

**ANDROGENOUS ABILITY OF HETEROZYGOUS WHEAT  
GENOTYPES AND CYTOLOGICAL CHARACTERISTICS  
OF GREEN REGENERANTS**

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Androgenous and regeneration abilities of 8 heterozygous wheat (*Triticum aestivum* L.) genotypes were analyzed in anther culture. Cytological characteristics of green regenerants were also analyzed. Significant differences were found among the genotypes in their androgenous and regeneration abilities. The average androgenous capacity of all genotypes was 4.4% ranging from 0.7% (Prima/Hueguen) to 11.0% (NS173-98/NS164-98). The average callus yield was 8.7%. The genotype Banks/F53-70 produced the highest number of green plants (33 green plants), while the genotype Banks/Pobeda showed no green plant regeneration ability. The frequency of albino plants ranged between 0% (Banks/Rodna) and 5.3% (Anastasija/Radika). A total of 61 green and 61 albino plants were regenerated. The results of cytological analysis showed that, from the total number of regenerated green plants, 40.9% were haploids and 59.1% were spontaneous double haploids.

*Key words:* androgenous ability, anther culture, cytological characteristics, *Triticum aestivum* L.

## INTRODUCTION

Most cereal breeding programmes are focused on producing superior homozygous genotypes and breeders, in conventional breeding, need many years for that. For this reason, they are interested in using methods that help them reach homozygosity rapidly.

Anther culture is one of the methods enabling the production of homozygous lines from heterozygous generations (most often from F<sub>1</sub>) within a short period of time (HENRY and DE BUYSER, 1990; ŠESEK *et al.*, 1994). The successful application of anther culture in wheat breeding programmes depends of many factors (KIM and BAENZIGER, 2005), but the most important one is a good androgenic response of genotypes (ŠESEK and KONDIĆ, 1996a).

This method is used for the production of haploid, spontaneous and induced double haploid plants from anthers in *in vitro* conditions. Haploid plants have one set of chromosomes in their cells and, therefore, express all their genetic traits. They are extremely valuable for genetic studies, plant breeding and genetic transformation experiments (CHU, 1996). Spontaneous chromosome doubling in wheat haploids may occur naturally. As result of that, spontaneous double haploids can be rise. These double haploid plants are used in selection as pure lines (ŠESEK and DENČIĆ, 1995; ZAMANI *et al.*, 2001).

The aims of this study were: to investigate the effect of genotype on androgenous and regeneration abilities of wheat and to detect the ploidy levels of regenerated green plants by cytological analysis.

## MATERIAL AND METHOD

Eight F<sub>1</sub> combinations of wheat (*Triticum aestivum* L.) were used in this experiment. Donor plants were grown under field conditions. The sampled spikes were exposed to cold pretreatment at 4°C for 5-10 days. Surface sterilization of the material was carried out according to the standard procedure (ŠESEK *et al.*, 1994). From each genotype, 600 anthers were isolated and placed onto a modified Potato-2 inductive medium (CHUANG *et al.*, 1978). The anthers were grown at 28-30°C in the dark. After 4-6 weeks, the calli were transferred onto the 190-2 regeneration medium (ZHUANG and JIA, 1980) and cultured with 16 h of photoperiod at 25-27°C. After approximately three weeks, green regenerants were transferred onto rooting medium and cultured under the same conditions (ŠESEK, 1989).

The traits studied were: androgenous capacity (number of responding anthers per 100 isolated anthers), callus yield (number of calli formed per 100 isolated anthers) and regeneration ability traits - frequency of green plants (number of regenerated green plants per 100 isolated anthers) and frequency of albino plants (number of regenerated albino plants per 100 isolated anthers).

Top of the root from each green regenerant was taken before their transfer to the flowerpot. Cytological analysis were done as described by PETROVIĆ and VUČKOVIĆ (1992).

The percentages of haploid and double haploid plants were determined by cytological analysis. Identified spontaneous double haploids were vernalized and grown first in the greenhouse.

The experimental data were statistically analyzed by ANOVA and the significance of differences between the values was determined by LSD test. The STATISTICA 7.1 computer program was used for statistical analysis.

## RESULTS AND DISCUSSION

Significant differences in androgenous and regeneration abilities were found between investigated heterozygous wheat genotypes. The genotype Prima/Hueguen had the lowest androgenous capacity and callus yield, both at 0.7%. The line NS173-98/NS164-98 had the highest androgenous capacity (11.0%) (Table 1). The highest callus yield of 20.3% was found in the genotype Anastasija/Radika. The average values of androgenous capacity and callus yield for all the genotypes were 4.4% and 8.7%, respectively (Table 1.).

*Table 1. - Anther response of eight heterozygous wheat genotypes*

Genotype	Androgenous capacity (%)	Callus yield (%)	Regeneration ability	
			Green plants (%)	Albino plants (%)
Prima/Hueguen	0.7	0.7	0.2	0.3
NS173-98/NS164-98	11.0	18.7	0.7	1.0
Mexico-3/Radika	4.3	7.0	2.2	2.0
Anastasija/Radika	9.8	20.3	0.7	5.3
Banks/PinzonInta	1.2	1.3	0.8	0.8
Banks/F53-70	4.3	14.7	5.5	0.5
Banks/Pobeda	1.3	3.5	-	0.2
Banks/Rodna	2.2	3.2	0.2	-
Mean	4.4	8.7	1.3	1.3

The frequency of green and albino plants produced was generally low. The average values of green and albino plants regeneration abilities for all genotypes were 1.3% in both cases (Table 1). The genotype Banks/F53-70 exhibited the best performance in anther culture (5.5 green plants per 100 isolated anthers). While the genotype Banks/Rodna produced only green plants, the genotype Banks/Pobeda showed no green plant regeneration ability. The highest number of albino plants per 100 isolated anthers was observed in the genotype Anastasija/Radika (5.3%) (Table 1.). A total of 61 green and 61 albino plants were regenerated. Twenty-one fertile green plants survived until maturity.

The results of the present study indicate that the genotype plays an important role in anther culture, which is in accordance with previous reports (ŠESEK and KONDIĆ, 1996a; ZAMANI *et al.*, 2003).

The ploidy levels of green plants was determined by cytological analysis. The results have shown that, out of the 61 green plants regenerated, 40.9% were haploids and 59.1% were spontaneous double haploids. SLUSARKIEWICZ-JARZINA and PONTIKA (2003.) obtained similar results in *Triticale*. The highest percentage of haploid plants was produced by the genotype Banks/F53-70 (51.5%). All green regenerants of the genotypes Prima/Hueguen and Banks/Rodna were spontaneous double haploids (100%) (Fig. 1). No other levels of ploidy were found, which is in agreement with the results of other authors (AHMED *et al.*, 1999; SLUSARKIEWICZ-JARZINA and PONTIKA, 2003).

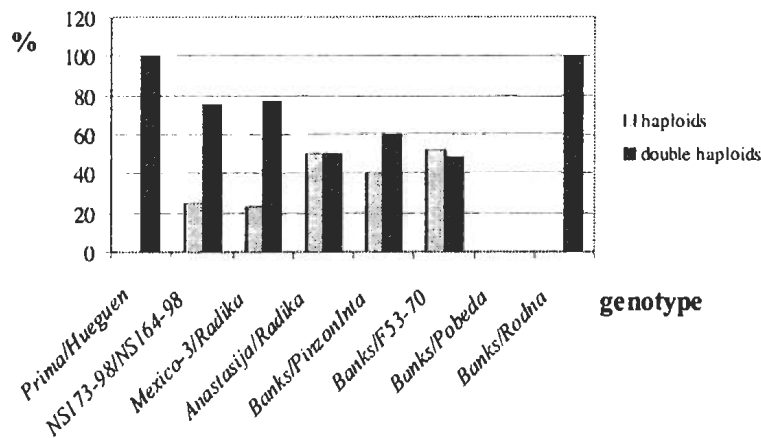


Fig. 1. - Cytological analysis of regenerated green plants obtained from wheat anther culture

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**ANDROGENEZA HETEROZIGOTNIH GENOTIPOVA PŠENICE I CITOLOŠKE  
KARAKTERISTIKE ZELENH REGENERANATA**

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**I z v o d**

Analizirana je androgena i regeneraciona sposobnost kod 8 heterozigotnih genotipova pšenice (*Triticum aestivum* L.) u kulturi antera. Urađena je i citološka analiza regenerisanih zelenih biljaka. Utvrđene su statistički značajne razlike između ispitivanih genotipova u pogledu androgene i regeneracione sposobnosti. Prosečna androgena sposobnost za sve ispitivane genotipove iznosila je 4,4%, a kretala se od 0,7% kod genotipa Prima/Hueguen do 11,0% kod genotipa NS173-98/NS164-98. Prosečan prinos kalusa bio je 8,7%. Najviše zelenih biljaka dao je genotip Banks/F53-70 (33 zelene biljke) dok genotip Banks/Pobeda nije regenerisao ni jednu zelenu biljku. Frekvencija albino biljaka kretala se između 0% (Banks/Rodna) i 5,3% (Anastasija/Radika). Ukupno je regenerisano 61 zelena i 61 albino biljka. Rezultati citološke analize pokazali su da su, od ukupnog broja regenerisanih zelenih biljaka, 40,9% bili haploidi, a 59,1% spontani dvostruki haploidi.

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