EMBRYOGENIC ABILITY AND ISOPEROXIDASE PATTERNS OF THE SCUTELLAR CALLI FROM IMMATURE HYBRID EMBRYOS *TRITICUM DURUM* x *SECALE CEREALE* AND THEIR PARENTAL FORMS

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ABSTRACT

Somaclonal variability has been suggested as a new, efficient method for increasing the frequency of recombinations in interspecific/intergeneric hybrids in cereals. In order to apply this method for producing durum wheat recombinant lines with improved traits introgressed from rye, a study concerning *in vitro* embryogenic ability of several parental lines and their F_1 hybrids (*Triticum durum* x *Secale cereale*) has been carried out. Among the parental lines (nine *Triticum durum* and five *Secale cereale*) and 45 resulting hybrid combinations, scutellar embryogenic calli were obtained in seven durum lines, one rye line and four durum x rye hybrids. A total of about 200 regenerated plants were obtained, 55 from hybrid cultures. The genotypes with the best tissue culture response were used for biochemical analysis, to reveal isoperoxidase (IPRXs) patterns, as affected by genotype and type of callus analyzed - embryogenic vs. nonembryogenic

Key words: Embryogenic ability, F_1 hybrids, isoperoxidase pattern, *Secale cereale*, *Triticum durum*.

INTRODUCTION

Interspecific and intergeneric crosses are at present the main possibility for producing new germplasm in cereals breeding, but the utilization of these methods is limited by the low recombination frequency.

In vitro culture of hybrid tissues has been recently suggested as a new, efficient method for overcoming this limitation via exploiting genomic rearrangements facilitated during cell growth (Larkin, 1985; Phillips, 1990).

Plant regeneration from hybrid callus cultures was reported for several species: *Hordeum vulgare* x *Hordeum procerum* (Jorgensen and Andersen, 1989), *Triticum aestivum* x *Elymus hispidus* (Larkin and Banks, 1991), *Triticum aestivum* x *T. durum* (Cialâcu et al., 1993).

In the present report, the first data obtained in a study concerning the embryogenic ability of several durum wheat lines, inbred rye lines and their hybrids are presented.

Although the recent years have revealed an increasing interest for biochemical and molecular studies of differentiation - dedifferentiation processes *in vitro* - for identifying markers of the embryogenic ability (Pedersen and Andersen, 1993), studies concerning the electrophoretic patterns of isozymes in cereals tissue cultures are relatively scarce.

Therefore we analysed the best tissue culture responding genotypes for revealing IPRXs patterns as affected by genotype and type of callus - embryogenic and nonembryogenic.

MATERIALS AND METHODS

Tissue culture: Nine *Triticum durum* and five *Secale cereale* lines (listed in Table 1) were selected for their agronomic traits as parental genotypes. The plants were grown in field and subjected to crossing.

Immature embryos (15-17 days after pollination) were used as explants. For each parental genotype - durum wheat and rye - a number of 30-40 immature embryos were planted on culture media. In the case of hybrids the number of plated embryos ranged between 5-80, as affected by the seed set.

Scutellar calli development and plant regeneration were obtained according to a method described previously by Cialâcu et al. (1993), using a multistep protocol, in order to facilitate somaclonal variability.

Briefly, scutellar calli were induced on MS (Murashige-Skoog, 1962) medium supplemented with 3.0 mg/l 2.4- dichlorophenoxyacetic acid (2.4-D). For the next subcultures the concentration of 2.4-D was gradually reduced (2.0 mg/l, then 1.0 mg/l). Plant regeneration was induced on MS plus 3.0 mg/l IBA (indolylbutiric acid).

The regenerated plants were subcultured for four weeks on MS half strength with 0.2 mg/l naphtylacetic acid (NAA) for promoting root development. Finally, the plants were transferred in soil, vernalized and grown to maturity in green house.

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The cultures were scored monthly and for each genotype several parameters were recorded: the number and percentage of explants forming callus (callus response), the number and percentage of embryogenic calli (embryogenic ability) and the total number of regenerated plants.

Statistic analysis of the data was performed using χ^2 test.

Biochemical analysis: Three F_1 hybrids - selected for having a good *in vitro* response (at least 17.5% embryogenic calli) - and their parental lines, CO 77/90, 420 DU 1, E 439/91 (durum lines) and Sv. 749-78 (rye), were used for biochemical analysis.

Tissue samples of both types of calli - embryogenic and nonembryogenic - were taken after three subcultures (12 weeks).

Vertical electrophoresis was performed using polyacrilamide gels (7%), in borax-borate buffer, pH 8.3. The enzymatic assay was performed using H_2O_2 (0.3%) and benzydine solution in acetate buffer 0.5 M, pH 4.7. The reaction was stopped with distilled water.

RESULTS AND DISCUSSIONS

***In vitro* response.** All the parental genotypes produced scutellar calli. For the durum lines, callus response ranged between 54.8-93.3%. Embryogenic ability and plant regeneration were recorded for seven genotypes, among them the line E 439/91 showing the best response (76.9% embryogenic calli and 32 regenerated plants) (Table 1).

Table 1. Embryogenic ability of nine *T. durum* lines and five *S. cereale* inbred lines

Genotype	Callus response %	Embryogenic ability %	χ^2 value	Regenerated plants no.
a) *Triticum durum*				
62 DU 2	93.3	-		-
Hordeiforme 1144-82	93.1	-		-
Coral Odeski	90.0	22.2		6
E 439/91	88.0	76.9		32
DF 42-87-31	83.3	50.0	40.6^{xxx}	16
420 DU 1	83.1	6.3		1
Leucurum 1226-83	73.3	10.5		2
DF 69-89	60.3	14.3		2
CO 77/90	54.8	40.0		12
b) *Secale cereale*				
S. montanum	86.4	-		-
Sv. 749-78	73.3	21.4		4
(Sv.6404-74/SxD_3)/Danae	51.0	-		-
Sv. 3-84	48.6	-		-
Sv. 6414-74-1	14.3	-		-

P 0.1%=34.5

In the case of rye, the callus response ranged between 14.3-86.4%, but embryogenic callus formation was recorded for a single genotype (Sv. 749-78).

Very significant differences concerning the embryogenic ability were found among parental genotypes, according to χ^2 values.

Among the 45 hybrid combinations obtained, only ten of them produced scutellar calli. Embryogenic ability was recorded in four combinations (Table 2). The highest embryogenic ability was obtained for the hybrid combination E 439/91 x Sv. 749-78 (71.4%).

Table 2. Embryogenic ability of ten durum wheat x rye hybrids

Hybrid combination	Callus response %	Embryogenic ability %	χ^2 value	Regenerated plants no.
DF 42-87-31 x Sv. 749-78	50.0	66.7		18
420 DU 1 x Sv. 749-78	42.9	33.3		5
Coral Odeski x Sv. 749-78	33.3	-		-
DF 42-87-31 x (Sv. 6404/SxD_3)/Danae	33.3	-		-
E 439/91 x Sv. 749-78	31.0	71.4	18.3^{x}	26
CO 77/90 x Sv. 749-78	17.5	-		-
Leucurum 1226-83 x Sv. 3-84	14.8	-		-
Hordeiforme 1144-82 x Sv. 3-84	11.1	-		-
62 DU 2 x Sv. 6414-74-1	6.1	-		-
DF 59-89 x *S. montanum*	1.2	43.2		6

P 5%=16.9

For the genotypes utilized in the present experiment, the embryogenic ability was usually associated with a lower callus response. The correlation between callus response and embryogenic ability remains to be established.

A total of about 200 plants were regenerated, 55 of them from hybrid cultures. The regenerated plants were successfully transferred into soil and grown to maturity in the greenhouse.

The electrophoretic analysis. We found between 9 and 11 IPRx in calli from the durum lines, each durum genotype expressing a specific zymogram (Figure 1A). The rye genotype analysis revealed 11 IPRx.

All the hybrids expressed 9 IPRx but different zymogram (Figure 1B), 2 fast IPRx being common for embryogenic calli.

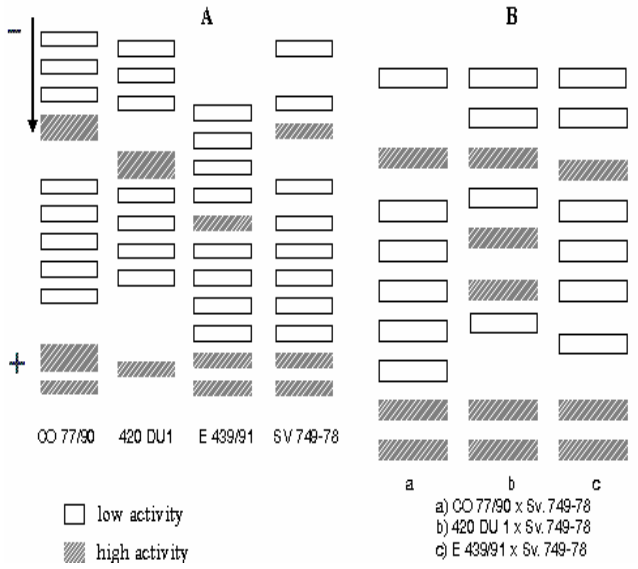

Figure 1. Peroxidase isozyme patterns analysed on PAGE
A - from embryogenic calli of the parental lines
B - from embryogenic calli of the F_1 hybrids

Embryogenic type callus differed from the nonembryogenic type by the number of IPRx. Usually a higher number of IPRx was detected in embryogenic cultures (Figure 2).

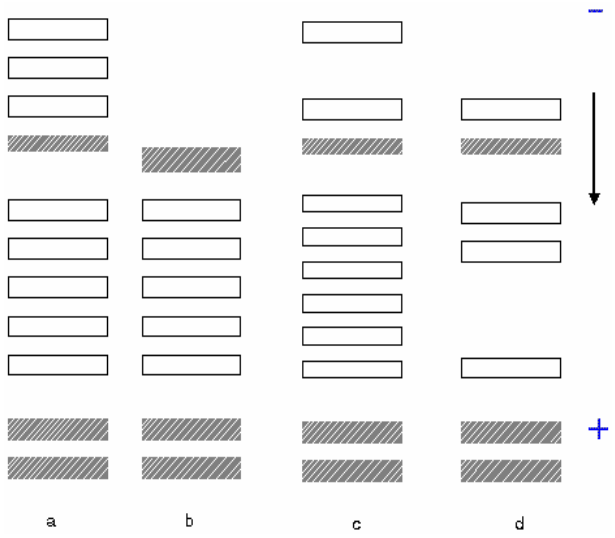

Figure 2. Isoperoxidase zymograms analysed from durum wheat line CO 77/90:
a - embryogenic calli; b - nonembryogenic calli, and from rye inbred line Sv. 749-78; c - embryogenic calli; d - nonembryogenic calli

Studies concerning isozymes patterns as affected by somatic embryogenesis in cereals are relatively scarce. Coppens and Dewitte (1990) proposed an enzyme-based marker system including esterase and peroxidase for embryogenic cultures of barley.

In maize, Rao et al. (1990) detected two cathodical IPRx specific for embryogenic callus. A similar study conducted in wheat revealed a higher number of IPRx in embryogenic calli than in nonembryogenic ones (Cialâcu et al., 1996).

In a study concerning the genetic control of IPRx in wheat, Müller et al. (1990) analysed the leaf tissue from plants regenerated in anther cultures of wheat-rye hybrids. The authors located the genes controlling the fast IPRx on the short arm of chromosome 1B. The slow isozymes were located on the rye chromosome 1RS.

The results obtained by Bai and Knott (1993) from a similar experiment directed at producing long-term regenerating calli from the hybrid *Triticum astivum* cv. Chinese Spring x *Thynopirum ponticum* underlined the potential of long-term cultures for introgression of useful characters from alien species into wheat.

CONCLUSIONS

Large differences were found among the nine durum lines, five rye inbred lines and their hybrids for callus response and embryogenic ability.

The electrophoretic analysis in the calli of three wheat-rye hybrids and their parental lines revealed a different number of IPRx and a specific zymogram for each genotype.

Our results are encouraging to continue the studies for applying *in vitro* culture of somatic hybrid tissues for obtaining recombinant lines in cereals.

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Table 2. Embryogenic ability of ten durum wheat x rye hybrids

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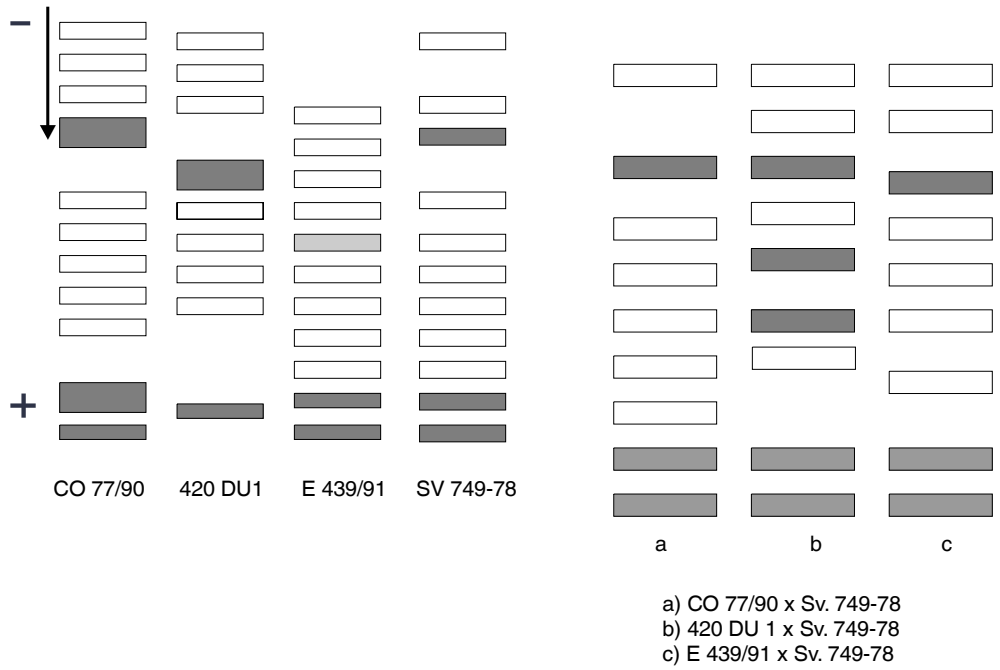

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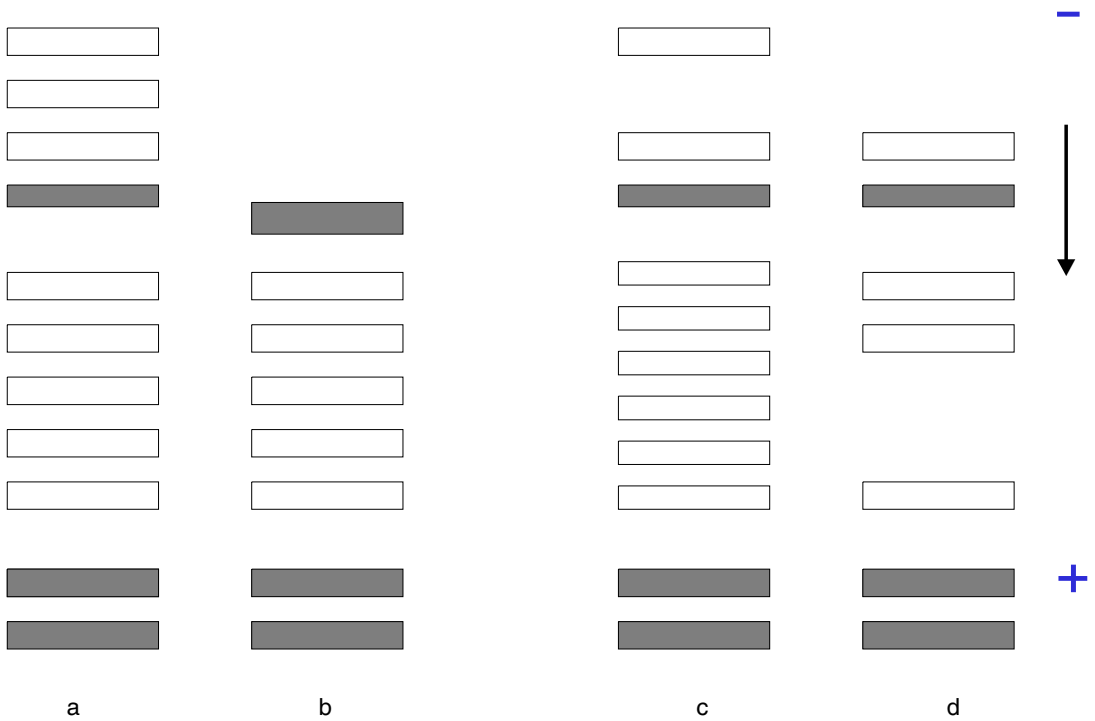

Figure 2: Isoperoxidase zymograms analyzed from durum wheat line CO 77/90 :
a - embryogenic calli; **b** - nonembryogenic calli and from rye inbred line Sv. 749-78:
c - embryogenic calli; **d** - nonembryogenic calli.

Table 2. Al - hematoxyline complexes formation on the root meristem surface.

Genotype	Hematoxylin stainability of Al - treated roots (Angular transformation)			Mean per genotype	Duncan test[1]
	0.03 mM Al	0.06 mM Al	0.09 mM Al		
Dayton	**0**	**21.92**	**72.61**	**31.51**	**A**
Smooth Awn	**0**	**23.99**	**69.27**	**31.01**	**A**
Sunrise	**0**	**27.09**	**71.10**	**32.73**	**A**
Volla	**0**	**39.82**	**66.55**	**35.48**	**B**
Gull	**0**	**40.15**	**65.95**	**35.36**	**B**
Bavaria	**0**	**43.37**	**62.38**	**35.25**	**B**
F 468 - 86	**58.12**	**66.47**	**81.61**	**68.73**	**C**
F 1385 - 90	**62.96**	**77.77**	**82.29**	**76.34**	**D**
Andra	**60.17**	**81.49**	**88.29**	**76.65**	**D**
Mean	**20.13a[1)]**	**46.87 b**	**73.97 c**	**-**	

1) Means without common letters are significantly different at $P \leq 0.05$