Acta Biol. Univ. Daugavp. 20 (2) 2020
ISSN 1407 - 8953

EFFECTIVENESS OF DOUBLED HAPLOIDS PRODUCTION BY ANTHER CULTURE FROM SELECTED WINTER WHEAT HYBRIDS

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Žagata K., Grauda D., Rashal I. 2020. Effectiveness of doubled haploids production by anther culture from selected winter wheat hybrids. *Acta Biol. Univ. Daugavp., 20 (2): 107 – 111.*

Using of doubled haploids (DH) allows shorten the crop breeding process by 3–4 years. Anther culture is one of the most effective methods of obtaining DH lines of different crops including wheat: doubling chromosome set of haploid plants, grown *in vitro* from pollen, resulting in homozygous plants. The objective of this work was obtaining DH lines by anther culture from winter wheat hybrids. For evaluation of embryos formation efficiency 60 different winter wheat hybrids were examined. Among them five winter wheat hybrids with the highest embryogenesis level were chosen for obtaining DH lines. From 8460 anthers placed on the induction medium 2113 embryos were obtained, which finally resulted in 505 plants-regenerants. Genotype effect on plants-regenerants production was observed. Obtained plants-regenerants were passed to Stende Research Centre of the Institute of Agricultural Resources and Economics for testing from the aspect of breeding.

Key words: DH lines, plants-regenerants, genotype effect.

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INTRODUCTION

In Latvia wheat account for more than 65% of the total sown areas of cereals, therefore, wheat in Latvia is considered to be one of the most important crops. The aim of crop breeding is to create high-yielding varieties that are disease-resistant and suitable for local agronomic and climatic conditions. Creating new crop varieties by traditional breeding methods takes 12–15 years, which is a rather long period of time in variable climatic conditions and in the situation of changeable impact of pathogens (Grauda et al. 2010). Using of doubled haploids (DH) in breeding is an important tool to accelerate the breeding of new varieties and allows shortening of the crop breeding process by 3–4 years (Kasha & Maluszynski 2003, Dwivedi et al. 2015, Lantos, Pauk, 2016).

Anther culture is one of the most effective methods for obtaining DH lines of different crops including wheat: it allows to double the chromosome set of haploid plants, grown *in vitro* from pollens, which resulted in complete homozygous plants (Forster et al. 2007). The main factors hindering the application of anther culture include very low or no response to androgenic induction and low rates of embryogenesis. Many physiological factors can influence *in vitro* cultures response

such as growth conditions of donor plants, developmental stage of pollens, type of spike pre-treatment, compositions of used mediums (Bajaj 1990, Touraev et al. 1996, Liu et al. 2002, Lantos & Pauk 2016). Thermal and chemical stress treatment allows conversion of microspores into embryos by diverting microspores from their normal gametophytic pathway into sporophytic development. The results obtained by different authors (Touraev et al. 1996, Hu and Kasha 1997, Liu et al. 2002, Barnabás 2003, Grauda et al. 2005, Grauda et al. 2010) suggest the high effects of genotype on formation of embryos (embryogenesis response) in anther culture. It is proven also by the influence of pollens nucleus development stage on embryogenesis response. It was found that for induction of embryogenesis in wheat anther culture, the best is the middle or late mono-nucleate stage (Barnabás 2003). In several investigations (Gustafson et al. 1995, Hu and Kasha 1999, Grauda et al. 2005, Grauda et al. 2010) it was shown that pre-treatment by short-term cold shock or mannitol is effective for wheat anther culture. It has also been observed that other stress treatment, for example, spikes treating by sterilizing solution, can play an important role in the increasing of numbers of green plantlets (Grauda et al. 2010, Slama et al. 2010).

Improvements in the composition of culture media have as well contributed to the progress of androgenesis and plant-regenerant producing. A positive influence of copper in concentrations 1.0–5.0 $mg \cdot l^{-1}$ on embryogenesis and increasing the number of plants-regenerants has been observed (Grauda et al. 2005, Jacquard et al. 2009, Makowska et al., 2017). Our previous investigations also showed the specificity of medium composition suitable for wheat anther culture of several wheat hybrids from Latvian breeding programme. For induction of embryogenesis AMC liquid medium with copper $CuSO_4 \times 5H_2O$ in concentration of 2.5 mg l^{-1} was used, for regeneration inducing solid regeneration – medium 190-2, and for plants-regenerants rooting effectively – solid MS medium with carbon (Grauda et al. 2010). For doubling of chromosome set in plants-regenerants, different concentrations of colchicine solution were applied (Tuvesson et al. 2003, Soriano et al. 2007), and the optimal concentration for the mentioned Latvian wheat breeding material was found to be 2.0 $mg \cdot l^{-1}$ (Grauda et al. 2010). An important stage of winter wheat development is vernalization, without this stage the formation of fertile winter wheat plants is impossible. Previous investigations have shown that vernalization of plants-regenerants placed in the growth chamber at low temperature (0 to +2 °C) for 16–24 weeks is suitable for Latvian winter wheat breeding material of winter wheat (Grauda et al. 2010).

For several years the anther culture in Latvian breeding programs was used for obtaining DH lines only in spring wheat. It is shown that in many cases effectiveness of anther cultures in the spring wheat are higher than in the winter wheat (Weight et al., 2020). The aim of this work was to describe the applied method of obtaining a reasonable number of DH lines for Latvian winter wheat breeding program.

MATERIAL AND METHODS

As the initial material for experiments there were used several winter wheat hybrids created at the Stende Research Centre of the Institute of Agricultural Resources and Economics (former State Stende Cereals Breeding Institute). Seeds of F_1 generation of those hybrids were grown in a growing chamber in partially controlled conditions (+17 °C to +20 °C at night, +20 °C to +24 °C at day, humidity ~70%, photoperiod 16 hours). Flowering spikes were detached from initial plants in the early mononuclear pollen nuclear stage. Pollen nuclear developmental stage was determined microscopically. For generation stress conditions in pollens, spikes were subjected to cold shock by placing in thermostat with temperature +4 °C for two weeks. After the effect of temperature shock, spikes were sterilized for 17 minutes by 50% water solution of bleach Belizna, and then rinsed four times with deionized, autoclaved water (Grauda et al. 2005).

For induction of embryogenesis, anthers were placed on liquid medium AMC with copper

Table 1. Hybrids having shown the highest potential of embryogenesis

Hybrid	**Cross combination**
11KL42	Nic99-3009B / Raduga / Galahad
11KL45	Schara / Nic04-4106B
11KL46	Premjera / Nic04-3241A
11KL49	Sepstra / Nic04-4106B
11KL51	Fantazia / Olivin

Table 2. Induction of embryogenesis and formation of plants-regenerants

Hybrid	**Anthers placed on the induction medium**	**Obtained embryos**	**Plants-regenerants**	**Green plants-regenerants**
11KL42	2340	559	132	90
11KL45	1530	258	56	17
11KL46	990	149	21	13
11KL49	1980	465	84	56
11KL51	1620	682	212	146
Total	8460	2113	505	322

($CuSO_4$ x $5H_2O$, concentration 2.5 $mg \cdot l^{-1}$). Anthers were cultivated in a thermostat at the temperature of +29 °C in dark. After 4–9 weeks of cultivation, clusters of closely formed embryos were placed on solid regeneration medium 190-2 (Tuvesson et al. 2003, Grauda et al. 2010). Clusters of embryos were divided into separate plantlets and placed again on solid regeneration medium 190-2. After 4–9 weeks of cultivation, the plants-regenerants were placed on rooting medium – solid MS medium enriched with coal (concentration 1.0 $g \cdot l^{-1}$). *In vitro* rooted plants-regenerants were transferred to soil in growing chambers with partially controlled growing conditions (+20 °C to +26 °C, humidity ~70%, photoperiod 16 hours).

Two weeks after transferring plants-regenerants to soil, doubling of plants chromosome set was performed: the roots were immersed in 0.2% colchicine solution for 4 hours (Grauda et al. 2014). After that plants were rinsed with tap water, planted in soil and cultivated in a growing chamber (+20 °C to +26 °C, humidity ~70%, photoperiod 16 hours).

Two weeks after the plant treatment with colchicine, plants-regenerants were placed in the growing chamber for vernalization (dim light, 0 to +2 °C). After 16–20 weeks of vernalization, they were transferred to greenhouse. Three weeks later, the plants were planted in the experimental fields of Stende Research Centre of the Institute of Agricultural Resources and Economics for multiplication of potential DH lines and further evaluation from the aspect of breeding.

RESULTS AND DISCUSSION

Most of the used hybrids had low viability: only about 25% of hybrids were suitable to establishment of anther culture. Anthers from successfully vernalized hybrids formed embryos in the range of 4 to 50%, but only from about one-third of these genotypes green plants-regenerants

were obtained. Five hybrids with the highest potential of embryogenesis and green plants regeneration capacity were chosen for obtaining of DH lines (Table 1).

Altogether 8460 anthers were placed on the liquid AMC medium for establishing of anther culture, in total 2113 embryos were obtained, which finally resulted in 322 green plants-regenerants (Table 2). High genotype influence was observed in all stages of forming of plants-regenerants: depending on the hybrid combination, the number of embryos per 100 anthers ranged from 15 to 42, while the number of plants-regenerants per 100 anthers ranged from 2 to 13 (Fig, 1, Table 2).

In comparison with the earlier used approach for spring wheat anther culture several points should be underlined as improvements of the method:

1. Addition of copper to the used mediums may increase the green plant formation efficiency.
2. Addition of coal to the rooting medium increase the rooting efficiency of plants-regenerants.
3. Preliminary testing of hybrids to find genotypes with a higher embryogenesis and green plant formation capacity can reduce non-effective laborious effort by excluding non-responding genotypes from the experiments.
4. Vernalization of plants-regenerants is necessary for producing fertile plants in case of winter wheat.

The complex of techniques applied in this investigation in different stages of producing green plant-regenerants from winter wheat anther culture allow developing a reasonable number of DH lines, useful for the breeding process.

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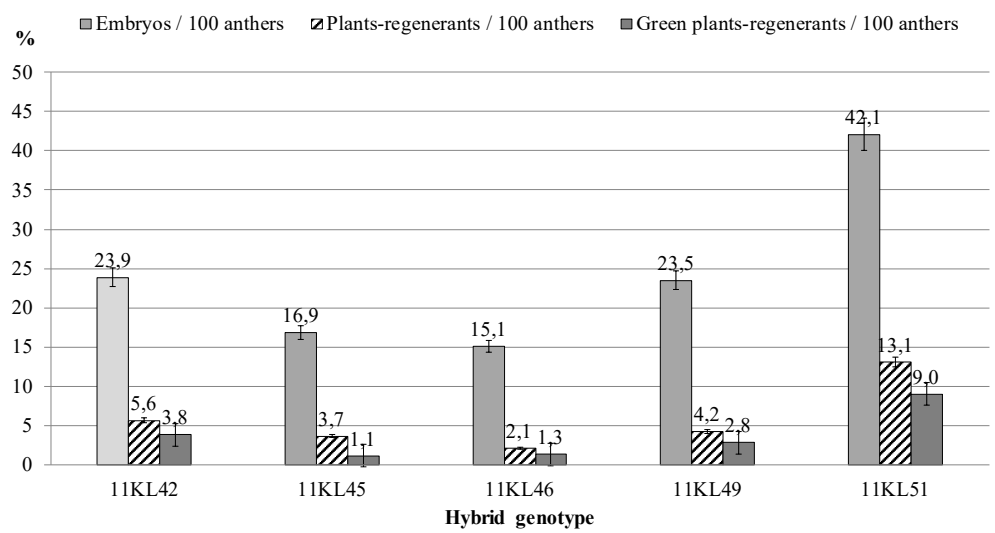

Fig. 1. Efficiency of formation of embryos and plants-regenerants.

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Received: 20.10.2020.
Accepted: 15.11.2020.