

## INHERITANCE OF *IN VITRO* RESPONSE IN WHEAT

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The inheritance of *in vitro* culture response was studied by using immature embryos from five wheat cultivars and their reciprocal hybrids. *In vitro* culture response was evaluated according to callus formation, percentage of regenerative calli and the number of plants per embryo. By crossing the cultivar Vesna (VS) with highest tissue culture response and the two cultivars with lowest response Leda (LD) and Zajecarska 65 (ZA), it was demonstrated that the regeneration potential was heritable. VS as female parent, enhanced regeneration response in hybrids VSxLD and VSxZA, while as a male parent, VS did not affect the regeneration ability of hybrids LD and ZA. However, hybrids having LD and ZA as a male parents exhibited a decreased regeneration potential, as compared to self-pollinated VS. The results suggest the presence of a class of extra-nuclear factors in the VS cultivar. They significantly account for relatively higher regeneration capacity in the hybrids having this cultivar as a female parent than in those where the VS was male parent.

*Key words:* *Triticum aestivum*, callus formation, *in vitro* regeneration potential, maternal effects, average dominance effects

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## INTRODUCTION

The most important prerequisite for applying *in vitro* techniques is the choice of lines and cultivars which possess good regeneration ability, or whose responsiveness could be improved. As in most crops, genetic factors are by far the most important for determining the regeneration potential in wheat (SEARS and DECKARD, 1982; MADDOCK *et al.*, 1983; MAËS *et al.*, 1996). The studies with reciprocal crosses indicated that in addition to nuclear genes, cytoplasmatic components might also exert a significant effect (MATHIAS *et al.*, 1986; OU *et al.*, 1989; BEBELI, 1995; BEN AMER and BÖRNER, 1997). As a part of long-term project on wheat genetic transformation, we found it necessary to investigate the tissue culture response of several wheat cultivars which represent potentially objects for genetic transformation. Callus formation and regeneration ability in immature embryos were studied. Since genotypic differences were evident, we further studied the transmission pattern of these traits in reciprocal crosses between the cultivars with high and low regeneration capacity.

## MATERIALS AND METHODS

Immature embryos were isolated from tree spring wheat cultivars: Vesna (VS), Leda (LD), and Zaječarska 65 (ZA), selected in the Center for Agricultural and technological Research in Zaječar, and two winter wheats, Beogradanka (BG) and Zemunka (ZM), obtained in Belgrade faculty of Agronomy (ANĐELKOVIĆ *et al.*, 1997). Immature caryopses were collected from plants grown in experimental field. All five cultivars were self-pollinated by hand and protected by paper bags till harvesting. Reciprocal crossing were performed in the same manner between spring cultivars in following combinations: VSxLD, LDxVS and VSxZA, ZA xVS. Immature seeds were collected 15 days after pollination and surface sterilized. Immature embryos, about 2 mm long, were isolated aseptically under stereomicroscope and cultured, with scutellar side up, on 20 ml of solid MS (MURASHIGE and SKOOG, 1962) medium containing 100 mg l<sup>-1</sup> casein hydrolysate, 30 g l<sup>-1</sup> sucrose, 0.45% agar (SIGMS Co.) and 2 mg l<sup>-1</sup> 2,4-dichlorophenoxyacetic acid (2,4-D). Obtained calli were subcultured on MS medium with decreased concentration of 2,4-D (0.5 mg l<sup>-1</sup>). Regenerated plants were transferred to the ½ MS medium without growth regulators. The pH of the media was adjusted to 5.8 prior to autoclaving, at 115°C for 20 min. The rooted plants were planted into pots with soil and acclimated in greenhouse. The *in vitro* response of five wheat cultivars and four hybrids was evaluated by measuring the following parameters: the percentage of developed calli, the percentage of regenerative calli, and the average number of plants per regenerating embryo.

The data obtained in three consecutive years represented three repetitions that were pooled together and analyzed using ANOVA and the random block system. The data expresses as percentage values were arcsin transformed. The statistical significance between the parameters of the groups was evaluated using the Tukey test (HADŽIVUKOVIĆ, 1991). For each tissue culture trait two features of the

transmission pattern were analyzed: 1) average dominance and 2) maternal effects. Maternal effects were inferred from the differences between reciprocal crosses, while average dominance effects were estimated from the differences between the pooled parents and pooled reciprocal crosses.

## RESULTS AND DISCUSSION

The *in vitro* responses of five wheat cultivars are presented in Table 1. All cultivars displayed a high potential for callus formation ranging from 79.0 to 91.7%, but the analysis of variance did not reveal a statistical significant differences in the mean value of this trait among cultivars. The five cultivars were classified into three groups, according to their regeneration capacity: a) VS with highest capacity, b) BG and ZM medium, and c) LD and ZA low. The Tukey test results corroborated this classification. The average number of plant regenerated per regenerating embryo also showed significant differences between the cultivar VS and the others (Table 1). In further experiments VS was crossed with the cultivars with lowest regeneration, LD and ZA, and *in vitro* response of hybrid embryos was assessed (Table 2).

Table 1. *In vitro* responses of immature embryos of five wheat cultivars; sample size (*n*), mean values (*X*) and standard errors (*SE*); different letters indicate significant differences ( $P \leq 0.05$ ) between cultivars, obtained using Tukey test

Cultivars	No. of cultivated embryos	Percent embryos forming calli	Percent of regenerable calli	No. of regenerated plants per embryo
	<i>n</i>	$\bar{X} \pm SE$	$\bar{X} \pm SE$	$\bar{X} \pm SE$
VS	216	91.2 $\pm$ 8.9a*	78.7 $\pm$ 0.5a	23.2 $\pm$ 2.4a
BG	126	87.3 $\pm$ 24.3a	55.3 $\pm$ 9.9b	10.9 $\pm$ 1.8b
ZM	120	86.3 $\pm$ 11.9a	50.3 $\pm$ 5.5b	12.6 $\pm$ 2.9b
LD	72	83.3 $\pm$ 6.8a	28.0 $\pm$ 1.5c	9.7 $\pm$ 0.2b
ZA	103	79.3 $\pm$ 14.7a	23.7 $\pm$ 2.7c	11.9 $\pm$ 4.1b

The mean percentage of callus formation was relatively higher in the cultivar VS than in its crosses, but all hybrid embryos also developed callus tissues in high percentage. Regeneration response of hybrid embryos was significantly increased, compared to LD and ZA, when VS was the maternal parent, although not to the level of self-pollinated VS. as a male parent, VS had no influence on regeneration ability in its hybrids with LD and ZA. On the other hand, in the crosses with LD and ZA as male parents the values on the mean percent of regenerable calli were intermediate between the two parental groups. As for the number of plants per embryo, the results were similar. VS as the maternal parent increased the average plant number per regenerating embryo in its hybrids, comparing to LD and ZA cultivars, but this effect was not observed when it was the male parent. The analyses of reciprocal crosses revealed that maternal effects significantly contrib-

uted to the phenotypic variation of the percent of regenerable calli and the number of regenerated plants per embryo in both crosses having VS as a seed-bearing plant (Table 2). In addition, an examination of the differences between the pooled parental cultivars and their pooled reciprocal crosses showed that significant dominance effects were exhibited for the number of plant per embryo in VSxLD hybrids, and for the percent of regenerable calli in the VSxZA crosses. Conversely, for all other traits, such as the percent of embryos forming calli, the percent of regenerable calli in VSxLD hybrids, and the number of regenerated plants per embryo in the VSxZA hybrids, there is no indication for directional dominance. This further suggests that additive gene effects were predominant components of genetic variance of these traits.

Table 2. Maternal and average dominance effects for in vitro embryo responses of wheat hybrids; P=significance levels taken from a Student's t-test of observed differences in trait means; s=significant at  $P < 0.05$ ; ns=non-significant

Trait	Maternal effects			Average dominance effect		
	VSxLD	LDxVS	P	Parental	Hybrids	P
Percent embryos forming calli	86.0 ± 5.0	85.0 ± 14.0	0.652ns	87.25	85.50	0.721ns
Percent of regenerable calli	63.0 ± 20.6	31.7 ± 2.1	1.000s	53.35	47.35	0.953ns
Regenerated plants/embryo	15.7 ± 1.6	13.3 ± 12.1	0.993s	16.50	14.50	0.958s
	'VS' x 'ZA'	'ZA' x 'VS'	P	Parental	Hybrids	P
Percent embryos forming calli	81.7 ± 1.4	80.0 ± 16.7	0.688ns	85.25	80.85	0.908ns
Percent of regenerable calli	57.0 ± 20.7	29.7 ± 14.9	0.999s	51.20	43.35	0.997s
Regenerated plants/embryo	17.7 ± 1.6	14.4 ± 0.8	0.999s	17.55	16.05	0.953ns

In conclusion, the cultivar VS was distinguished by its high regeneration potential with respect to all parameters measured. This feature seems to be due to maternal effects, transmitted to hybrids when VS was the female parent. While the male component of Vs did not contribute to a higher regeneration capacity, other male parents (LD and ZA) transmitted a negative effect to VS as female. The observed "hybrid inferiority" is likely caused by a breakage of coadapted gene complexes existing in parental cultivars. Therefore, the cultivar VS seems to be the most suitable for further work concerning in vitro wheat improvement. It may also

be useful as the female parent, when the response of other cultivars needs to be increased by hybridization.

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**NASLEĐIVANJE *IN VITRO* ODGOVORA KOD PŠENICE**

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## Izvod

Nezreli embrioni pšenice (*Triticum aestivum* L.) gejeni su u kulturi *in vitro* i proučavan je njihov *in vitro* odgovor, na osnovu procenata embriona koji formiraju kalus, procenta regenerativnih kalusa i broja biljaka dobijenih po regenerativnom embrionu. Proučavanja su obuhvatila pet domaćih sorti pšenice i njihove recipročne hibride. Formiranje kalusa i regeneracija su indukovani na podlogama sa visokom koncentracijom 2,4-D, a zatim bez regulatora rasta. Na osnovu procenta regenerativnih kalusa sorte su podeljene u tri grupe: Vesna (VS) sa visokim potencijalom (78.8%), Beograđanka (BG) i Zemunka (ZM) sa srednjim (50.3-55.3%), i Leda (LD) i Zaječarska 65 (ZA) sa niskim potencijalom (23.7-28.0%). Ukrštena je sorta sa najvišim potencijalom (VS) sa dvema sortama sa najnižim potencijalom (LD i ZA) i pokazano je da je potencijal za regeneraciju nasledna osobina. Kada je 'VS' bila ženski roditelj, hibridi "VSxLD" i "VSxZA" su imali znatno viši regenerativni odgovor, nego samooprašene "LD" i "ZA" sorte. Međutim, "LD" i "ZA" kao muške komponente su uticale na smanjenje regenerativnog odgovora kod hibrida sa "VS" sortom kao primaocem polena, u poređenju sa samooprašenom "VS". Rezultati ukazuju na postojanje klase ne-jedarnih činilaca (genetičkih i sredinskih) u kultivaru "VS". Oni značajno doprinose relativno višoj regenerativnoj sposobnosti kalusa hibrida čiji je "VS" ženski roditelj, u poređenju sa onima kod kojih je "VS" muški roditelj.

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