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Obtaining of doubled haploid lines by anther culture method for the Latvian wheat breeding

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Abstract. Methods of modern biotechnology, like double haploids (DH), could highly contribute improving efficiency and speeding up the breeding process. Aim of the present work was to elaborate most effective protocol of obtaining DH lines by spring and winter wheat anther culture. As initial material 10 spring and 4 winter wheat F_2 hybrids were used. The cold (4ºC) pre-treatment of spikes was applied, and spikes were sterilized by 50% solution of bleach for 17 min. Isolated anthers were cultivated on the different induction media: 190-0, AMC, and AMC with addition of 2.5 mg l^{-1} $CuSO_4$ x $5H_2O$. The most suitable induction medium for obtaining DHs from used wheat hybrids was the AMC medium with copper. Produced DH lines were multiplied and tested in the field conditions.

Key words: wheat, double haploids, breeding

INTRODUCTION

The spring and winter wheat breeding programmes are established at the State Stende Cereals Breeding Institute with the goal to create new varieties with high grain yield and quality, conforming industry requirements, as well with the resistance to lodging and main diseases and excellent winter hardness for the winter type. It is advisable that the new varieties are suitable for cultivation in the organic agriculture.

To speed up the breeding process, plant tissue culture methods could be used effectively. Among them, the generation of double haploid (DH) plants by anther culture is an important method, which enables significant shortening the breeding process (Kasha & Maluszynski, 2003; Belchev et al., 2004). Doubled haploid lines are homozygous, and it allows evaluating the material in rather short time. By this method it is possible to obtain new varieties even in 5–7 years, while conventional breeding usually takes 10–15 years.

Different investigations have been carried out to clarify mode of inheritance of anther culture response (for example, Torp et al., 2001; Xynias et al., 2001; Zamani et al., 2003) and to find out the ways to increase percentage of green plant-regenerants. Copper is a substance used for this reason both during seeds pre-treatment and as an

addition to the embryo induction medium. In the investigations carried out already in 1970s was proven that although plants accumulate copper only in small amounts, this element is involved in plant metabolism with great importance (Ozoliņa et al., 1975; Rashal et al., 1987). For a long time copper deficiency is known as a reason for male sterility, especially in cereals (Jewell et al., 1988). Copper is required for chlorophyll biosynthesis and photosynthesis (Maksymiec, 1997). The positive influence of copper while obtaining DH plants by the anther culture is expressed both in reducing the number of albino plants and in increased numbers of green plants-regenerants. These effects are related to improved survival of microspores during the different tissue culture stages and with the synchronization of the first microspore symmetric division (Wojnarowiez et al., 2002; Jacquard et al., 2009).

For several years anther culture has been applied to obtain wheat DH lines from Latvian wheat hybrids. The goal of this investigation was to improve the protocol of wheat anther culture for obtaining DH lines from the material used in Latvian breeding programmes.

MATERIALS AND METHODS

Ten spring and four winter wheat hybrids created at the State Stende Cereals Breeding Institute were used in the research (Table 1). Donor plants were grown in a greenhouse (17 to 20ºC at night, 25 to 30ºC at day, humidity ~70%). Spikes were collected and preserved at +4°C. After two weeks the developmental stage of microspores was determined by squashing in the acetic carmine on a glass slide (Jacquard et al., 2003). Spikes with the optimal mid- or late-uniculate stages of microspores (Barnabás, 2003) were used. Spikes were sterilized by 50% bleach 'Beļizna' water solution for 17 minutes, and then rinsed in sterile conditions 4 times by deionized and autoclaved water (Grauda et al., 2005). Anthers were separated from the spikes and put on the induction medium. Three different induction media were used – AMC, AMC with addition of copper (2.5 mg l^{-1} $CuSO_4$ x $5H_2O$), and 190-0.

After four weeks of cultivation obtained embryos were transferred on the regeneration medium 190-2. When embryos started to develop green plantlets, they were transferred on the rooting medium MS with coal (1 g l^{-1}). After 2–4 weeks of cultivation on the rooting medium, when the development of leaves and roots were observed, plantlets were planted into autoclaved sand. In two weeks, for the doubling of chromosome number, roots were immersed in 0.3% colchicine solution for 5 hours. Then plants were rinsed with water, planted into soil and cultivated in a growing room (20 to 26°C, humidity ~70%, 16 hours photoperiod). Spring wheat hybrids were grown in the mentioned conditions till maturity, but winter wheat hybrids after 3 weeks were transferred either to the field conditions or for vernalization to a cold chamber (1 to 7°C, deem light). After 2 months, plants from a cold chamber were moved to a growing room (20–26°C, humidity ~70%, 16 hours photoperiod) and grown till maturity.

One leaf from each plant-regenerant was collected for the ploidity determination using flow cytometry (Partec flow cytophotometer CyFlow Space).

Table 1. Spring and winter wheat hybrids used for DH obtaining.

	Spring wheat hybrids	Hybrid combination
1.	75KV8	Dragon/ Anniina
2.	75KV9	Selpex/ Banti// Picolo
3.	75KV10	BOR24201/ BOR25581
4.	75KV11	Std 95-64-1021/ Zebra
5.	75KV12	Anniina/ Daur
6.	75KV13	BOR25577/ Vinjett// BOR25759
7.	75KV14	Remia/ Vinjett// Anniina
8.	75KV15	Zebra/ Vinjett
9.	75KV16	Jasana/ BOR25113
10.	75KV17	Zebra// Anniina/ BOR25113
	Winter wheat hybrids	
11.	7KL1	Nadia/ 3942 (ASV)
12.	7KL2	Zernogradka/ 953 (Banga/ Krista)
13.	7KL3	Hana/ Koc 2520/97
14.	7KL4	Natalka/ Pamjati Fedina

Obtained DH lines were sown in the field conditions at the State Stende Cereals Breeding Institute for field evaluation. The winter hardiness of winter wheat, as well as disease and lodging resistance of all DH lines were determined. Standard error was calculated at the level $P = 0.05$.

RESULTS AND DISCUSSION

The embryogenesis was remarkably higher on the AMC medium in comparison with both other induction media (Fig. 1). Only two wheat hybrids did not develop embryos on this medium (75KV13 and 7KL3). On the medium AMC with copper embryos were obtained from 10 wheat hybrids but with significantly lower frequency. Hybrids on the medium 190-0 showed very low embryogenesis capacity. In general, winter wheat hybrids had lower embryogenesis ability than spring wheat hybrids.

Cultivation on the regeneration medium produced green plants from six hybrids – 4 of spring wheat (75KV8, 75KV10, 75KV12, 75KV15) and 2 of winter wheat (7KL3 Designation of wheat hybrid numbers as in the Table 1and 7KL4).

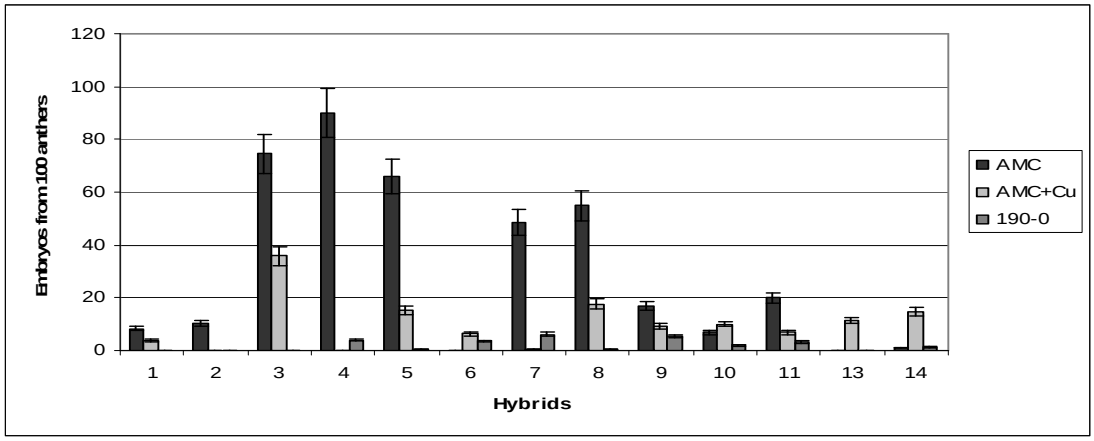
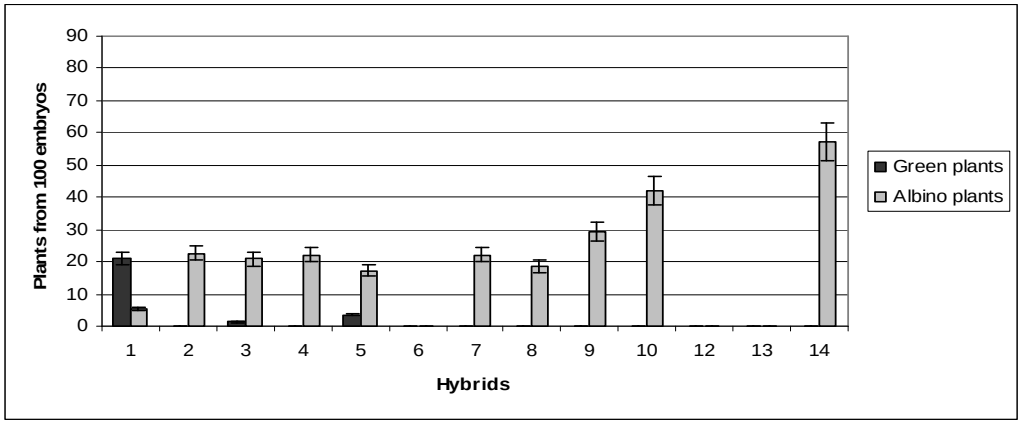


Figure 1. Embryogenesis of hybrid wheat anthers on different induction media.

Developing of green plants strongly depended on the induction media on which embryos were obtained. From this point of view the most effective was induction medium AMC with copper, both taken into account the number of hybrids, developed green plants and total number of such plants (Fig. 2). It is clear from the results that genotype influences the regeneration effectiveness. Hybrid 75K8 was most responsive to embryo cultivation on the both media. Three hybrids (75KV15, 7KL3, and 7KL4) produced green plants only on the medium AMC with copper. One hybrid (75KV12) produced small number of green plants on the AMC medium but not any on the medium AMC with copper. It is important to note, that the medium AMC without copper had higher embryogenic ability, nevertheless embryos, cultivated on this medium, had much lower capacity to develop green plants. No green plants were obtained from anthers cultivated on the medium 190-0.

It was found that all plants-regenerants had diploid cells. Out of them 34% were diploid (2n) plants, but 66% plants were mixaploids, beside diploid, also cells of different ploidity (3n, 4n, and 6n). Haploid plants-regenerants were not detected.

a



b

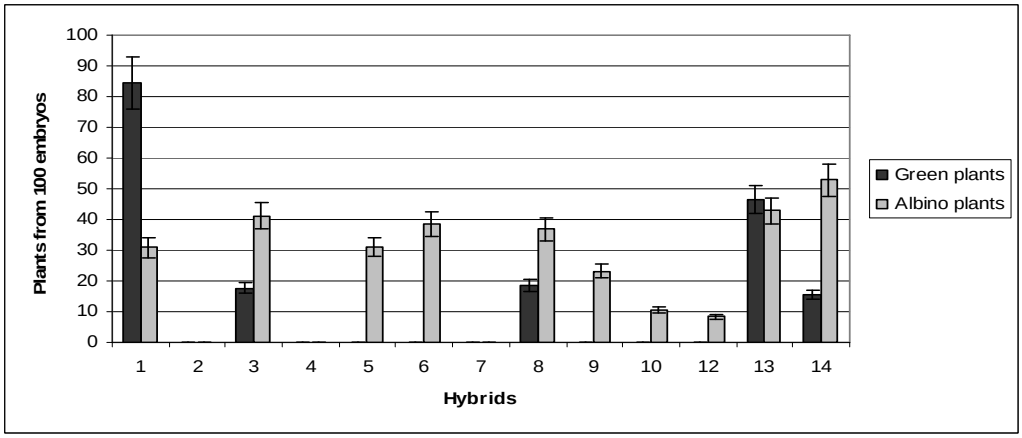


Figure 2. Efficiency of green and albino plant development from embryos obtained on either AMC (a) or AMC with copper (b) induction media. Designation of wheat hybrid numbers as in the Table 1.

In total, 14 winter and 50 spring wheat DH lines were obtained, which produced seeds (Table 2). After first year of the field evaluation 10 winter wheat DH lines with good yield, disease resistance and winter hardiness as well as 14 spring wheat DH lines with good yield and disease resistance were selected for the next breeding steps.

Table 2. Obtained DH lines from spring and winter wheat hybrids.

Spring wheat hybrids	Number of obtained DH lines	Number of selected lines after first year field trials
75KV8	6	1
75KV10	41	13
75KV12	2	0
75KV15	1	0
Winter wheat hybrids		
7KL3	10	6
7KL4	4	4

Results of the experiment showed that embryogenesis was remarkably higher on the AMC medium without copper. Nevertheless, the presence of copper obviously reduced the embryogenesis, but sufficiently increased development of green plants. Used concentration of copper (2.5 mg l^{-1}) was chosen according to the investigation, carried out on barley (Jacquard et al. 2009), which showed, that this concentration was the optimal for different varieties of barley. Our results demonstrated that the mentioned concentration was also effective for wheat in combination with the AMC medium.

Obtained results concerning medium 190-0 dramatically differed from those obtained with both other media (AMC and AMC with copper). Although 190-0 is a widely used medium (Barnabás, 2003; Tuveson et al., 2003), it was not suitable for wheat hybrids used in this study.

All plants-regenerants after treatment by colchicine had diploid cells, but the greatest part of the plants contained also cells with higher ploidity level. Nevertheless, the treatment gave positive effect in the production of DH lines. Our previous experiments have shown that without colchicine treatment only about 10% of wheat plants-regenerants produced seeds. In the current study the use of colchicine increased this rate, depending of the genotype, till 20–70%.

Most of the winter wheat DH lines demonstrated better winter hardiness, than parent varieties, which were crossed to create hybrids for DH production. At the same time hybrid families of F2 obtained from the same crosses showed very low survival ability during winter. It means that use of those DH lines gives a possibility to involve in the breeding process a new material with agronomical important traits, but rather low winter hardiness. After one year of field trials DH lines with good yield and disease resistance were selected from such crosses.

CONCLUSIONS

It was shown that the most suitable induction medium for obtaining DHs from wheat hybrids used in Latvian wheat breeding programmes was AMC with addition of copper (2.5 mg l^{-1}). Although the embryogenesis was higher on the AMC induction medium, obtained embryos had low capacity to develop green plants. Medium 190-0 was not suitable for investigated wheat hybrids. Therefore, this induction medium will not be used anymore for producing of wheat DH lines for breeding purpose. All plants-regenerants contained diploid cells, but the greatest part from them had also cells with higher ploidity level. The use of DH lines gives a possibility to involve quickly novel genes in the breeding.

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