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Genotipic Specificity of F_1 Wheat Hybrids in Doubled Haploid Production via Anthers Cultures

- Original scientific paper -

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Abstract: The objective of the study was to investigate the production of homozygous doubled haploid (DH) lines from heterozygous genotypes of wheat (*Triticum aestivum* L.) using an anther culture. Anthers were isolated from 20 randomly selected F_1 wheat combinations and grown *in vitro* on a modified Potato-2 inductive medium. Plant regeneration was performed on the modified 190-2 medium.

The results have shown that all genotypes had the ability to produce pollen calli, as well as, to regenerate green plants. The hybrid Mex.3 x MV 18 had the highest value for androgenous capacity (40.6%), while the combination Kutječanka x Slavija had the lowest androgenous capacity (6.3%). The average androgenous capacity of all genotypes was 19.4%. The regeneration frequency ranged from 1.3 to 21.6 green plants per 100 isolated anthers, with an overall mean of 9.6%. Out of a total of 582 regenerated green plants 279 (47.9%) were spontaneous double haploids. The average production of double haploids of wheat in this study amounted to 4.6 per 100 cultured anthers.

Key words: Androgenesis, double haploid, *in vitro*, wheat.

Introduction

Wheat anther cultures have a history of more than 30 years and are now employed efficiently in many countries of the world for the development of doubled haploid (DH) lines for breeding, ***Barnabas et al.***, 2001. An efficient DH technology can greatly reduce the time and the cost of the cultivar development, ***Šesek et al.***, 1994, ***Kertész et al.***, 2001, ***Kondić-Špika*** and ***Šesek***, 2002, ***Kim*** and ***Baenziger***, 2005.

The anther culture method, in addition to wide hybridisation, is a common way to produce DH plants in cereals, ***Kiviharju et al.***, 2005. Microspore and anther

culture methods have the potential to produce more than a thousand haploid plants per cultured anther; all other methods are limited to one haploid plant per floret. However, androgenesis induction, which to a great extent depends on a genotype, may be affected by various factors which cause a low induction efficiency, ***Dunwell***, 1985. Also, low regeneration rates have restricted the use of this potentially powerful technique, ***Liu et al***., 2002.

Studies concerning the wheat anther culture produced significant results and it is now possible to use this technique for haploid breeding, selection, various genetic and physiological studies, ***Tyankova et al***., 2004, ***Ljevnaić***, 2007. The objective of the study was to investigate the production of homozygous DH lines, using the anther culture technique, from F_1 wheat combinations developed through breeding programmes of Institute of Field and Vegetable Crops, Novi Sad.

Material and Methods

Twenty randomly selected F_1 combinations of winter wheat (*Triticum aestivum* L.) were used as the plant material in this study. F_1 combinations were produced in the trial fields of the Institute at Rimski Šančevi, within a breeding programme of the Department of Small Grains. The combinations were randomly selected according to their androgenic ability. As androgenic abilities of parent genotypes were not known it was not possible to estimate androgenic ability of the selected combinations. The F_1 combinations were selected by breeders for their agronomic rather than androgenic traits.

Anther-donor plants were grown under field conditions following the conventional sowing and growing methodology for the breeding material. The sampled spikes were cold pretreated at 4-6º C for 5-10 days. After sterilisation in 1.3% NaOCl, anthers in the mid- or late uninucleate stage of microsporogenesis were isolated. Three hundred anthers from each combination were isolated and transferred in tubes (25 x 100 mm) with the modified potato-2 induction medium, ***Chuang et al***., 1978, (30 anthers per tube). Ten tubes totalling 300 anthers per combination were placed in a culture room according to the randomised block system. During their cultivation on the induction medium, the anthers were grown in the dark at 28-30ºC.

After four to six weeks of growing on the induction medium, the developed embryogenic calli were transferred to the modified 190-2 regeneration medium, ***Zhuang*** and ***Jia***, 1980, and cultured at 25-27ºC, with a 16/8 hr photoperiod and 2500-3000 lx light intensity. After approximately three weeks, calli with green shoots were transferred to a rooting medium and cultured under the same conditions. For the development of the root system, a semi-solid (5 g l^{-1} 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 the fact that one the concentration of auxins and cytokinines in this medium was reduced from 0.5 to 0.1 mgl^{-1}.

Plants that had a well-developed root system were transplanted into containers with the sterilised substrate, consisting of garden soil, compost and sand in

a 3:2:1 ratio. Prior to transferring, five to six root tips were taken from each plant and checked for a chromosome number by the standard acetocarmine squash method, described for the anther culture by ***Šesek***, 1995. After acclimatisation and vernalisation 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 analysed:
- androgenous capacity (number of androgenous anthers per 100 cultivated anthers)
- frequency of green plants (number of green plants per 100 anthers)
- frequency of DH plants (number of DH plants per 100 anthers).

Results and Discussion

The results on the androgenic capacity have shown that all the genotypes had the ability to form calli by growing anthers in the *in vitro* culture. The androgenic capacity ranged from 6.3% in combination Kutječanka x Slavija to 40.6% in Mex.3 x MV 18. The average androgenic capacity of all the genotypes was 19.4% (Table 1).

Orlov et al., 1993, proposed a 4%-level of the responding anthers as a criterion for genotype fitness in common wheat breeding. In this experiment, all studied genotypes exceeded this level. In our previous experiment, ***Kondić*** and ***Šesek***, 1999, with 10 randomly selected heterozygous wheat genotypes, 13.8% of anthers were responsive. Similar results (18.0% of responding anthers) were obtained by ***Kim*** and ***Baenziger***, 2005, but in their study genotypes were not selected randomly.

The regeneration ability was found in all of the genotypes. A total of 582 green plants originating from 6,000 isolated F_1 microspores were produced during the study. The frequency of green plants produced per 100 cultured anthers varied from 1.3% (30-Sc.Smoc.88-89 x Hays2) to 21.6% (Mex.3 x MV 18), with an overall mean of 9.6% (Table 1).

According to ***Šesek*** and ***Denčić***, 1996, the anther culture technique can be successfully utilised in genotypes with the ability of green plant regeneration at 2.5% or more. In our experiment, 80% of the genotypes tested had this or higher level of green plant regeneration ability.

The studies with a large number of tested wheat genotypes show that this number of homozygous green plants is obtainable in general, which makes the anther culture technique applicable in wheat breeding. ***Šesek*** and ***Denčić***, 1996, testing 160 heterozygous wheat genotypes obtained on average 2.3 green plants per 100 cultured anthers. However, an average green plants frequency in responsive genotypes (68%) amounted to 3.4%. ***Tuvesson et al***., 2000, in their screening study with 91 F_1 wheat combinations obtained very promising results: 5.6 green plants per 100 anthers and 4.7 green plants per spike.

Out of a total of 582 regenerated green plants 279 (47.9%) were spontaneous DHs. The number of DH plants ranged from 0 to 14.3 per 100 anthers. The final average

Table 1. Androgenous and Regeneration Abilities of F_1 Wheat Combinations
Androgena i regeneraciona sposobnost F_1 kombinacija pšenice

F_1 combination F_1 kombinacija	Androgenous capacity Androgeni kapacitet	Frequency of green plants Frekvencija zelenih biljaka	Frequency of DH plants Frekvencija DH biljaka
	(%)	(%)	(%)
Ana x NS 0-691	15.0	7.9	6.3
Balkan x Košuta	28.2	5.0	2.7
CHI 6 x Tiha	11.0	2.3	0.3
CHI 6 x Sremica	15.3	2.0	0.7
Kutječanka x Slavija	6.3	5.7	3.7
Mex.3 x Tiha	29.0	13.0	5.3
Mex.3 x NS 55-25	28.3	14.3	7.0
Mex.3 x MV 18	40.6	21.6	14.3
NS 33-90 x Fawwon-138	9.0	8.7	4.7
NS 38-93 x Rusija	12.3	16.0	5.0
NS 38-93 x Košuta	29.3	13.0	4.0
NS 92-205 x Tiha	9.0	8.3	3.0
NS 95-95 x Tiha	12.0	5.0	2.0
NS 95-95 x NSP 11	29.7	2.3	0.7
NS 111-95 x Tiha	17.7	12.0	4.7
NS 111-95 x Renesansa	11.0	6.3	2.7
NS 111-95 x Ana	33.0	18.7	8.7
NS 111-95 x Sremica	26.3	21.3	13.0
NSP 41 x NS 0-649	12.3	7.3	4.3
30-Sc.Smoc.88-89 x Hays2	13.3	1.3	-
F_1 (mean/prosek)	19.4	9.6	4.6
LSD 0.05	2.06	1.18	0.74
LSD 0.01	2.76	1.58	0.99

yield obtained in the study was 4.6 DH plants per 100 cultured anthers (Table 1).

Some authors considered that the wheat x maize hybridisation technique is more effective in DH production than the anther culture method, ***Fedak et al***., 1997, ***Sadasivaiah et al***., 1999, ***Singh et al***., 2001. ***Sadasivaiah et al***., 1999, used this hybridisation technique and reported that 6.29 haploid plants had been produced per 100 pollinated florets. In the present study, 9.6 green plants per 100 isolated anthers were produced, which is a significantly higher result than the one obtained by ***Sadasivaiah et al***., 1999. Also, 42.9% of produced green plants in anther the were spontaneous double haploids, which did not need colchicine treatment for chromosome doubling.

Conclusion

In this study, 4.6 DH plants per 100 anthers were produced from combinations with unknown androgenic ability. This number of DH plants can be considered as sufficient for the successful use of the anther culture as an additional method in wheat breeding programs. Also, it is possible to double this efficiency by treating haploid plants with colchicine, since more than a half of regenerated plants were haploids.

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Genotipska specifičnost F_1 hibrida pšenice u pogledu proizvodnje dvostrukih haploida metodom kulture antera

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R e z i m e

Cilj rada bio je da se ispita mogućnost proizvodnje homozigotnih linija dvostrukih haploida (DH) iz heterozigotnih genotipova pšenice (*Triticum aestivum* L.) korišćenjem kulture antera. Antere su izolovane iz 20 slučajno odabranih F_1 kombinacija pšenice i gajene u kulturi *in vitro* na modifikovanoj potato-2 indukcionoj podlozi. Regeneracija biljaka vršena je na modifikovanoj 190-2 podlozi.

Rezultati su pokazali da su svi genotipovi posedovali sposobnost da proizvedu kaluse iz polena, kao i da regenerišu zelene biljke. Hibrid Mex.3 x MV 18 je imao najvišu vrednost za androgeni kapacitet (40,6%), dok je kombinacija Kutječanka x Slavija imala najniži androgeni kapacitet (6,3%). Prosečan androgeni kapacitet za sve genotipove bio je 19,4%. Frekvencija za regeneraciju zelenih biljaka kretala se od 1,3 do 21,6 zelenih biljaka na 100 izolovanih antera, sa prosekom od 9,6%. Od ukupno 582 regenerisane zelene biljke, 279 (47,9%) su bile spontani dvostruki haploidi. U ovom istraživanju prosečno je proizvedeno 4,6 dvostrukih haploida pšenice na 100 izolovanih antera.

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