	90.92	1.49 C	58.31 ABCD
CEP 23	9.00	94.37	1.83 BC	64.07 ABCD
CEP 25	7.75	84.87	1.60 C	67.66 ABCD
EMB 17	7.33	83.42	2.20 ABC	74.56 ABC
EMB 18	8.48	91.22	2.52 AB	76.88 AB
IAPAR 23	8.61	86.92	2.22 ABC	58.58 ABCD
IAPAR 54	8.56	91.19	2.73 A	79.36 AB
PFT 107	8.80	90.67	2.13 ABC	69.94 ABC
PFT 116	8.81	90.56	1.67 BC	45.09 D
PFT 218	8.31	84.46	2.06 BC	57.35 BCD
PFT 8922	8.93	97.14	1.81 BC	80.94 A
TCEP 9038	8.77	87.69	1.35 C	53.05 CD
TCEP 904	8.75	97.50	1.70 BC	62.63 ABCD
Mean	**8.69 a**	**90.83**	**1.91 a**	**64.18 a**
Wheat				
BR 23	9.40	94.80	0.99 B	43.05 B
BR 35	9.22	96.67	2.61 A	83.43 A
EMB 16	8.91	90.00	0.25 C	12.91 C
Mean	**9.17 a**	**93.60**	**1.24 b**	**44.94 b**

*Means followed by the same letter in a column, with capital letters referring to within-species differences, and lower case letters to between-species differences, did not differ significantly by the Duncan test (5%).

The evaluation of calli as regenerating or not permitted a complementary and reliable evaluation.

The wheat genotypes showed much greater differences: whereas BR 35 showed a high rate of plant regeneration that was superior to triticale, BR 23 showed intermediate regeneration and EMBRAPA 16 showed very low regeneration (Table II). These data demonstrate that the variability of wheat for this trait is more marked, a fact that may, based on the genotype used in this study, be explained by the origin and evolution of the two species.

On average, the triticale genotypes proved to be superior to the wheat genotypes in terms of plant regeneration, with no significant differences in terms of callus induction (Table II). This greater capacity of the triticale genotypes studied to induce embryogenesis, which is expressed as plant regeneration (the lowest value was 45% regenerating calli), may be explained by the presence of the R genome of rye which could possess genes that, alone or interacting with genes of the wheat genome, confer better growth and greater regenerability in triticale.

With respect to the different 2,4D concentrations tested in the induction medium (2 and 4 mg/l culture medium), no significant differences were observed in any of the species tested (wheat and triticale) or in the callus induction and plant regeneration traits. Thus, we recommend the use of MS2 medium (MS containing 2.0 mg 2.4 D/l) as more economic and less toxic.

RESUMO

Com o objetivo de identificar o comportamento *in vitro* de genótipos brasileiros de triticale, foram semeados, em junho de 1994, 16 genótipos de triticale e três genótipos de trigo a serem usados como testemunhas. Os explantes utilizados foram embriões imaturos. Além do teste de genótipos, foram avaliados também dois meios de cultura de indução de calos: MS (Murashige and Skoog, *Physiol. Plant. 15*: 473-497, 1962) com 2,0 e 4,0 mg de 2,4D/l, respectivamente. O protocolo de regeneração de plantas usado foi o mesmo utilizado pelo Laboratório de Cultura de Tecidos do Departamento de Plantas de Lavoura da Universidade Federal do Rio Grande do Sul para trigo. Tanto no triticale como no trigo foram observadas diferenças entre genótipos nos escores que se referem à regeneração de plantas. De uma forma geral o triticale se mostrou melhor regenerante que o trigo. Não foram observadas diferenças entre os meios de indução de calos, sendo recomendado o uso de 2,0 mg de 2,4D/l.

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