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HIGH PRODUCTION OF WHEAT DOUBLE HAPLOIDS VIA ANTHER CULTURE

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Androgenous and regeneration abilities of 14 randomly selected F_1 hybrids of wheat (*Triticum aestivum* L.) were analyzed. Anthers were grown *in vitro* on a modified Potato-2 inductive medium.

The hybrid NS111-95/Ana had the highest average values for androgenous capacity (33%) and callus yield (119%), while the hybrid NS 92-250/Tiha had the lowest values for these traits (9 and 21%, respectively).

Seven genotypes (50%) had a frequency of green plants relative to the number of isolated anthers of over 10%, with the highest frequency of 21.3% (NS111-95/Sremica). This hybrid produced 12.8 doubled haploid (DH) lines per spike used for isolation. In the other genotypes, the number of produced DH lines per spike ranged from 1 (30-Sc.Smoc.88-89/Hays-2) to 11.2 (NS111-95/Ana).

As half of the randomly selected genotypes exhibited high green plant regeneration ability and a high production of DH lines per spike, it

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can be concluded that *in vitro* anther culture can be successfully used in breeding programs for rapid production of homozygous wheat lines.

Key word: anther culture, doubled haploid, wheat

INTRODUCTION

The anther culture is one of the most efficient methods for the production of haploids and homozygous doubled haploids. However, wheat was known as a recalcitrant species with regard to *in vitro* androgenesis techniques such as anther and microspore culture. In general, many wheat genotypes, especially when they were randomly selected, respond poorly. In particular green plant regeneration is low, either because regeneration of albino plantlets occurs or no regeneration at all (LJEVNAIĆ, 2006).

Studies concerning the wheat anther culture technique produced significant results and it is now possible to use this technique for haploid breeding, selection, various genetic and physiological studies (WANG *et al*., 2002; SALOMON *et al*., 2003; TYANKOVA *et al*., 2004;).

Using the combination of this technique with the conventional breeding methods commercial wheat cultivars have already been developed and released (KERTESZ *et al*., 2001; SADASIVAIAH *et al*., 2004; WEYEN, 2006).

The objective of this study was to prove that this method could produce a sufficient number of DH plants for its successful use as an additional method in wheat breeding. In order to do this, we studied the androgenic and regeneration abilities of 14 randomly selected wheat genotypes, i.e. we studied their androgenic capacity, callus yield, frequency of green regenerates, and the number of DH plants per spike.

MATERIAL AND METHODS

In this study 14 randomly selected F_1 wheat hybrids were used as a material for anther isolation. The material was produced at the experimental fields of the Small Grains Department of the Institute of Field and Vegetable Crops in Novi Sad.

Donor plants were grown under field conditions. Spikes were taken at the middle uninuclear stage of microsporogenesis, which is considered the optimum time for isolation. After a low temperature pre-treatment, the sterilization of the material was carried out and the anthers were isolated under aseptic conditions.

The Potato-2 inductive nutrient medium (CHUANG *et al*., 1978) was used for callus induction. Plant regeneration from formed embryogenic calluses was performed on the modified 190-2 (ZHUANG and JIA, 1980) medium. For the development of the root system a semi-solid agar medium was used, which also contained the 190-2 mineral solution. The only difference between this medium and the one used for plant regeneration was that in this one the concentration of

auxins and cytokinines was reduced from 0.5 to 0.1 mgl^{-1}.

Plants that had a well-developed root system were transplanted into containers with the sterilized substrate. After acclimatization and vernalization periods, further plant growth and development until full maturity took place under field conditions. The plants of the H_1 generation were harvested in early July.

During the study the following traits were analyzed:

- anrdogenous capacity (no of androgenous anthers per 100 cultivated anthers)
- callus yield (no of calluses per 100 anthers)
- frequency of green regenerants (no of green plants per 100 anthers)
- success (no of DH plants per spike)

RESULTS AND DISCUSSION

The results on the androgenic capacity show that all the genotypes had the ability to form callus tissue by anther growing in the in vitro culture. The androgenic capacity ranged from 9% in combination NS 92-205/Tiha to 33% in NS 111-95/Ana. The average androgenic capacity of all the combinations was 17.7% (Table 1), which is a high value considering that the genotypes was selected randomly.

Other authors who also used a randomly selected genotypes obtained lower androgenic capacity values: ZAMANI et al. (2003) – 6.83%; LJEVNAIĆ (2006) – 5.78%; TERSI et al. (2006) – 6.62%. Higher values are reported mostly in studies in which genotypes were not selected randomly but because they were known to have high values of certain androgenesis components. Using this approach, BARNABAS et al. (1991) obtained an average androgenic capacity of 20.3%, while BRUINS and SNIJDERS (1995) and KIM and BAENZIGER (2005) report obtaining 23.0 and 18.0%, respectively.

In all of the genotypes, around 80-90% of androgenic anthers formed a number of calluses, while the rest of the anthers formed only a single callus each. For this reason, the highest callus yield (found in the genotype NS 111-95/Ana) exceeds 100% (119), since each anther produced more than one callus. The lowest callus yield (21%) was found in NS 111-95/Tiha (Table 1).

The average callus yield in the experiment as a whole was 50%, which is close to the results obtained by BARNABAS *et al.* (1991) -41%, HE *et al.* (1993) – 57.4% and MARCINIAK *et al.* (2003) –45%. Lower values for callus yield were reported by BELCHEV *et al.* (2000) –23.5% and LJEVNAIĆ (2006) -8.88%.

The regeneration ability was found in all of the genotypes to a lesser or greater extent. A total of 407 green plants originating from F_1 microspores were produced during the study. The frequency of green regenerants ranged from 1.3% in 30-Sc.Smoc.88-89/Hays2 to 21.3% in NS 111-95/Sremica (Table 1). Looking at all the regenerants taken together, the average frequency was 9.7%.

Table 1. Androgenous and regeneration abilities of 14 randomly selected F_1 wheat hybrids.

Genotype	Androgenous capacity (%)	Callus yield (%)	Green plants (%)	Success (DH plants/ spike)
Mex.3/Tiha	29.0	69.7	13.0	7.8
Mex.3/NS 55-25	28.3	67.0	14.3	8.4
NS 95-95/Tiha	12.0	22.3	5.0	3.0
NS 95-95/NSP 11	29.7	92.7	2.3	1.4
NS 111-95/Tiha	17.7	21.0	12.0	7.2
NS 111-95/Renesansa	11.0	25.0	6.3	3.8
NS 111-95/Ana	33.0	119.0	18.7	11.2
NS 111-95/Sremica	26.3	54.0	21.3	12.8
CHI 6/Tiha	11.0	27.7	2.3	1.4
CHI 6/Sremica	15.3	30.0	2.0	1.2
NS 38-93/Rusija	12.3	35.0	16.0	9.6
NS 38-93/Kosuta	29.3	82.7	13.0	7.8
NS 92-205/Tiha	9.0	32.0	8.3	5.0
30-Sc.Smoc.88-89/Hays2	13.3	33.7	1.3	1.0
F_1 (mean)	17.7	50.8	9.7	5.8

Similar results were obtained by EKIZ and KONZAK (1994) - 13.7 green plants per 100 isolated anthers, and KIM and BAENZIGER (2005) -10 green plants per 100 anthers. Lower averages than this were obtained by HOLME *et al*. (1999) – 2%; TUVESSON *et al*. (2000) –3.3%; TERSI *et al*. (2006) –3.65%.

The number of DH plants ranged from 1 to 12.8, averaging 5.8 (Table 1). It should be emphasized that eight of the 14 genotypes, had five or more regenerated DH plants per spike. This can be considered a very good result, since the numbers reported in the literature are by and large significantly lower. ŠESEK (1989), for example, obtained 1.8 DH plants per spike on average, while TUVESSON *et al*. (2000) got 2.1 DH plants per spike.

Significant differences between our and results of other authors could be explained using different genetic material, number of genotypes, methods of sampling, growing conditions of donor plants, culture conditions, inductive media, etc.

CONCLUSION

According to the fact that more than 10 DH plants per spike were produced in some of the genotypes it can be concluded that wheat anther culture technique is effective enough to be used successfully as an additional method to supplement the classical wheat breeding methods. Low susceptible seedlings which

also have some qualitative characteristics will be use as raw material to obtain quality peach genotypes, low susceptible to leaf curl pathogen.

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VISOKA PRODUKCIJA DVOSTRUKIH HAPLOIDA PŠENICE KORIŠĆENJEM KULTURE ANTERA

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I z v o d

Analizirana je androgena i regeneraciona sposobnost 14 slučajno odabranih F_1 hibrida pšenice (*Triticum aestivum* L.). Antere su gajene u *in vitro* uslovima na modifikovanoj Potatao-2 podlozi.

Hibrid NS111-95/Ana imao je najviše prosečne vrednosti za androgeni kapacitet (33%) i prinos kalusa (119%), dok je hibrid NS 92-250/Tiha imao najniže vrednosti za ove osobine (9% i 21%).

Sedam genotipova (50%) imalo je frekvenciju zelenih biljaka u odnosu na broj izolovanih antera preko 10%, sa najvišom vrednošću od 21.3% (NS11-95/Sremica). Kod istog hibrida (NS11-95/Sremica) proizvedeno je 12.8 linija dvostrukih haploida (DH) po klasu, korišćenom za izolaciju. Kod drugih genotipova, broj proizvedenih DH linija po klasu varirao je od 1 (30-Sc.Smoc.88-89/Hays-2) do 11.2 (NS111-95/Ana).

S obzirom na to da je polovina slučajno odabranih genotipova ispoljila visoku sposobnost za regeneraciju zelenih biljaka i visoku produkciju DH linija po klasu, *in vitro* kultura antera može se uspešno koristiti u oplemenjivačkim programima za brzu proizvodnju homozigotnih linija pšenice.

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