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ANTHER CULTURE EFFECTIVENESS IN PRODUCING DOUBLED HAPLOIDS OF CEREALS

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Our goal was to improve the method of obtaining double haploids (DH) by anther culture from Latvian breeding material for several agriculturally important cereals in Latvia: barley, spring and winter wheat. Hybrids from Latvian breeders were initial material. It was found that copper (2.5 mg/l) added to barley anther pre-treatment media and to both barley (C3) and wheat (liquid AMC) induction media, resulted in a higher percentage of green plants-regenerants. In general, the winter wheat hybrids exhibited lower embryogenesis than spring wheat hybrids. Obtaining a large number of DH lines from barley and wheat hybrids with unknown androgenesis response can be organized in two stages: first — selection from breeding initial material hybrids responsive in anther culture, which enables production of green plants-regenerants, and second — production of DH lines in a large scale from selected hybrids.

Key words: *DH lines, breeding, barley, wheat, copper.*

INTRODUCTION

Plant tissue culture methods can be used more effectively to speed up the breeding process. Among them, the production of doubled haploid (DH) plants by anther culture is the most significant method used for considerably shortening the breeding process period (Kasha and Maluszynski, 2003; Belchev *et al.,* 2004; Grauda *et al.,* 2009; El-Hennawy *et al.,* 2011). Doubled haploid lines are homozygous, which allows to evaluate breeding lines in a rather short time and to quickly involve novel genes in breeding. By this method it is possible to obtain new varieties even in 5–7 years, while conventional breeding usually takes up to 10–15 years (Grauda *et al.,* 2010; Tadesse *et al.,* 2012).

Ability of anther cultures to form haploid plants is influenced by genotype of used hybrids, donor plant growth conditions, the developmental stage of microspores, spike pre-treatment, and media components (Kasha and Maluszynski, 2003). Research has been carried out to clarify the mode of inheritance of microspore regeneration capacity in anther culture and *de novo* shoot organogenesis (see, for example, Torp *et al.,* 2001; Xynias *et al.,* 2001; Zamani *et al.,* 2003; Jacquard *et al.,* 2009; Ferrie and Caswell, 2010; El-Hennawy *et al.,* 2011, Duclercq *et al.,* 2011; Rubtsova *et al.,* 2012) and to find ways to increase percentage of green plants-regenerants. Copper (usually $CuSO_4$) has been used for this purpose in spike pre-treatment and as an add-on to the embryo induction medium. Investigations carried out already in the 1970s showed that, although plants accumulate copper only in small amounts, this element has great importance in plant metabolism (Озолиня и др., 1971; Рашаль и др., 1987). Copper deficiency is a reason for male sterility, especially in cereals (Dell, 1981; Jewell *et al.,* 1988). Copper is required for chlorophyll biosynthesis and photosynthesis (Maksymic, 1997), copper deficiency induces chlorosis in leaves, and results in decrease of chlorophyll content (Deriu *et al.,* 2007). In anther culture, copper deficiency is associated with increased formation of albino plants (Jacquard *et al.,* 2009). The positive influence of copper on obtaining DH plants by the anther culture can occur by reduction of the number of albino plants and in increased numbers of green plants-regenerants. These effects are related to improved survival of microspores during tissue culture stages and with the synchronisation of the first microspore symmetric division (Jewell *et al.,* 1988; Wojnarowiez *et al.,* 2002; Jacquard *et al.,* 2009).

Nevertheless, genotype still is the main limiting factor of androgenesis in tissue culture, and even the use of copper for overcoming this factor not always gives positive effect (Grauda *et al.,* 2010; El-Hennawy *et al.,* 2011). The goal of this study was to develop an anther culture based complex approach for obtaining a reasonable number of DH lines for Latvian barley and wheat breeding programmes.

MATERIALS AND METHODS

Plant material. Spring barley, and spring and winter wheat F_1 and F_2 hybrids created at the State Stende Cereals Breeding Institute, and spring barley hybrids created at the State Priekuļi Plant Breeding Institute were included in the study. Donor plants were grown in greenhouse (+17 ^{o}C to +20 ^{o}C at night, +25 ^{o}C to +30 ^{o}C at day, humidity ~70%) or in room conditions (+20 ± 2 ^{o}C, humidity ~40%, 16 hours photoperiod). Seedlings of winter genotypes, three weeks after sowing, were vernalised at +4 ^{o}C for 8 weeks. Spikes of barley were collected when most microspores were at the mid-uninucleate stage, spikes of wheat when most microspores were in the early or mid-uniculate stage. The developmental stage of microspores was determined by squashing in acetic carmine on a glass slide (Barnabás, 2003; Jacquard *et al.,* 2003).

The investigation was performed in two steps: first, the influence of copper on barley and wheat anther culture of selected hybrids was studied, in the second step — anther response of breeder hybrids.

Effect of copper on microspore regeneration capacity. Seven barley and five wheat hybrids (Tables 1 and 2) with previously determined different anther response were used in evaluation of effect of copper on embryogenesis and plant regeneration capacity.

Barley spikes were sterilised with 70% ethanol for 5 minutes and then rinsed four times with de-ionised and autoclaved water (Grauda *et al.,* 2005). Anthers were separated from the spikes and put on 0.3 M mannitol with addition of 2.5 mg/l $CuSO_4 \times 5H_2O$ (Jacquard *et al.,* 2003) or without addition of Cu. Before transferring to the induction medium anthers were pre-treated at +4 ^{o}C for three days.

Table 1

ANTHER CULTURE RESPONSE AND GREEN PLANTS-REGENERANTS FORMATION FROM 7 BARLEY HYBRIDS WITH AND WITHOUT COPPER SULPHATE SUPPLEMENTATION DURING PRE-TREATMENT

Hybrid	Supplementation of $CuSO_4$, mg/l	Number of plated anthers	Number of embryos/100 anthers	Number of plants-regenerants/ 100 anthers	Green plants-regenerants, %
Ansis/Dziugiai	0	640	2.5	62.5	40.0
	2.5	600	9.0	48.2	92.3
Dziugiai/Ansis	0	490	26.9	14.4	0
	2.5	600	11.5	23.2	100.0
Primus/Anni	0	600	11.0	72.7	0
	2.5	620	9.1	21.4	33.3
Saana/Maja	0	600	6.0	25.0	44.4
	2.5	610	6.6	20.1	100.0
Aura/Abava	0	610	4.6	50.0	14.3
	2.5	600	25.0	40.0	80.0
Merlin/SW1291/Danuta	0	300	50.3	14.8	18.5
	2.5	360	46.1	24.9	53.0
Danuta/6131	0	300	90.7	30.9	2.9
	2.5	300	44.0	27.4	18.2

Table 2

ANTHER CULTURE RESPONSE AND GREEN PLANTS-REGENERANTS FORMATION FROM 5 WHEAT HYBRIDS WITH AND WITHOUT COPPER SULPHATE SUPPLEMENTATION OF INDUCTION MEDIUM

Hybrid	Supplementation of $CuSO_4$, mg/l	Number of plated anthers	Number of embryos/100 anthers	Number of plants-regenerants/ 100 anthers	Green plants-regenerants,%
Natalka/Pamjati Fedina*	0	552	5.1	2.9	0
	2.5	1097	14.7	10.0	22.7
Dragon/Anniinia**	0	230	8.3	2.1	80.0
	2.5	348	3.7	4.3	84.1
BOR24201/BOR25581**	0	431	74.5	16.5	5.9
	2.5	942	35.8	21.0	29.8
Anniinia/Daur**	0	335	66.0	1.3	21.1
	2.5	378	10.1	7.1	3.7
Zebra/Vinjett**	0	186	54.8	10.2	0
	2.5	215	17.7	9.8	33.3

* winter wheat; ** spring wheat

After pre-treatment, anthers were placed on C3 induction medium as described by Jacquard *et al.* (2003), which included also 2.5 mg/l $CuSO_4$. Petri dishes with anthers were maintained in a growth chamber at +26 ^oC in the dark. After four weeks of cultivation, the obtained embryos, about 2 mm in diameter, were transferred on M1 regeneration medium (Jacquard *et al.,* 2003). Embryo cultures were grown under light (+24 ^oC, 16 hours photoperiod, light intensity 3000 lx). When embryos started to develop green plantlets, they were transferred on rooting medium: MS (Murashige and Skoog, 1962) (3% sucrose, 0.6% agar) with 1 g/ml activated charcoal (Nichterlein, 2003). Plantlets were grown under light (+24 ^oC, 16 hours photoperiod, light intensity 3000 lx).

Collected wheat spikes were maintained at +4 °C for two weeks. Spikes were sterilised with 50% commercial bleach (4% of hypochlorite content) water solution for 17 minutes, and then rinsed four times with de-ionised and autoclaved water (Grauda *et al.,* 2005). Anthers were separated from spikes, and, in equivalent amounts, put on solid or liquid induction medium AMC (Kunz *et al.,* 2000) with or without 2.5 mg/l $CuSO_4$ x $5H_2O$ (Grauda *et al.,* 2005). Petri dishes with anthers were maintained in a growth chamber at +29 ^oC in dark conditions. After four weeks of cultivation, obtained embryos, about 2 mm in diameter, were transferred onto regeneration medium 190-2 (Tuvesson *et al.,* 2003). Embryos were cultivated under light (+24 ^oC, 16 hours photoperiod, light intensity 3000 lx). When embryos started to develop green plantlets, they were transferred on rooting medium: MS (3% sucrose, 0.6% agar) with 1 g/ml activated charcoal. Plantlets were grown under light (+24 ^oC, 16 hours photoperiod, light intensity 3000 lx).

Both barley and wheat plantlets after 2–4 weeks cultivation on rooting medium, in cases when development of leaves and roots was observed, were planted into an autoclaved mixture of soil and sand (1:1) and grown in a growing room (+20 ± 2 ^oC, humidity ~40%, 16 hours photoperiod). For the first days plantlets were covered with glass jars to maintain high humidity. Two weeks after transferring plants-regenerants to soil, one leaf from each plant was collected for ploidy determination using flow cytometry (Partec Flow Cytometer) or microscopy (Pauk *et al.,* 2003). For the doubling of the chromosome set, roots of plantlets were immersed in 0.2% colchicine solution for four hours. Colchicine treatment was not performed for diploid plantlets. Colchicined plantlets were rinsed by water, re-planted into soil and cultivated in a growing room in the same conditions. Spring wheat DH lines were grown in the growing room till maturity. Winter wheat DH lines, after three weeks of treatment by colchicine, were transferred for vernalisation to a growth chamber (+1 °C to +7 °C, dim-light). After eight weeks of vernalisation, survived plantlets were moved to a growing room with the conditions given above and grown till maturity.

Evaluation of anthers response of breeder hybrids. Anther response of 24 spring barley hybrids (Table 3) and five spring and 16 winter wheat hybrids (Table 4) received from the State Stende Cereals Breeding Institute was evaluated. Thirty anthers from about each of ten to twenty five spikes of each hybrid were used for anther culture, described above. Based on the first step of the investigation, barley anther pre-treatment in mannitol with 2.5 mg/l $CuSO_4$ × $5H_2O$ and wheat anther cultivation in induction medium AMC supplemented with 2.5 mg/l $CuSO_4$ x $5H_2O$ were chosen. The same methods of anther cultivation and plantlet growing conditions as in the first step were applied.

In both steps, after establishing anther culture, the number of planted anthers, the number of embryos per 100 planted

Table 3

EMBRYO AND PLANTS-REGENERANTS FORMATION EFFECTIVENESS OF BREEDER BARLEY HYBRIDS

Hybrid	Number of embryos/ 100 anthers	Number of green plants-regenerants/ 100 anthers	Number of green plants-regenerants/ 100 embryos
Beatrix/Conchita	6.0	0	0
Alexis/Anni	101.6	0	0
12688/Ivana	9.9	0.6	5.6
Alliot/Cellar	19.3	0	0
Aricada/Effendi	72.9	0.1	0.1
Merlin/Sw1291/3/Danuta/ L-30008//L3101	82.0	3.2	3.9
Roxane/Danuta//L-3101/3/ Lawina/Azhul	114.3	0.4	0.4
F1-02-23/G136	35.0	2.4	3.8
Kristaps/Evelin	21.3	0.7	3.1
Šikara/Ohara	67.0	1.0	1.4

Table 4

EMBRYO AND PLANTS-REGENERANTS FORMATION EFFECTIVENESS OF BREEDER WHEAT HYBRIDS

Hybrid	Number of embryos/100 anthers	Number of green plants-regenerants/ 100 anthers	Number of green plants-regenerants/ 100 embryos
UK-8/Tarso*	1.9	0.1	5.9
Inna/Sakta/Akteur*	0.7	0.3	50.0
Olivin/Maltop/1020*	2.1	0.6	27.8
Tiger/Vergas/Tarso*	5.3	1.1	21.6
Kijevskaja 6/Ranger/Finezja*	12.4	0.5	4.2
Nic99-3009B / Raduga / Galahad*	23.9	5.6	23.6
Schara / Nic04-4106B*	16.9	3.7	21.7
Premjera / Nic04-3241A*	15.1	2.1	14.1
Sepstra / Nic04-4106B*	23.5	4.2	18.1
Fantazia / Olivin*	42.1	13.1	31.1
Vinjett/Picolo/Helle/Vinjett**	17.4	2.3	13.2
SWŽura/Trisso**	1.5	0.4	28.0
Spektr/SWŽura**	49.5	8.7	17.6
Rollo/Vinjeet**	1.6	0.6	38.9
Venera/Anniina/Picolo**	12.8	0.6	4.4

* winter wheat; ** spring wheat

anthers, the number of plants-regenerants per 100 anthers and percent of green plants-regenerants from all plants-regenerants were recorded. Microsoft Excel software was used for data calculation.

RESULTS

Effect of copper on microspore regeneration capacity. The use of 2.5 mg/l copper sulphate for all seven barley hybrids during pre-treatment with mannitol had positive effect on the development of microspore-derived green plants-regenerants (Table 1). The results were particularly important for those hybrids producing exclusively albino plants with mannitol pre-treatment without copper sulphate (Dziugiai/Ansis and Primus/Anni): the percentage of green plants reached 100 and 33.3%, respectively, despite decrease of the proportion of responding anthers. For some hybrids (Danuta/6131 and Dziugiai/Ansis), copper sulphate considerably decreased embryo formation. Nevertheless, even for those hybrids, as in other cases, the improved green plant production was sufficient. Increased green plant percentage in most cases resulted in increased DH plant production (data are not shown).

Spring and winter wheat hybrid microspores had various effect of $CuSO_4$ addition to induction medium (Table 2). Liquid embryo induction medium showed considerable higher embryogenesis with subsequent plants-regenerants formation, than on solid induction medium with agar. No positive effect on formation of embryos on solid medium was observed (Fig. 1). Only one spring wheat hybrid (Natalka/Pamjati Fedina) showed positive effect of copper: increased microspore embryogenesis effectivity from 5 to 15%. For other wheat hybrids no positive effect of copper on embryogenesis was found and addition of copper to the induction media even decreased regeneration capacity. Nevertheless, positive effect of copper on green plant regeneration capacity was observed, excepting for one hybrid (Anniinia/Daur). The embryos developed on induction medium with copper more often formed green plants-regenerants. Two wheat hybrids (Natalka/Pamjati Fedina and Zebra/Vinjett) formed green plants-regenerants only after cultivation on induction medium with copper. Addition of copper was tested both on liquid and solid induction media.

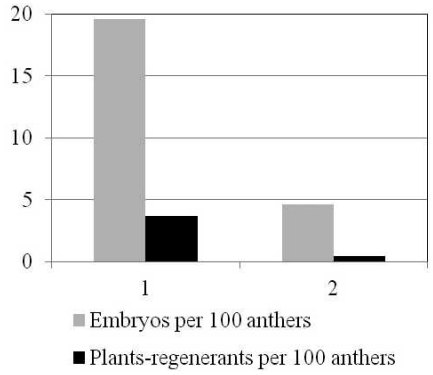

Fig. 1. Formation of wheat embryos and green plants-regenerants on solid and liquid induction medium. **1.** Liquid induction medium AMC with $CuSO_4$; **2.** Solid induction medium AMC with $CuSO_4$.

Evaluation of anthers response and green plants-regenerants formation of breeder hybrids. In barley, 41.7% of evaluated hybrids developed embryos; the embryo formation capacity ranged from 6 to 114.3 embryos on 100 anthers (Table 3). Embryos of some hybrids (Beatrix/Conchita, Alexis/Anni and Alliot/Cellar) formed fast growing calluses, but which did not respond after planting on regeneration medium. Embryo capacity to develop green plants-regenerants ranged from 0 (Beatrix/Conchita, Alexis/Anni and Alliot/Cellar, Aricada/Effendi) to 5.6% (12688/Ivana).

All evaluated spring and 63% of winter wheat hybrids (Table 4) formed embryos and green plants-regenerants. Anther response ranged from 0.75 (Inna/Sakta/Akteur) to 49.5% (Spektr/SWŽura), and green plants-regenerants formation from 100 anthers varied between 0.1 (UK-8/Tarso) and 13.1% (Fantazia/Olivin).

All wheat plants-regenerants had diploid cells, but 60% of those were mixoploids (2n, 3n, 4n and 6n). Of barley plants-regenerants, 80% were spontaneous diploids. The loss of both barley and wheat plants-regenerants during transfer from *in vitro* culture to soil was about 10%, and no effect of genotype was observed. No damage to winter wheat plants-regenerants during vernalisation was observed. After growth in a growth room, plants-regenerants were ready for transfer into soil in either greenhouse or field conditions.

DISCUSSION

Use of barley and wheat anther culture technology in breeding programmes is associated with relatively low yield of pollen-derived embryos and green plants-regenerants and high frequency of albino plants-regenerants (Jacquard *et al.,* 2009). Therefore, various modifications have been made to improve this method, particularly regarding effective pre-treatment methodology (Touraev *et al.,* 1997; Hu and Kasha, 1999; Xynias *et al.,* 2001; Grauda *et al.,* 2005; 2009; 2010). Increased microspore survival during cultivation and synchronisation of the first microspore symmetric division (Wojnarowiez *et al.,* 2002) reduces albino formation and increases number of green plants-regenerants, and, in general, increases number of obtained DH plants.

The barley and wheat hybrids used in Latvian breeding programmes have quite low embryogenesis and embryo regeneration capacity in anther culture and need additional attention during all stages of anther culture, starting from pre-treatment till planting plants-regenerants into soil. This investigation showed that supplementation of copper in mannitol at time of barley pollen pre-treatment decreases embryogenesis capacity of anther cultures, but increases percentage of green plants-regenerants. In the case of wheat, it was previously found that the most effective method for Latvian breeder hybrids is cold pre-treatment of spikes for two weeks (Grauda *et al.,* 2005). However, selection of the best mediums for anther cultivation is also im-

portant. It was found (Grauda *et al.,* 2010) that the widely used cultivation mediums used to establish anther culture from hybrids included in Latvian breeding programmes are not suitable for barley and wheat (Barnabás, 2003; Tuveson *et al.,* 2003). This may be due to genetic differences between genotypes involved in the breeding in different regions. For the investigated barley hybrids of Latvian origin, medium C3 with copper was effective. The concentration of copper sulphate (2.5 mg/l) was found to be optimal for different varieties of barley in previous experiments (Jacquard *et al.,* 2009), in which liquid AMC induction media with copper in the same concentration was effective for obtaining wheat plants-regenerants. The optimal concentration for wheat might differ from barley, but additional experiments are needed. In general, winter wheat hybrids had lower embryogenesis than spring wheat hybrids.

Flow cytometry showed high percentage of spontaneous diploidisation of barley plantlets. Therefore, for obtaining DH for breeding purpose, we did not use ploidy determination and treatment with colchicine. The role of colchicine for wheat DH lines production is an open question. All wheat plants-regenerants in our study had diploid cells, but 60% of those plantlets were mixoploid (2n, 3n, 4n and 6n). Our previous experiments showed that, without colchicine treatment, only about 10% of wheat plants-regenerants produced seeds. In the current study, the use of colchicine increased the rate of fertile plants, depending on the genotype, up to 20–70% (data are not shown).

The use of copper sulphate during barley pre-treatment and in wheat embryo induction media allowed generally to promote the development of microspore derived green plants-regenerants from the tested hybrids. Nevertheless, obtaining of large numbers of DH by anther culture for barley and wheat breeding programmes is still problematic. The main reason is for this the effect of genotype on anther culture results (Zamani *et al.,* 2003; Grauda *et al.,* 2010). One option is use of anther culture only for responsive genotypes (Irikova *et al.,* 2011; Murovec and Bohanec, 2012). In this case, obtaining a high number of DH lines from barley and wheat hybrids with unknown androgenesis response should be organised in two stages. The first stage implies selection from breeding initial material hybrids which are responsive in anther culture and which produce embryos able to regenerate green plants. The second stage involves production of DH lines in a large scale from selected hybrids by anther culture. Besides applying an optimal timeline of obtaining barley and wheat DH plantlets in the laboratory, acclimatisation and planting in field conditions is also considerable important for decreasing expenses of DH line production, especially for winter wheat, and to determine important traits already in the first grown season.

REFERENCES

Barnabás, B. (2003). Protocol for producing doubled haploid plants from anther culture of wheat (*Triticum aestivum* L.). In: Maluszynski, M., Kasha, K. J., Forster, B. P., Szarejko I. (ed.). *Doubled Haploid Production in Crop Plants* (pp. 65–70). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Belchev, I., Tchorbadjieva, M., Pantchev I. (2004). Effect of 5-azacytidine on callus induction and plant regeneration potential in anther culture of wheat (*Triticum aestivum* L.). *Bulgarian J. Plant Physiol*., **30** (1–2), 45–50.

Dell, M. (1981). Male sterility and anther structure in copper-deficient plants. *Ann. Bot.*, **48** (5), 599–608.

Deriu, D., Calace, N., Bianca, M. Pietroletti, M. (2007). Morphological and physiological responses of barley plants grown on soils characterized by metal toxicity and metal deficiency. *Ann. Chim.*, **97**, 153–162.

Duclercq, J., Sangwan-Norreel, B., Catterou, M., Sangwan, R. S. (2011). *De novo* shoot organogenesis: From art to science. *Trends Plant Sci.*, **16** (11), 597–606.

El-Hennawy, M. A., Abdalla, A. F., Shafey, S. A., Al-Ashkar, I. M. (2011). Production of doubled haploid wheat lines (*Triticum aestivum* L.) using anther culture technique. *Ann. Agri. Sci.*, **56** (2), 63–72.

Ferrie, A. M. R., Caswell, K. L. (2010). Isolated microspore culture techniques and recent progress for haploid and doubled haploid plant production. *Plant Cell Tissues Org. Cult.*, **104** (3), 301–309.

Grauda, D., Keiša, A., Rashal, I. (2005). Obtaining of doubled haploid lines for Latvian barley and wheat breading programs by anther culture method. *Sordiaretus ja Seemnekasvatus,* **9**, 209–216.

Grauda, D., Lepse, N., Strazdiņa V., Kokina, I., Lapiņa, L., Miķelsone, A., Ļubinskis, L., Rashal, I. (2010). Obtaining of doubled haploid lines by anther culture method for the Latvian wheat breeding. *Agron. Res.*, **8** (3), 545–552.

Grauda, D., Strazdiņa, V., Kokina, I., Lapiņa L., Miķelsone, A., Rashal, I. (2009). Extension of spring wheat breeding using haploids technology. *Acta Biol. Univ. Daugavpiliensis*, **9** (2), 1407–8953.

Hu, T., Kasha, K.J. (1999). A cytological study of pretreatments used to improve isolated microspore cultures of wheat (*Triticum aestivum* L.) cv. Chris. *Genome,* **42** (3), 432–441.

Irikova, T., Grozeva, S., Popov, P., Rodeva, V., Todorovska, E. (2011). *In vitro* response of pepper anther culture (*Capsicum annuum* L.) depending on genotype, nutrient medium and duration of cultivation. *Biotechnol. Biotechnol. Equip.*, **25** (4), 2604–2609.

Jacquard, C., Wojnarowiez, G., Clément, C. (2003). Anther culture in barley. In: Maluszynski, M., Kasha, K.J., Forster, B. P., Szarejko, I. (eds.), *Doubled Haploid Production in Crop Plants* (pp. 21–27). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Jacquard, C., Nolin, F., Hécart, C., Grauda, D., Rashal, I., Dhondt-Cordelier, S., Sangwan, R. S., Devaux, P., Mazeyrat-Gourbeyre, F., Clément, C. (2009). Microspore embryogenesis and programmed cell death in barley: Effects of copper on albinism in recalcitrant cultivars. *Plant Cell Rep.*, **28**, 1329–1339.

Jewell, A. W., Murray, B. G., Alloway, B. J. (1988). Light and electron microscope studies on pollen development in barley (*Hordeum vulgare* L.) grown under copper-sufficient and deficient conditions. *Plant Cell Environ.*, **11** (4), 273–281.

Kasha, K. J., Maluszynski, M. (2003). Production of doubled haploids in crop plants. In: Maluszynski, M., Kasha, K. J., Forster, B. P., Szarejko, I. (eds.). *Doubled Haploid Production in Crop Plant (*pp. 1–4). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Kunz C., Islam, S. M. S., Berberat, J., Peter, S. O., Büter, B., Stamp, P., Schmid, J. E. (2000). Assessment and improvement of wheat microspore derived embryo induction and regeneration. *J. Plant Physiol.,* **156,** 190–196.

Maksymic, W. (2007). Effects of methyl jasmonate and excess copper on root and leaf growth. *Biol. Plant.*, **51**, 322–326.

Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.,* **15** (3), 473–497.

Murovec, J., Bohanec, B. (2012). Haploids and doubled haploids in plant breeding. In: Abdurakhmonov, I. Y. (ed). *Plant Breeding* (pp. 87–101). Rijeka: InTech.

Nichterlein, K. (2003). Anther culture of linseed *(Linum usitatissimum)*. In: Maluszynski, M., Kasha, K. J., Forster, B. P., Szarejko, I. (eds.). *Doubled Haploid Production in Crop Plants* (pp. 249–254). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Pauk, J., Mihaly R., Puolimatka M. (2003). Protocol for wheat (*Triticum aestivum* L.) anther culture. In: Maluszynski, M., Kasha, K. J., Forster, B. P., Szarejko, I. (eds.). *Doubled Haploid Production in Crop Plants (*pp. 249–254). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Rubtsova, M., Gnad, H., Melzer, M., Weyen, J., Gils, M. (2012). The auxins centrophenoxine and 2,4-D differ in their effects or non-directly induced chromosome doubling in anther culture of wheat (*T. aestivum* L.). *Plant Biotechnol. Rep.*, 1–9. DOI 10.1007/s11816-012-0256-x.

Tadesse, W., Inagaki, M., Tawkaz, S., Baum, M., van Ginkel, M. (2012). Recent advances and application of doubled haploids in wheat breeding. *African J. Biotechnol.*, **11** (89), 15484–15492.

Torp, A. M., Hansen, A. L. (2001). Chromosomal regions associated with green plant regeneration in wheat (*Triticum aestivum* L.) anther culture. *Euphytica*, **119**, 377–387.

Touraev, A., Vicente, O., Heberle-Bors, E. (1997). Initiation of microspore embryogenesis by stress. *Trends Plant Sci.*, **2** (8), 297–302.

Tuvesson, S., von Post, R., Ljungberg, A. (2003). Wheat anther culture. In: Maluszynski, M., Kasha, K. J., Forster, B. P., Szarejko I. (eds.). *Doubled Haploid Production in Crop Plants* (pp. 71–76). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Wojnarowiez, G., Jacquard, C., Devaux, P., Sangwan, R.S., Clément, C. (2002). Influence of copper sulfate on anther culture in barley (*Hordeum vulgare* L.). *Plant Sci.*, **162**, 843–847.

Xynias, I. N., Zamani, I. A., Gouli-Vavdinoudi, E., Roupakias, D. G. (2001). Effect of cold pretreatment and incubation temperature on bread wheat (*Triticum aestivum* L.) anther culture. *Cereal Res. Comm.*, **29** (3–4), 331–338.

Zamani, I., Gouli-Vavdinoudi, E., Kovacs, G., Xynias, I., Roupakias, D., Barnabás, B. (2003). Effect of parental genotypes and colchicine treatment on the androgenic response of wheat F1 hybrids. *Plant Breed.,* **122** (4), 314–317.

Озолиня Г. Р., Лапиня Л. П. (1971). Особенности физиологического действия меди при обогащении ею семян [Ozoliņa, G., Lapiņa, L. Particularities of physiologic influence of copper during the seeds enrichment]. В кн.: Пейве Я. В., Жизневская Г. Я., Айзупиет И. П., Озолиня Г. Р., Упитис В. В. (ред.). *Микроэлементы — регуляторы жизнедеятельности и продуктивности растений* (с. 65–92). Рига: Зинатне (in Russian).

Рашаль И. Д., Озолиня Г. Р., Лапиня Л. П. (1987). Зависимость продольного роста корней ячменя от генотипа при воздействии солями меди и серебра [Rashal, I. D., Ozolinya, G. R., Lapinya L. P. Dependence of the longitudinal growth of barley roots on the genotype under the effect of copper and silver salts]. *Физиология и биохимия культурных растений*, **19** (5), 505–509 (in Russian, summary in English).

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PUTEKŠŅU KULTŪRAS EFEKTIVITĀTE GRAUDAUGU DUBULTOTO HAPLOĪDU IZVEIDOŠANĀ

Darba mērķis bija uzlabot dubultoto haploīdu (DH) līniju iegūšanas metodiku ar putekšnīcu kultūru palīdzību Latvijā svarīgiem graudaugiem: miežiem, vasaras un ziemas kviešiem. Kā izejas materiāls pētījumam kalpoja F_1 un F_2 paaudzes selekcionāru iegūtie hibrīdi. Pētījuma rezultāti parādīja, ka vara sulfāta pievienošana koncentrācijā 2,5 mg/l miežu pirmapstrādes barotnei un gan miežu (C3), gan kviešu (šķidrā AMC) kallusu indukcijas barotnēm paaugstina zaļo augu-reģenerantu iznākumu. Liela skaita DH līniju iegūšanu ar putekšnīcu kultūras palīdzību var organizēt divos etapos: pirmajā nosaka hibrīdu atsaucību putekšnīcu kultūrā un otrajā masveidā izmanto tikai atlasītos hibrīdus ar augstu spēju veidot zaļus augus-reģenerantus.