

# Influence of Zeatin on Wheat Regeneration from Immature Embryos

Snježana KEREŠA

Marijana BARIĆ

Hrvoje ŠARČEVIĆ

Jerko GUNJAČA

## SUMMARY

Immature embryo culture of nine Croatian winter wheat genotypes were performed in order to determine the influence of zeatin, as an exogenous growth regulator, on regeneration capacity of callus and number of regenerated shoots. Immature embryos 0.5-1.5 mm in size were aseptically isolated and plated with the scutellum exposed on modified MS medium containing MS salts and vitamins supplemented with 2 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid) and 30 g/l sucrose. For shoot initiation, calli were transferred on two different regeneration media: MSZ0 medium (MS salts and vitamins supplemented with 0.2 mg/l 2,4-D and 30 g/l sucrose) and MSZ5 medium (MS salts and vitamins supplemented with 0.2 mg/l 2,4-D, 5 mg/l zeatin and 30 g/l sucrose) and incubated at 26°C with 16/8 h light/dark photoperiod. Mean regeneration capacity of callus was higher on MSZ5 medium compared to MSZ0 medium (48.0% and 33.6% respectively). For all genotypes, except Lipa, regeneration capacity of callus was higher on MSZ5 medium. However, mean number of regenerated shoots was similar on MSZ5 compared to MSZ0 medium (1.6 and 1.3 respectively). The highest number of regenerated shoots per callus induced was found for genotype Žitarka (4.39), followed by genotypes Barbara (2.76), Lipa (2.61), line ZG1 (2.04) and Edita (1.67).

## KEY WORDS

embryo culture, medium, plant regeneration, winter wheat, *Triticum aestivum* L

Department of Plant Breeding, Genetics and Biometrics, Faculty of Agriculture  
University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia  
E-mail: skeres@agr.hr

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## INTRODUCTION

Plants regenerated from callus cultures after cell transformation or after changes caused by somaclonal variation could provide useful germplasm for plant breeding programs. However, selection at the callus level requires an effective system of initiating, maintaining and subsequently regenerating plants from callus. Establishing efficient tissue culture techniques has been more difficult in monocotyledonous species, particularly in *Gramineae* family, than in dicotyledons. In wheat, immature embryos and scutella have been the preferred tissues for transformation because they are the most reliable source for regenerating whole plants (Barcelo and Lazzeri 1995; Barro et al. 1999; Kereša et al., 2003). In addition, genotype and medium composition are important factors influencing embryogenic response and regeneration (Madock et al. 1983; Mathias and Simpson 1986). For callus induction, immature embryos should be placed on medium containing auxin, mostly 2,4-D (2,4-dichlorophenoxyacetic acid) or picloram. However, for shoot regeneration, some authors use media with cytokinins (Barro et al. 1999; He and Lazzeri 2001; Fennell et al. 1996), while others do not (Özgen et al. 1996; Cheng et al. 1997). Barro et al. (1999) found that cytokinin zeatin had clear positive effect on plant regeneration from immature inflorescences and used zeatin in concentration of 5 mg/l in (for wheat regeneration from immature embryos as well). The aim of this study was therefore to explore whether zeatin added as exogenous growth regulator has influence on regeneration capacity of callus and number of regenerated shoots of nine Croatian winter wheat (*Triticum aestivum* L.) genotypes.

## MATERIAL AND METHODS

Nine Croatian genotypes (Kuna, Banica, Lipa, Magdalen, Žitarka, Edita, Hana, Barbara and line ZG1) of winter wheat (*Triticum aestivum* L.) were grown in the field and used to collect immature embryos. Immature seeds, 14-18 days after anthesis, were surface sterilised with 70% ethanol for 5 min and 1.5% sodium hypochlorite solution with 0.1% Tween 20 for 30 min, followed by four changes of sterile distilled water. Immature embryos 0.5-1.5

mm in size were aseptically isolated from seeds under a stereo dissecting microscope and plated with the scutellum exposed onto modified MS medium (Murashige and Skoog 1962) containing MS salts and vitamins supplemented with 2 mg/l 2,4-D and 30 g/l sucrose. Embryos were reared in disposable plastic Petri dishes (90 x 15 mm). All media were solidified with 0.25% Gerlite and the pH adjusted to 5.8 prior to autoclaving. Numbers of calli induced were recorded after the incubation of the cultures at 26°C in the dark for 3 weeks. For shoot initiation, calli were transferred onto two different regeneration media: MSZ0 (MS salts and vitamins supplemented with 0.2 mg/l 2,4-D and 30 g/l sucrose) and MSZ5 (MS salts and vitamins supplemented with 0.2 mg/l 2,4-D, 5 mg/l zeatin and 30 g/l sucrose) and incubated at 26°C with 16/8 h light/dark photoperiod. Number of regenerable calli were counted after 4 weeks of culturing the calli on the shoot initiation medium, while the number of regenerated shoots per callus was calculated from 3rd till 6th week of culturing on the same medium. As regenerable calli were considered embryogenic calli on which shoots appeared, or which had green spots – potential spots for regeneration. Regeneration capacity of callus was assessed as the number of regenerable calli / number of calli induced x 100. For all traits one-way analysis of variance was performed.

## RESULTS

The first observed stage in the production of callus tissue was enlargement of the scutellar surface, followed by the production of embryogenic callus after 2-3 weeks. Genotype had significant influence on efficiency of callus induction (Tab. 1). Mean callus induction for all genotypes was 90.7% and varied among genotypes from 78.5% (Kuna) to 96.9% (Hana) (Fig. 1).

Not all of the calli produced were embryogenic, which accounts for the differences observed between the percentages of embryos producing calli and the proportion of calli producing shoots or having green spots (regenerable calli). For regeneration capacity of callus, genotype, medium, as well as their interaction were significant (Tab. 1). Mean regeneration capacity

Table 1. Results of ANOVA for three traits for nine wheat genotypes

Source of variability	df	F Value		
		Callus induction (%)	Regeneration capacity of callus (%)	Number of shoots regenerated per callus induced
Genotype	8	3.862**	9.65**	8.93**
Medium	1		12.57**	2.22 ns
Genotype x Medium	8		0.95**	0.032*

\* significant at p=0.05; \*\* significant at p=0.01; ns not significant

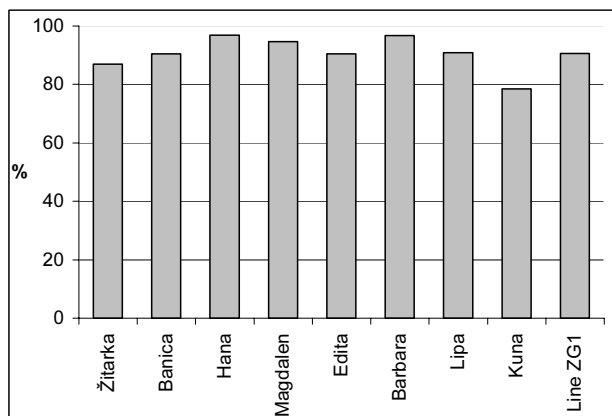


Figure 1. Callus induction for nine wheat genotypes

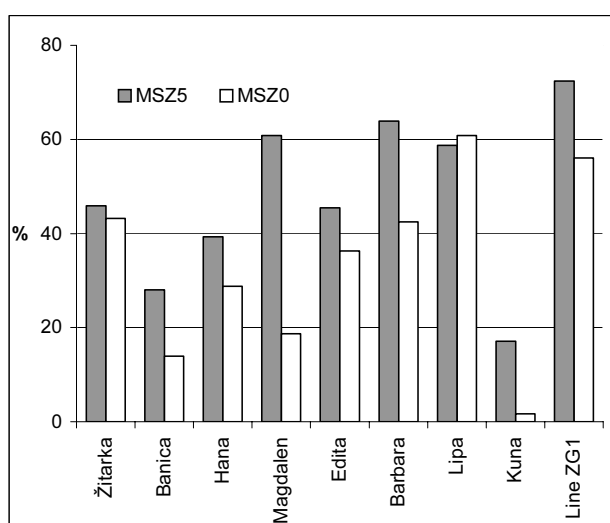


Figure 2. Regeneration capacity of callus (%) for nine wheat genotypes cultured on regeneration medium with (MSZ5) or without (MSZ0) zeatin.

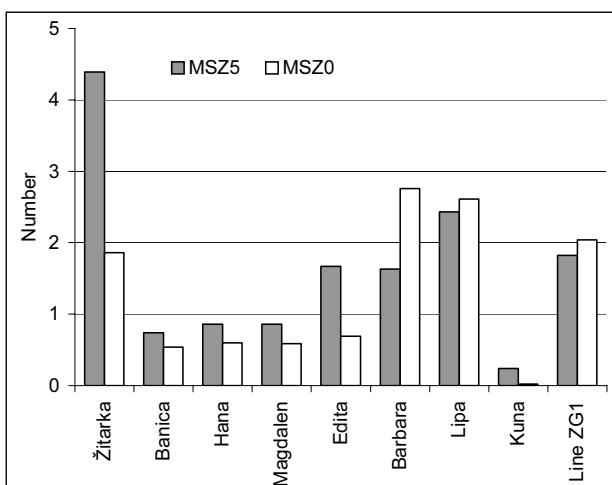


Figure 3. Number of shoots regenerated per number of calli induced for nine wheat genotypes cultured on regeneration medium with (MSZ5) or without (MSZ0) zeatin.

of callus across genotypes was higher on MSZ5 medium compared to MSZ0 medium (48.0% and 33.6% respectively). Regeneration capacity of callus on MSZ5 medium varied from 17.1% (Kuna) to 72.4% (ZG1) (Fig. 2). On the MSZ0 medium regeneration capacity of callus varied from 1.7% (Kuna) to 60.8% (Lipa). For all genotypes, except Lipa, regeneration capacity of callus was higher on MSZ5 medium. Genotypes Kuna and Magdalen showed few times lower regeneration capacity of callus on MSZ0 than on MSZ5 medium.

First shoots appeared on calli approximately 14 days after transferring the calli onto the regeneration medium. For the number of regenerated shoots per calli induced genotype and genotype x medium interaction were significant, while medium was not (Tab. 1). Number of regenerated shoots per calli induced varied on MSZ5 medium from 0.24 (Kuna) to 4.39 (Žitarka) (Fig. 3). On MSZ0 medium it varied from 0.02 (Kuna) to 2.76 (Barbara). Six genotypes (Žitarka, Banica, Hana, Magdalen, Edita and Kuna) produced higher number of shoots on MSZ5 medium, while three genotypes (Barbara, Lipa and ZG1) had higher number of shoots on MSZ0 medium. After six week on the shoot regeneration medium, calli reared on the MSZ5 medium were still viable producing shoots, while many of calli on MSZ0 medium become necrotic, specially for genotypes which showed low regeneration capacity of callus (Kuna, Banica and Magdalen).

DISCUSSION

After efficient callus induction and embryogenesis on auxin containing medium, shoot initiation requires low auxin content, or even auxin free medium. However, cytokinins included in regeneration medium could improve shoots regeneration. Some authors use 6-benzylaminopurine (BAP), others zeatin for that purpose. In the present study the addition of zeatin in regeneration medium significantly improved regeneration capacity of callus which is consistent with findings of Barro et al. (1999).

However, for the number of shoots regenerated per number of calli induced the advantage of the medium with zeatin was not detected. This is not strange, knowing that as regenerable calli were counted also calli with green spots from which shoots can regenerate, but this does not happen necessarily.

Final counting of the regenerated shoots in current investigation was done after six weeks of culturing on regeneration media, that is, after second passage on these media. Calli of genotypes grown on MSZ5 were viable and still producing shoots after this period, while many of calli on MSZ0 medium become necrotic. Similar was found by He and Lazzeri (2001) who noticed for durum wheat increased regeneration

frequency by the second passage on medium containing zeatin. Counting of the shoots for a longer time in current investigation would probably result in higher final number of regenerated shoots on MSZ5 medium. Remaining a long period of regeneration capability, behind the high regeneration efficiency, is very important for regeneration of transgenic plants or somaclonal variants.

Concerning the regeneration efficiency of particular genotype, the highest number of regenerated shoots per calli induced was found for genotype Žitarka (4.39), followed by genotypes Barbara (2.76), Lipa (2.61), line ZG1 (2.04) and Edita (1.67). These results are consistent with results of Kereša et al. (in press) where Žitarka, Edita and Lipa were found as the genotypes with the best regeneration capacity from immature embryos.

## CONCLUSION

According to presented results it is possible to conclude that regeneration medium containing zeatin improved regeneration capacity of callus and retained calli viable after six week of culturing. Genotypes showed different ability of callus induction, regeneration capacity of callus, and shoots regeneration.

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