International Journal for Research in Applied Sciences and Biotechnology

ISSN: 2349-8889
Volume-7, Issue-6 (November 2020)

www.ijrasb.com

https://doi.org/10.31033/ijrasb.7.6.2

Transferring of Hybrid F1 of Spring Wheat into Dihaploid Basis Using Andgrogen Thechnologies

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ABSTRACT

Anther culture is the most technologically advanced method of obtaining doubled haploids. Dihaploid lines are required to speed up the breeding process. From 7 hybrids of first-generation (F1) of spring wheat, 15 dihaploid culture lines were obtained by anther culture. An average of 23.7 embryo-like structures was obtained on 150 anthers. The percentage of plant regeneration was 49.1%, of which there were 39.9% albino plants and 9.1% green plants. The percentage of spontaneous chromosome doubling among the obtained green plants was 35.7.

Keywords- Anthogenesis, Anther Culture, Embryogenesis, Regeneration.

I. INTRODUCTION

Haploid is one of the most popular tissue culture methods in plant breeding. This method ensures the rapid and effective achievement of plant homozygosity. The main selection advantage of using haploids comes from the possibility of one-stage obtaining homozygotes, which allows you to quickly fix the morph physiological parameters of adaptability and shorten the time for creating varieties adapted to the harsh conditions of Kazakhstan and numerous diseases, capable of consistently forming high grain yields and meeting all the needs of the modern market.

In haploids, each gene is represented by a single allele, and the recessive alleles of some genes appear along with the dominant alleles of others. Genetic cleavage when using haploids is less difficult (in fact, it does not exceed the number of gamete classes) and a relatively small population is needed to isolate a certain combination of genes [1].

To obtain wheat haploids in mass quantities, methods of distant hybridization with subsequent selective elimination of chromosomes of the pollinator species and androgenesis methods are used. Anther cultures and isolated microspores are the most technologically advanced androgenetic methods today. These are reliable and effective methods for producing doubled haploids.

In this work, the method of anther culture is used to obtain dihaploid homozygous wheat lines from the hybrid F1 generation in order to accelerate the breeding process.

II. METHODOLOGY

7 hybrids of the first generation (F1) of spring soft wheat, obtained by the laboratory of biotechnology of the Kazakh Research Institute of Agriculture and Crop Production, in consultation with breeders, served as the material for research.

Research methods donor plants for androgenic wheat technology were grown at the field station of the department of grain crops of Kazakh Research Institute of Agriculture and Crop Production.

Immature inflorescences were taken from donor wheat plants, in the phase of the flag leaf, which did not come out of the sheath, with microspores at the middle and late mononuclear stages of development. Spring wheat plants of the first sowing date approached the required flag leaf phase on June 2-4, 2019.

Assessment of the stage of development of microspores was determined according to the generally accepted technique of temporary squashed preparations [2].

To increase the frequency of calli release and spontaneous doubling of chromosomes, the ears of donor plants were subjected to cold stress in a refrigeration unit at a temperature of +2 - + 3 ° for 14 days [3].

Wheat ears that have undergone cold treatment were sterilized with 20% NaOCl solution with a drop of Tween-80 for 10 minutes on a shaker, and then washed three times with sterile distilled water in a laminar flow hood, 3 minutes each.

Anthers were cultivated according to Rubtsova et al., 2012 [4]. Anthers were isolated from the spike under aseptic conditions and placed in plastic Petri dishes 60 mm in diameter (100-150 anthers / Petri dish), which contained 6 ml of liquid culture medium for induction. The anthers were incubated in the dark at 32 ° C for the first 3 days, and then transferred to a thermostat with a temperature of 28 ° C until neoplasms appeared.

During the isolation process and after transfer to the culture medium, the state of microspores was observed on a Meiji Techno MT4000 microscope.

Determination of ploidy was carried out by the method of squashed preparations on the roots of 2-3 day old seedlings [2].

For the induction of embryogenesis, the AP medium was used, developed in the framework of the Kazakh-Australian project (Ismagul A. et al., 2013) with

the addition of 50 g of Ficoll 400 [5].

Regeneration was performed using standard MS medium supplemented with kinetin 2 mg / l, zeatin 3 mg / l, 30 g / l sucrose, and 3 g / l PhytogelTM.

For root formation, standard MS medium supplemented with 0.5 g / L of casein hydrolyzate, 20 g / L of sucrose, 2 mg / L of IAA, 4 g / L of PhytogelTM.

All green plants at the three-leaf stage were transplanted into potting soil. Peat, vermiculite and sand (1: 1: 1) were taken for the soil.

The planted plants were placed in climatic chambers, where conditions were created for their adaptation - the temperature regime was maintained at 23-24 ° C, lighting at 8-10 thousand. lux and 80% humidity. During the first two weeks (adaptation period), the regenerated plants were sprayed with a phytohormone solution.

III. OUR APPROACH

After the introduction of anthers into an in vitro culture on a liquid nutrient medium, cytological observations were carried out for 4 weeks, counting the division of microspores and the development of calli and embryo structures. The observation results showed that the spontaneous release of microspores from the pollen sac into the nutrient medium proceeded very quickly and amounted to 75-85%. Cytological observations of the state of microspores showed a high percentage of microspore viability (70-80%) on the first and second days. Subsequently (7 days), the percentage of microspore viability decreased to 27-35%.

The yield of embryo- structures (ES) varied from 2 to 110 ES / 150 anthers from one Petri dish, Figure 1.

The results of the assessment of embryogenesis in the culture of wheat anthers of 7 hybrids of the first generation are presented in Table 1. The highest level of formation of embryoid structures among the studied wheat hybrids was recorded in the F 1 Maira x Baiterek hybrid, where 186 ES were formed, which averaged 46.5 ES per Petri dish. The hybrid combination Maira x Chelyaba 2, in which the formation of ES 42.5 / Petri dish, was also shown to be highly responsive to the anther culture.

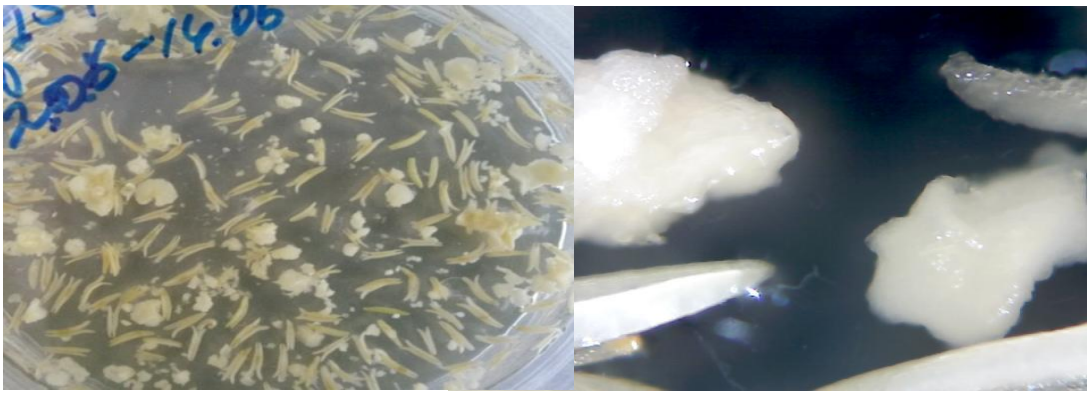

Figure 1: Embryogenesis in the anther culture of the F1 hybrid Mayra x Bayterek 1 on the induction medium (AP with Ficoll).

Table 1: The results of the assessment of embryogenesis and regeneration of plants of the first-generation wheat hybrids in the anther culture.

Name	Number of planted anthers, pcs	Total number of formed ES / average number per 150 anthers, pcs	Number of transplanted ES, pcs	Plant regeneration, pcs	
				albino	green
South 12 x Karabalyk 92	600	6/3	0	0	0
Tertsiya x Baiterek	400	0	0	0	0
Mayra x Chelyaba2	600	170/42,5	151	45	17
Mayra x Baiterek	600	186/46,5	124	64	12
Simbirskaya 17 x Akmola 2	600	58/29	29	12	0
Simbirskaya 17 x MIS	600	93/23,5	81	21	7
Irtysh 97 x TR1	600	80/20	73	41	6
Total		595/23,7	458	183	42

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In the F1 hybrid Tertia x Baiterek, the formation of ES was not recorded. The hybrid South 12 x Karabalykskaya 92 showed a low responsiveness to the anther culture technology, in which, on average, from 0 to 6 ES from one Petri dish was formed from microspores as a result of division.

Embryo structures that reached 2-2.5 mm were transplanted onto a medium for regeneration in Petri dishes of 90 mm in diameter in an amount of 18-20 ES. Smaller ES were left in the medium for further growth. In general, according to experience, 458 ES were transplanted onto solid medium for regeneration.

Evaluation of the regeneration of the first generation hybrids showed that the total yield of albino plants (chlorophyll-free seedlings) was 183 pcs, which is 39.9%. Observations and analysis of the yield of green plants showed that 42 green plants have been obtained in the entire experiment, which is 9.1%. All green plants - regenerates were transplanted onto a medium for rooting (Figure 2). Albino plants were discarded.

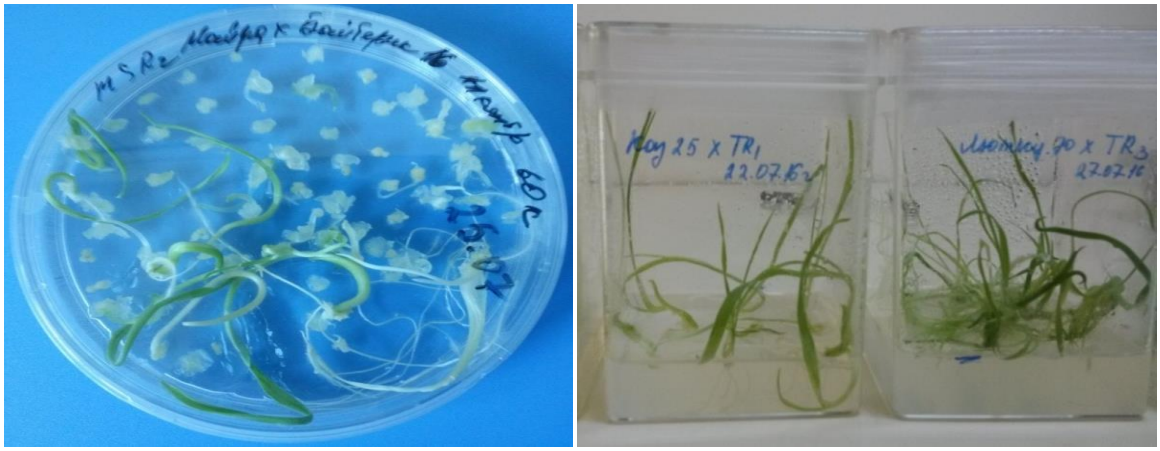

Figure 2: Regeneration of green wheat plants

The resulting green plants (42) were subjected to ploidy determination. Before planting in the ground, the roots of the plants were split off and ploidy was determined by the method of crushed preparations [2]. Determination of ploidy in the resulting 42 regenerated spring wheat plants showed that spontaneous doubling was recorded in 15 plants, which is 35.7%. 27 green plants are haploid.

Dihaploid plants were placed in climatic chambers, where conditions were created for their adaptation - the temperature regime was maintained at 23-24 ° C, lighting at 8-10 thousand lux and 80% humidity. During the first two weeks (adaptation period), the regenerated plants were sprayed with a phytohormone solution.

The level of adaptation was high. All 15 dihaploid plants adapted to the ground.

IV. CONCLUSION

According to the results of the induction of embryogenesis of 7 hybrids of the first generation (F1) of spring soft wheat, 595 embryo structures were obtained in the anther culture. The yield of embryoid structures per 150 anthers averaged 23.7.

The percentage of regeneration of wheat plants from calli and embryoid structures was 49.1%. From the transplanted 458 ES, 183 albino plants (39.9%) and 42 green plants (9.1%) were obtained.

The analysis to determine the ploidy in the obtained 42 green regenerated plants of spring wheat showed that spontaneous doubling was recorded in 15 plants, which is 35.7%. 27 green plants were haploid.

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