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EFFICIENCY OF ANTHER CULTURE TECHNIQUE IN THE PRODUCTION OF WHEAT DOUBLE HAPLOIDS

ABSTRACT: The objective of the study was to investigate efficiency of anther culture in the production of spontaneous double haploids from randomly selected heterozygous genotypes of wheat (*Triticum aestivum* L.). Anthers of 20 F_1 wheat combinations were grown *in vitro* on a modified Potato-2 medium.

All of the examined genotypes have shown the ability to produce pollen calluses as well as to regenerate green plants. On average for the whole experiment material, 47.2 calluses were produced per 100 cultured anthers. The green plant regeneration ranged from 0.8 to 13.4 green plants per spike, with an overall mean of 5.8. From the total of 582 regenerated green plants, 47.9% (279) were spontaneous double haploids. The final average yield from the study was 2.8 double haploids per spike.

KEY WORDS: androgenesis, double haploid, *in vitro*, *Triticum aestivum* L.

INTRODUCTION

Double haploid techniques provide plant breeders with pure lines in a single generation, which may save considerable time in the breeding of new cultivars. There are two major techniques for haploid production in cereals — anther/microspore culture and chromosome elimination using wide hybridizations. The former technique is usually considered as simpler, more efficient, and more cost-effective than the latter (Ljevnaić, 2007).

The most important advantage of the anther culture method is occurrence of spontaneous chromosome doubling which results in production of homozygous DHs. Those spontaneous DHs are fertile and cytologically stable, except for a small percentage of them, which exhibit chromosomal abnormalities (Ahmed et al., 1999). However, the frequency of spontaneous DH plants depends of several factors and it is usually low (Armstrong et al., 1987; Ahmed et al., 1999).

Wheat in particular is known as a recalcitrant species with regard to *in vitro* androgenesis techniques such as anther and microspore culture. Use of anther culture in wheat breeding programs is limited by strong genotype specificity, low frequency of haploids, and a high rate of albinism in regenerants (Kisana et al., 1993; Sadasivaiah et al., 1999). Despite these problems, the anther culture technique has been successfully applied in some wheat breeding programs, resulting in new cultivars (Pauk et al., 1995; Kertesz et al., 2001; Sadasivaiah et al., 2004).

It is known that anther culture response is highly genotype-specific (Orlov et al., 1993; Moieni and Sarafi, 1995; Kondić and Šesek, 1999) and typically, it would produce many individuals from only a few selected crosses. One suggestion has been to use the anther culture technique only for breeding combinations in which at least one parent line is highly responsive (Zhou and Konzak, 1992; Tuvesson et al., 2000). The goal of this study was to investigate if it is possible to produce a large number of DHs from randomly selected wheat breeding combinations using the anther culture method.

MATERIAL AND METHODS

In this study, 20 randomly selected F_1 wheat combinations were used for anthers isolation. The breeding 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. Five spikes were taken from each combination at the mid- or late uninuclear stage of microsporogenesis. After a temperature pre-treatment, sterilization of the material was carried out as described in Šesek and Denčić (1996) and anthers were isolated under aseptic conditions.

The Potato-2 inductive nutrient medium (Chuang et al., 1978) was used for callus induction. During their culturing on the inductive medium, anthers were kept in the dark and at 28—30°C. Plant regeneration from formed embryogenic calluses was performed on the modified 190-2 medium (Zhuang and Jia, 1980). This medium contained 190-2 mineral salt solution as well as some other components, namely (in gl^{-1}) agar (5), sucrose (30), and (in mgl^{-1}) glycine (2), thiamine-HCl (1), pyridoxine-HCl (0.5), nicotinic acid (0.5), myo-inositol (100), NAA (0.5) and kinetin (0.5). When green shoots reached 5—10 mm after approximately three weeks, calluses with green shoots were transferred to the rooting medium. A semi-solid agar medium was used for the development of the root system. It 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 NAA and kinetin was reduced from 0.5 to 0.1 mgl^{-1}. During the plant regeneration and root development period, the temperature in the growing chamber was maintained at 25—27°C. The intensity of white fluorescent illumination was 2500—3000 lx, with a photoperiod of 14 hours of light.

Plants that had a well-developed root system were transplanted into containers with the sterilized substrate. Prior to transplanting, five to six root tips were taken from each plant and checked for chromosome number by the standard acetocarmine squash method. 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:

— callus yield (CY — no. of calluses per 100 anthers)
— green plants yield (GP — no. of green plants per spike)
— DH plants yield (DH — no. of DH plants per spike)

RESULTS AND DISCUSSION

All genotypes had the ability to form callus tissue by growing anthers in the *in vitro* culture. The highest callus yield (found in the combination NS 111-95/Ana) exceeds 100% (119%), since each androgenic anther produced more than one callus. The lowest callus yield (21%) was found in NS 111-95/ Tiha (Tab. 1).

Tab. 1 — Callus yield and numbers of green and DH plants per spike of 20 randomly selected F_1 wheat combinations

Genotype		CY (%)	GP (No.)	GP/ spike	DH (No.)	DH/ spike
Ana/NS 0-691		35.9	27	5.4	19	3.8
Balkan/Košuta		34.3	15	3.0	8	1.6
CHI 6/Tiha		27.7	7	1.4	1	0.2
CHI 6/Sremica		30.0	6	1.2	2	0.4
Kutječanka/Slavija		26.7	17	3.4	11	2.2
Mex.3/Tiha		69.7	39	7.8	16	3.2
Mex.3/NS 55-25		67.0	43	8.6	21	4.2
Mex.3/MV 18		85.1	67	13.4	43	8.6
NS 33-90/Fawwon-138		29.0	26	5.2	14	2.8
NS 38-93/Rusija		35.0	48	9.6	15	3.0
NS 38-93/Košuta		82.7	39	7.8	12	2.4
NS 92-205/Tiha		32.0	25	5.0	9	1.8
NS 95-95/Tiha		22.3	15	3.0	6	1.2
NS 95-95/NSP 11		92.7	7	1.4	2	0.4
NS 111-95/Tiha		21.0	36	7.2	14	2.8
NS 111-95/Renesansa		25.0	19	3.8	8	1.6
NS 111-95/Ana		119.0	56	11.2	26	5.2
NS 111-95/Sremica		54.0	64	12.8	39	7.8
NSP 41/NS 0—649		21.3	22	4.4	13	2.6
30-Sc.Smoc.88—89/Hays2		33.7	4	0.8	—	—
F_1 (mean)		47.2	29.1	5.8	13.9	2.8
LSD	0.05	3.31		0.89		0.66
	0.01	4.43		1.19		0.89

The average callus yield in the experiment on the whole was 47.2%, which is close to the results obtained by Barnabas et al. (1991) and Marciniak et al. (2003) — 41% and 45%, respectively. Ekiz and Konzak (1994) as well as Bruins and Snijders (1995) reported significantly higher average values (70.9 and 77.8%, respectively), but they did not use randomly selected genotypes in their studies.

Regeneration ability was found to exist in all of the genotypes. A total of 582 green plants originating from 6000 isolated F_1 microspores were produced during the study. The number of green plants produced per spike varied from 0.8 (30-Sc.Smoc.88—89/Hays2) to 13.4 (Mex.3/MV 18). The average response per spike was 5.8 green plants (Tab. 1).

Studies with a large number of tested wheat genotypes have shown that such a large number of green plants is obtainable in general, which makes the anther culture technique applicable in wheat breeding. Tuvesson et al. (2000) in their screening study with 91 F_1 wheat combinations obtained very promising results: 4.7 green plants per spike.

From the total of 582 regenerated green plants, 279 (47.9%) were spontaneous DHs. The final average yield for this study was 2.8 DH plants per spike. The range was from 0 (30-Sc.Smoc.88—89/Hays2) to 8.6 (Mex.3/MV 18) DH plants per spike (Tab. 1).

Similar results were obtained by Šesek (1989) — 1.8 DH plants per spike, Snape et al. (1986) — 2.2 DH plants per spike and Tuvesson et al. (2000) — 2.1 DH plants per spike.

The results showed that significant genotypic differences have been found for callus yield, regeneration of green plants and DH production. It is in agreement with results of other authors (Tuvesson et al., 2000; Tersi et al., 2006; Ljevnaić, 2007). Among the large number of cultivated genotypes, the number of cultivars exhibiting a good level of androgenic response is usually small (Zamani et al., 2003). According to the results of Tuvesson et al. (2000), the ability to respond in anther culture is present in more modern cultivars compared with older material. The year of testing and the land of origin seem not to be important factors in determining the degree of response. Therefore, the results are of general value to wheat breeders. They also suggest that expensive tissue culture programmes should be concentrated on responsive breeding combinations, while unresponsive material should be improved via crossing.

CONCLUSION

Since the average production in this study was 5.8 green plants per spike and 2.8 spontaneous DH plants per spike, the efficiency of anther culture in DH production could be improved by inducing chromosome doubling using colchicine treatment. It will improve this technique enough for its effective use as an additional method in wheat breeding programs.

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ЕФИКАСНОСТ ТЕХНИКЕ КУЛТУРЕ АНТЕРА У ПРОИЗВОДЊИ ДВОСТРУКИХ ХАПЛОИДА ПШЕНИЦЕ

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Резиме

Циљ рада био је да се испита ефикасност културе антера у производњи спонтаних двоструких хаплоида из случајно одабраних хетерозиготних генотипова пшенице (*Triticum aestivum* L.). Антере из 20 F_1 комбинација пшенице гајене су *in vitro* на модификованој Потато-2 подлози.

Сви испитивани генотипови показали су способност да произведу калусе, као и да регенеришу зелене биљке. У просеку за цео експериментални материјал, произведена су 47.2 калуса на 100 изолованих антера. Регенерација зелених биљака кретала се од 0.8 до 13.4 зелене биљке по класу, са укупним просеком од 5.8. Од укупно 582 регенерисане зелене биљке, 47.9% (279) биле су спонтани двоструки хаплоиди. У просеку, у овом истраживању, произведено је 2.8 DH биљака по класу.