DOI: 10.12731/2658-6649-2025-17-6-2-1542 EDN: NOSNRM
UDC 633.18: 57.085.1: 57.017.64: 631.527.6: 631.524.5

Original article

ACCELERATED DEVELOPMENT OF RICE POPULATIONS USING ANTHER CULTURE *IN VITRO* METHOD

E.G. Savenko, V.A. Glazyrina, L.A. Shundrina, Zn.M. Mukhina, L.V. Esaulova

Abstract

Background. Inclusion of doubled haploids in the breeding process allows increasing the speed and reliability of selection of desired forms with smaller population volumes. To stabilize the genotype, anthers of hybrid combinations of F_2, F_3, F_4 and F_5 generations, developed in crossings of white-grained samples with traits of high nutritional value of grain, and anthers of combinations of F_4 generation from crossings of varieties contrasting in pericarp color and amylose content, were introduced into the culture. The responsiveness to gamete technologies was studied in 21 hybrid combinations. New genetically stable material (DH - doubled haploids) was developed. Phenotyping was carried out in conditions of a vegetation experiment for economic and biological traits and elements of plant productivity in four populations, which included 45 DH lines. Variability was noted in a number of traits within the DH line populations. Based on the results of the biometric analysis of the regenerated populations, 6 sources with a "1000 grain mass" of more than 30 grams were identified.

The study was carried out with the financial support of the Kuban Science Foundation and the Russian Science Foundation within the framework of the scientific project No. 25-16-20103 "Application of the genomic approach in rice breeding for high technological grain quality"

Purpose. To study the response of hybrids obtained from crossing contrasting rice samples to in vitro pollen culture, to accelerate the creation of DH line populations based on the studied genotypes, and to phenotype them.

Materials and methods. The research was conducted at the Laboratory of Biotechnology and Molecular Biology at the Federal Research Center for Rice, using the culture of isolated anthers in vitro according to the generally accepted method of R.G. Butenko (1990).

Results. The genetic determinism of the "regeneration" trait in the donor plants used in the crossbreeding was noted. The genotypes of the crossbreeding involving the varieties Favorit and Azovsky proved to be the most productive in terms of the output of highly morphogenic calluses and androgenic lines. Phenotypic analysis revealed significant diversity among the plants in the individual DH populations in terms of the shape of the panicle, the angle of the flag leaf deviation, the length of the growing season, the weight of 1,000 grains, the height of the plants, and the length of the growing season.

Conclusion. Phenotypic analysis of the DH lines' traits showed that their genesis originates from microspores, thus these lines are a valuable genetic resource. The inclusion of doubled haploids in the breeding process will help to facilitate the assessment of recombinant genotypes arising from the cross, will allow to detect rare recessive alleles, will increase the speed and reliability of the selection of desired forms with smaller population volumes.

Keywords: Oryza sativa L. rice; in vitro pollen culture; doubled haploid (DH) lines; economic and biological traits; productivity elements

For citation. Savenko, E. G., Glazyrina, V. A., Shundrina, L. A., Mukhina, Zn. M., & Esaulova, L. V. (2025). Accelerated development of rice populations using anther culture *in vitro* method. *Siberian Journal of Life Sciences and Agriculture*, *17*(6-2), 149-164. https://doi.org/10.12731/2658-6649-2025-17-6-2-1542

Научная статья

УСКОРЕННОЕ РАЗВИТИЕ ПОПУЛЯЦИЙ РИСА С ИСПОЛЬЗОВАНИЕМ КУЛЬТУРЫ ПЫЛЬНИКОВ

Е.Г. Савенко, В.А. Глазырина, Л.А. Шудрина, Ж.М. Мухина, Л.В. Есаулова

Аннотация

Обоснование. Включение удвоенных гаплоидов в селекционный процесс способствует повышению скорости и надежности отбора желаемых форм при меньших объемах популяции. Для стабилизации генотипа в культуру введены пыльники гибридных комбинаций F_2, F_3, F_4 и F_5 поколений, созданных в скрещиваниях белозерных образцов с признаками высокой пищевой ценности зерна и пыльники комбинаций F_4 поколения от скрещиваний сортов контрастных по окраске перикарпа и содержанию амилозы. Изучена отзывчивость на гаметные технологии у 21 гибридной комбинации. Выделены отзывчивые на

культуру пыльников *in vitro* генотипы. Создан новый генетически стабильный материал. В условиях вегетационного опыта изучены морфологические признаки растений 45 удвоенных гаплоидных линий риса четырех популяций. Отмечена вариабельность по ряду признаков внутри популяций DH линий. По результатам биометрического анализа регенерантных популяций выделено 6 источников с «Массой 1000 зерен» более 30 грамм.

Цель. Изучение отзывчивости гибридов, полученных от скрещивания контрастных образцов риса, на культуру пыльников *in vitro,* ускоренное создание популяций DH линий на основе изучаемых генотипов и их фенотипирование.

Материалы и методы. Исследования проводились в лаборатории биотехнологии и молекулярной биологии ФГБНУ «ФНЦ риса» с использованием культуры изолированных пыльников *in vitro* по общепринятой методике Бутенко Р.Г. (1990).

Результаты. Отмечена генетическая детерминированность по признаку «регенерация» вводимых в скрещивания растений – доноров. Наиболее продуктивными по выходу высоко морфогенных каллусов и андрогенных линий оказались генотипы скрещивания с участием сортов Фаворит и Азовский. Фенотипический анализ выявил существенное разнообразие растений в линиях индивидуальных популяций DH по форме метелки, углу отклонения флагового листа, длине вегетационного периода, массе 1000 зерен, высоте растений и длине вегетационного периода.

Заключение. Фенотипический анализ признаков линий DH показал, что их генезис происходит от микроспор, таким образом, эти линии являются ценным генетическим ресурсом. Включение удвоенных гаплоидов в селекционный процесс поможет облегчить оценку рекомбинантных генотипов, возникающих в результате скрещивания, позволит обнаружить редкие рецессивные аллели, повысит скорость и надежность отбора желаемых форм при меньших объемах популяции.

Ключевые слова: рис *Oryza sativa* L.; культура пыльников in vitro; удвоенные гаплоидные (DH) линии; хозяйственно биологические признаки; элементы продуктивности

Для цитирования. Савенко, Е. Г., Глазырина, В. А., Шудрина, Л. А., Мухина, Ж. М., & Есаулова, Л. В. (2025). Ускоренное развитие популяций риса с использованием культуры пыльников. *Siberian Journal of Life Sciences and Agriculture*, *17*(6-2), 149-164. https://doi.org/10.12731/2658-6649-2025-17-6-2-1542

Introduction

To select varieties with economically valuable traits, it is necessary to replenish the genetic base using not only hybridization, but also new biotechno-

logical methods. These methods make it possible to create lines with a high level of homozygosity in one year, instead of 8-10 years. Currently, there is an urgent need to create varieties with accelerated breeding traits. [1].

The efficiency of breeding can be increased by stabilizing the genetic material using gamete technologies *in vitro*.

Doubled haploid lines play an important role in breeding due to their phenotypic homogeneity, wide range of variations, and the manifestation of both dominant and recessive traits. [2-5].

The offspring of regenerants is studied to identify new phenotypic traits and gametoclonal variations [6; 7]. Gametoclonal variability is observed in many economically valuable traits, including biochemical ones. Most often, genetic variability manifests itself in changes in the level of ploidy, chromosome and gene mutations. This phenomenon is successfully used by breeders in programs aimed at developing varieties with improved characteristics. The use of doubled haploids in breeding has a positive effect [8]. Useful variations in individual traits and 100% homozygosity have been found in androgenic lines of wheat, potatoes, teff, and rice. Based on gametoclonal variants, the Dam variety, which is resistant to pyricularia and has good culinary qualities, has been developed. The Federal Research Center for Rice has studied a variety of factors to develop an effective protocol for the regeneration of rice anthers in vitro in the japonika and indika subspecies. The effectiveness of the rice anther culture in vitro scheme has been confirmed by practical results: thousands of doubled haploids of rice have been created, which have been studied by the breeding departments of the research center in the field to test their morphological uniformity and evaluate their economically important characteristics. Based on the selected homozygous DH lines, the following rice varieties have been registered in the State Register of Breeding Achievements of the Russian Federation, zoned, and approved for use in the North Caucasus region: Sonet, Sonata, Privolny 4, Ivushka, and Vodopad. When cultivated in the Krasnodar rice-growing zone, these varieties consistently demonstrate high yields, resistance to lodging, shedding, and pyricularia, as well as excellent grain quality. The culture of gametes in vitro includes two stages. The first stage is the induction of organo/embryogenic calluses, and the second stage is the regeneration of green plants [9]. This strategy helps to reduce the selection process, increases the efficiency of selection, and increases genetic diversity [10]. DH populations do not split and are used in various molecular genetic studies for mapping fixed homozygotes [11; 12]. They are also an ideal material for genetic mapping of morphological and complex traits [13].

Thus, plants derived from gametes have characteristics that differ from those of their donors, making them attractive for use in breeding programs and genetic engineering [14; 15].

The goal of the research was to study the responsiveness of hybrids obtained from crossing contrasting rice samples to *in vitro* anther culture and to accelerate the development of DH line populations based on the studied genotypes.

Purpose. To study the response of hybrids obtained from crossing contrasting rice samples to in vitro pollen culture, to accelerate the creation of DH line populations based on the studied genotypes, and to phenotype them.

Materials and methods

The research was conducted at the Federal State Budgetary Scientific Institution "Federal Scientific Rice Centre" using standard methods according to Butenko R.G. (1990) [16]. To stabilize the genotype, the culture includes the anthers of hybrid combinations of F2, F3, F4, and F5 generations, created by crossing white-grained samples with high grain nutritional value, and the anthers of F4 combinations from crossing varieties with contrasting pericarp color and amylose content. The anthers were inoculated under aseptic conditions in a laminar flow hood using the Blades basic agar medium with phytohormones (17). Calluses were cultured on Murashige and Skoog medium until green shoots appeared, which were transferred to a hormone-free MS medium for rooting (18).

Results

To stabilize the genotype, anthers of hybrid combinations of F_2, F_3, F_4 and F_5 generations, developed in crossings of white-grained samples with traits of high nutritional value of grain, and anthers of combinations of F_4 generation from crossings of varieties contrasting in pericarp color and amylose content, were introduced into the culture. Anthers were inoculated onto Blaydes induction medium (0-passage) with an increased content of 2,4-D (4.0 mg/l) until calli appeared, then calli were transferred to Blaydes subcultivation medium with a reduced concentration of 2,4-D (2.0 mg/l), enriched with proline 500.0 mg/l and glutamine 500.0 mg/l. Cultivated for 20-30 days (1st passage). For further incubation and stimulation of regeneration processes, the calli were transferred to MS medium + 1.0 mg/l α-NUA + 5.0 mg/l kinetin + 500.0 mg/l proline + 500.0 mg/l glutamine + 30.0 g/l sucrose.

When cultivating anthers of rice hybrids of F_2, F_3, F_4 and F_5 generations, developed by crossing white-grained samples with traits of high nutritional value of grain, high responsiveness of all studied genotypes for the trait of callusogen-

esis induction was noted. The maximum result (13.72%) was shown by the F_5 hybrid Yakhont / Azovsky. The minimum indicator was demonstrated by the F_4 hybrid Atlant / Sprint - 2.35% ($LSD_{0.05}$ = 0.48) (see Fig. 1).

The formed callus lines had a high regenerative capacity. Genetic determination by the "regeneration" trait of the donor plants introduced into the crossing was noted. The most productive in terms of the yield of androgenic lines were the genotypes of the crossing involving the varieties Favorit and Azovsky: F_3 Favorit / Azovsky (60 lines), F_3 Favorit / Sonet (31 lines), F_3 Apollon / Favorit (49 lines), F_4 Atlant / Azovsky (14 lines), F_2 Neve / Azovsky (79 lines), F_5 Yakhont / Azovsky (8 lines).

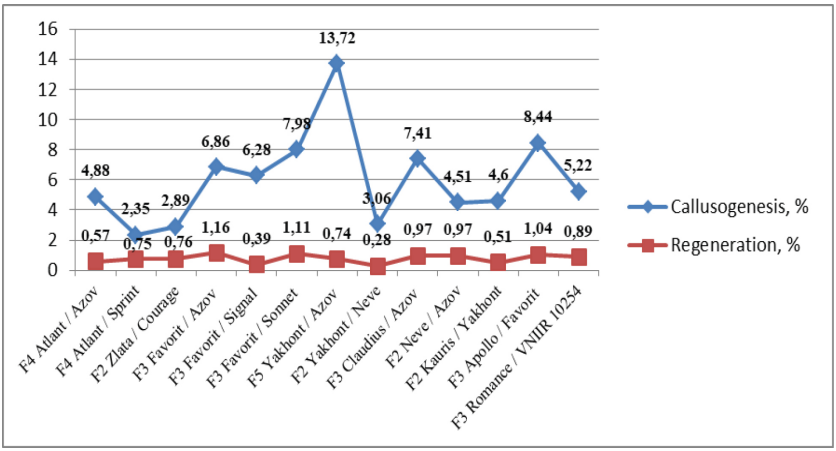

Fig. 1. Callusogenesis and regeneration in anther culture *in vitro* of hybrid rice combinations of F_2, F_3, F_4 and F_5 generations developed in crossing white-grained samples with traits of high nutritional value of grain

When cultivating anthers of rice hybrids of the F_4 generation from crossing samples contrasting in pericarp color and amylose content, all samples also showed high responsiveness to callus induction from 5.42% to 20.39% (see Fig. 2).

The maximum result of callusogenesis was noted in genotypes involving the variety Violetta in the crossing: F_4 Violetta / Zlata and F_4 Violetta / CRLB-1 −17.10% and 20.39%, respectively. The indicators for these genotypes were at the same level (LSD_{05} = 3.24).

2950 morphogenic callus lines were obtained. During the cultivation, 52.8% of the callus lines were realized by gemmogenesis and gemmorhizogenesis into 1557 full-fledged plants (see Fig. 3).

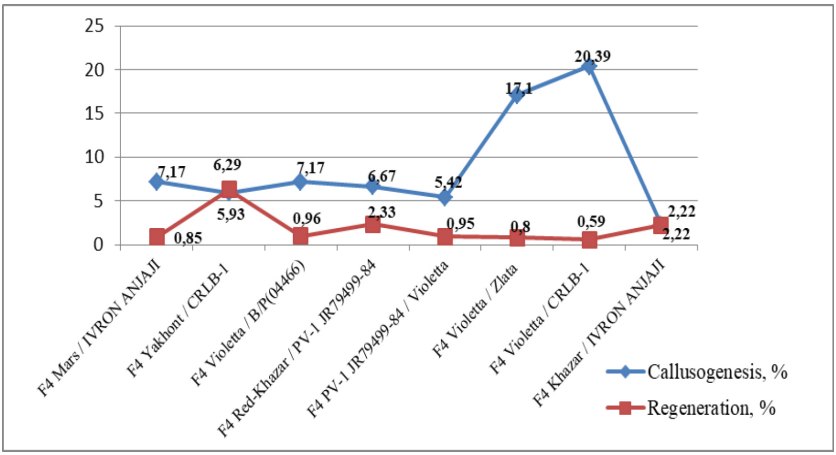

Fig. 2. Callusogenesis and regeneration in anther culture *in vitro* of hybrid combinations of rice F_4 generation from crossings rice samples contrasting in pericarp color and amylose content

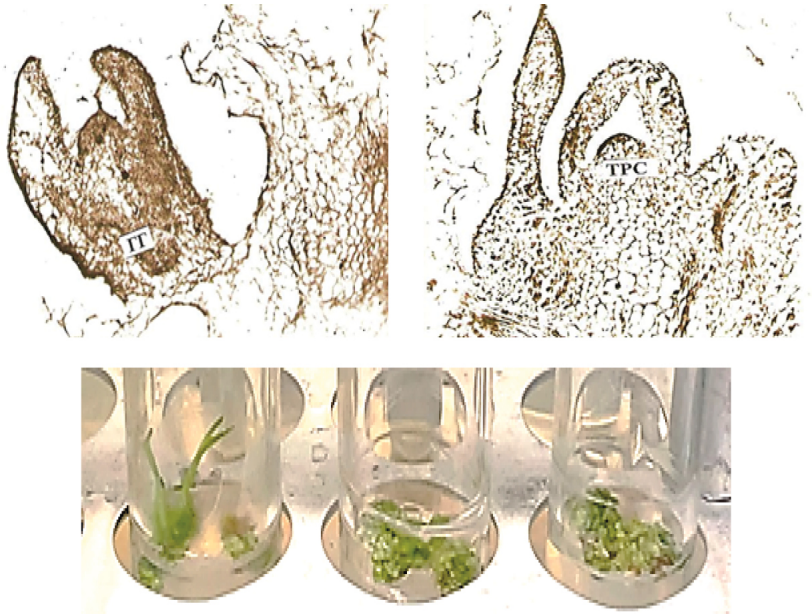

Fig. 3. Gemmogenesis processes in rice callus F_5 hybrid Yakhont / Azov (macro photo)

Regenerants were planted in specialized plant growth chambers under conditions of high humidity and controlled temperatures to adapt to *ex vitro* conditions. After spontaneous chromosome doubling in some callus lines, the cells proliferated into doubled haploids, which produced seed progeny.

Under the conditions of a pot experiment, phenotyping of plants from four populations, which included 45 DH lines, was carried out for a number of vegetative, morphological and biological traits.

Phenotypic analysis in DH line populations revealed significant diversity in panicle shape. In the F_1 Violetta/Zlata population and in the F_1 Violetta/CRLB-1 population, all plants had an upright compact panicle. In the F_1 Mars/IVRON ANJAJI population, plants with an upright compact panicle, with a weakly spreading panicle, and lines with a weakly spreading inclined panicle were identified. In the F_1 Violetta/B/P (04466) population, various forms of a weakly spreading panicle were noted in the lines: drooping, inclined, vertical (see Fig. 4).

F_1 Violetta / B/R (04466) F_1 Mars / IVRON ANJAJI F_1 Violetta/ CRLB-1

Fig. 4. Variability in the shape of panicles in populations of doubled haploid lines

Within the populations, the lines varied in the length of the growing season; early ripening lines– 110 days, mid-ripening lines – 112 days, and late ripening lines – 130 days were identified. No lines with a growing season of less than 100 days were identified.

Plants of lines within populations differed in the angle of deviation of the flag leaf; there were individuals with a semi-vertical angle of 30-45^0, an intermediate angle of 50-70^0, and a horizontal angle of 80-90^0. DH lines with a vertical

angle of deviation of the flag leaf from the stem of up to 30^0 were identified, and line No. 5 DH F1 Violetta / B/P (04466) had an angle of 20^0.

Dwarfy lines (60-70 cm) were identified. Tall plants (125-135 cm) were noted in all lines of the F_1 Mars/IVRON ANJAJI population. Most of the plants in the populations were medium-sized, from 80 to 102 cm, with a low mass of 1000 grains (20-25 g). The F_1 Violetta/CRLB-1 population lines had a high mass of 1000 grains - over 30 g (min – 32.1 g, max – 35.4 g (LSD 1.2)); these lines also had a strong stem resistant to lodging (1 point).

Discussion

The main limiting factor in obtaining a large number of androgenic plants is the genotype. To overcome this problem, it is necessary to develop a reliable and efficient system for pollen culture regeneration, which will allow the production of genetically stable rice lines with targeted traits from hybrids. Although cultured cells are usually able to synthesize all the necessary amino acids, the addition of proline and glutamine to the medium can increase the growth rate of cells. It has been reported that the use of proline and glutamine amino acids in the medium has a positive effect on the frequency of callus formation and regeneration of rice (Chowdhry et al., 1993; Ge et al., 2006; Shahsavari, 2011). The addition of proline and glutamine to the induction medium reduced the time from anther inoculation to callus formation and improved the quality of the callus. This is because amino acids are an easily accessible source of nitrogen for growing tissue cells and can maintain a high rate of callus cell growth for an extended period. On callus obtained on medium supplemented with proline and glutamine, the intensity of green plant regeneration was higher. This is in line with the studies of Chowdhry et al. (1993) and Shahsavari (2011), who also reported that these amino acids increase the percentage of plant regeneration. In our experiment, all rice genotypes were responsive to anther culture. However, the frequency of callus formation and plant regeneration varied greatly among the genotypes. Ge et al. (2006) also reported different potential of rice genotypes in inducing callus and regeneration.

The maximum callus formation rates were noted in the F_5 Yakhont/Azov hybrid (13.7%) and genotypes involving the variety Violetta variety in the crossing: F_4 Violetta/Zlata and F_4 Violetta/CRLB-1 - 17.10% and 20.39%, respectively, which was associated with the high combining ability of the crossing samples for the “callus formation” trait.

The ability to regenerate depended on the quality and degree of development of the callus tissues, as well as the presence of auxin-type hormones in

the nutrient medium, as at a certain stage, their cells no longer needed certain auxins, as they began to produce them on their own.

During long-term cultivation of calluses on inducing media without subculturing and subsequent transfer to regeneration media, the cells of androgenic structures (gemm) stopped proliferating and underwent dedifferentiation, i.e., the differentiation of the morphogenic complex stopped, and the cells transformed from meristematic to parenchymatous. The studies have shown that it is particularly successful to cultivate the anthers until calluses appear, followed by subculturing the newly formed calluses on a nutrient medium with a reduced concentration of 2,4-D for 20-30 days, and then culturing them for 30-45 days on regeneration media, which ensures maximum yield of regenerated plants.

The analysis of economically valuable traits and productivity elements of androgenic lines revealed their variability among populations. The similarities and differences in the morphological traits of DH lines in populations were expressed in the uniformity of plants within a single line and the diversity in economic and biological characteristics and biometric traits between lines within a single population.

The biometric trait "mass of 1000 grains" is an important element of productivity, negatively correlating with the number of spikelets on a panicle and positively - with the yield of the variety. In the studied samples, the value of the trait "mass of 1000 grains" varied from 17.4 to 35.4 grams. All domestic rice varieties have an average grain size (26-30 grams). Based on the results of the biometric analysis of regenerated populations, 6 sources of large grains with a "mass of 1000 grains" over 30 grams, with an average panicle length from 13.5 to 14.9 cm were identified, all of them are lines of the F_1 Violetta / CRLB-1 population.

According to breeders, a highly productive rice morphotype should have an erectoid (up to 15^0) or vertical arrangement of leaves ($20\text{-}30^0$). Thus, the development of samples with such an angle of deviation of the flag leaf from the stem is an important trait, since varieties with vertical leaves allow thickening crops and increasing productivity. During phenotyping, variation of this trait in populations from $80\text{-}90^0$ (horizontal) to $20\text{-}30^0$ (vertical) was noted. Forms with an erectoid arrangement of leaves were not identified.

Most DH lines developed on the basis of anther culture had a compact vertical panicle, which domestic breeders prefer in the original forms, which is associated with the weather and climatic conditions of rice cultivation in Russia.

Conclusion

The obtained data of biometric analysis and economic-biological characteristics indicate gametoclonal variability in populations of doubled haploids,

which is based on the isolated anthers culture *in vitro*, which contributes to the development of genetic diversity of the source material. Phenotypic analysis of the DH lines showed that their genesis comes from microspores. Thus, these lines are a valuable genetic resource. Inclusion of doubled haploids in the breeding process will help to facilitate the evaluation of recombinant genotypes arising from crossing, will allow detection of rare recessive alleles, will increase the speed and reliability of selection of desired forms with smaller population volumes. In self-pollinating cereals, one homozygous genotype often carries successful single traits or combinations of traits and can be used to improve the variety or potentially become a new variety.

References

1. Dwivedi, S. L. (2015). Haploids: Constraints and opportunities in plant breeding. *Biotechnology Advances*, *33*, 812–829. https://doi.org/10.1016/j.biotechadv.2015.07.001
2. Wedzony, M., Foster, B. P., Zur, I., Golemiec, E., Szechynska-Hebda, M., Dubas, E., & Gotebiowska, G. (2009). Chapter 1. Progress in doubled haploid technology in higher plants. In: *Advances in haploid production in higher plants* (pp. 1–35). Springer Science + Business Media B.V.
3. Weyen, J., Touraev, A., Foster, B. P., & Jain, E. M. (2009). Chapter 15. Barley and wheat doubled haploids in breeding. In: *Advances in haploid production in higher plants* (pp. 179–189). Springer Science + Business Media B.V.
4. Urazaliev, K. R., Orsini, Kh. M., Abekova, A. M., Bazylova, T. A., & Daniyarova, A. K. (2013). Accelerating wheat breeding using dihaploids obtained by microspore culture. *Bulletin of KazNU. Ecological Series*, *2/2*(38), 369–374.
5. Mishra, R., Gundimeda, J., & Narashima, R. (2016). In vitro androgenesis in rice: Advantages, constraints and future prospects. *Rice Science*, *23*, 57–68. https://doi.org/10.1016/j.rsci.2016.02.001
6. Bernardo, R. (2009). Should maize double haploids be induced among F1 or F2 plants? *Theoretical and Applied Genetics*, *119*, 255–262. https://doi.org/10.1007/s00122-009-1034-1. EDN: https://elibrary.ru/EKWFIE
7. Zheng, M. Y. (2003). Microspore culture in wheat (*Triticum aestivum*): Doubled haploid production via induced embryogenesis. *Plant Cell, Tissue and Organ Culture*, *73*, 213–230. EDN: https://elibrary.ru/EQLTAR
8. Maluszynski, M., Kasha, K. J., & Szarejko, I. (2003). Published doubled haploid protocols in plant species. In: *Doubled haploid production in crop plants* (pp. 309–335). Springer: Dordrecht, the Netherlands. https://doi.org/10.1007/978-94-017-1293-4_46. ISBN 978-94-017-1293-4. EDN: https://elibrary.ru/XWCXXB

9. Li, W., Gang, L., Deming, Z., Feng, W., & Jiabin, C. (2011). Tissue culture system for different hybrid of indica rice. *Northeast Agricultural University Journal*, *18*, 13–17.
10. Wren, J., Wu, P., Trumpe, B., Tian, H., Lubberstedt, T., & Chen, S. (2017). New technologies in the extraction of doubled haploid lines. *Plant Biotechnology*, *15*, 1361–1370.
11. Forster, B. P., Heberle-Bors, E., Kasha, K. J., & Touraev, A. (2007). Revival of haploids in higher plants. *Trends in Plant Science*, *12*(8), 368–375. https://doi.org/10.1016/j.tplants.2007.06.007. EDN: https://elibrary.ru/MMXAAH
12. Bishnoy, U., Jain, R. K., Rohilla, J. S., Chowdhury, W. K., Gupta, K. R., & Chowdhury, J. B. (2000). Another culture of recalcitrant indica rice hybrids × Basmati. *Euphytica*, *114*, 93–101. EDN: https://elibrary.ru/AGJSPB
13. Dwivedi, S. L., Britt, A. B., Tripathi, L., Sharma, S., Upadhyaya, H. D., & Ortiz, R. (2015). Haploids: Limitations and opportunities in plant breeding. *Biotechnology Advances*, *33*, 812–829.
14. Belicuas, P. R., Guimarães, C. T., Paiva, L. V., Duarte, J. M., Maluf, W. R., & Paiva, E. (2007). Androgenetic haploids and SSR markers as tools for the development of tropical maize hybrids. *Euphytica*, *156*, 95–102. https://doi.org/10.1007/s10681-007-9356-z. EDN: https://elibrary.ru/XPVIZH
15. Germana, M. A. (2011). Anther culture for haploid and doubled haploid production. *Plant Cell, Tissue and Organ Culture*, *104*, 283–300. https://doi.org/10.1007/s11240-010-9852-z. EDN: https://elibrary.ru/VXVTHW
16. Butenko, R. G., & Tikhonovich, G. I. (1990). Cultivation of isolated cells and tissues in plant breeding. In: *Fundamentals of agricultural biotechnology* (pp. 162–165).
17. Blaydes, D. F. (1966). Interaction of kinetin and various inhibitors in the growth of soybean tissue. *Physiologia Plantarum*, *19*, 748–753.
18. Murashige, T. A., & Skoog, F. (1962). Revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, *15*, 473–497.

Список литературы

1. Dwivedi, S. L. (2015). Haploids: Constraints and opportunities in plant breeding. *Biotechnology Advances*, *33*, 812–829. https://doi.org/10.1016/j.biotechadv.2015.07.001
2. Wedzony, M., Foster, B. P., Zur, I., Golemiec, E., Szechynska-Hebda, M., Dubas, E., & Gotebiowska, G. (2009). Chapter 1. Progress in doubled haploid technology in higher plants. In: *Advances in haploid production in higher plants* (pp. 1–35). Springer Science + Business Media B.V.

3. Weyen, J., Touraev, A., Foster, B. P., & Jain, E. M. (2009). Chapter 15. Barley and wheat doubled haploids in breeding. In: *Advances in haploid production in higher plants* (pp. 179–189). Springer Science + Business Media B.V.
4. Уразалиев, К. Р., Орсини, Х. М., Абекова, А. М., Базылова, Т. А., & Данияров, А. К. (2013). Ускорение селекции пшеницы с использованием дигаплоидов, полученных методом культуры микроспор. *Вестник КазНУ. Серия экологическая*, *2/2*(38), 369–374.
5. Mishra, R., Gundimeda, J., & Narashima, R. (2016). In-vitro androgenesis in rice: Advantages, constraints and future prospects. *Rice Science*, *23*, 57–68. https://doi.org/10.1016/j.rsci.2016.02.001
6. Bernardo, R. (2009). Should maize double haploids be induced among F1 or F2 plants? *Theoretical and Applied Genetics*, *119*, 255–262. https://doi.org/10.1007/s00122-009-1034-1. EDN: https://elibrary.ru/EKWFIE
7. Zheng, M. Y. (2003). Microspore culture in wheat (*Triticum aestivum*): Doubled haploid production via induced embryogenesis. *Plant Cell, Tissue and Organ Culture*, *73*, 213–230. EDN: https://elibrary.ru/EQLTAR
8. Maluszynski, M., Kasha, K. J., & Szarejko, I. (2003). Published doubled haploid protocols in plant species. In: *Doubled haploid production in crop plants* (pp. 309–335). Springer: Dordrecht, the Netherlands. https://doi.org/10.1007/978-94-017-1293-4_46. ISBN: 978-94-017-1293-4. EDN: https://elibrary.ru/XWCXXB
9. Li, W., Gang, L., Deming, Z., Feng, W., & Jiabin, C. (2011). Tissue culture system for different hybrid of indica rice. *Northeast Agricultural University Journal*, *18*, 13–17.
10. Wren, J., Wu, P., Trumpe, B., Tian, H., Lubberstedt, T., & Chen, S. (2017). New technologies in the extraction of doubled haploid lines. *Plant Biotechnology*, *15*, 1361–1370.
11. Forster, B. P., Heberle-Bors, E., Kasha, K. J., & Turaev, A. (2007). Revival of haploids in higher plants. *Trends in Plant Science*, *12*(8), 368–375. https://doi.org/10.1016/j.tplants.2007.06.007. EDN: https://elibrary.ru/MMXAAH
12. Bishnoy, U., Jain, R. K., Rohilla, J. S., Chowdhury, W. K., Gupta, K. R., & Chowdhury, J. B. (2000). Another culture of recalcitrant indica rice hybrids × Basmati. *Euphytica*, *114*, 93–101. EDN: https://elibrary.ru/AGJSPB
13. Dwivedi, S. L., Britt, A. B., Tripathi, L., Sharma, S., Upadhyaya, H. D., & Ortiz, R. (2015). Haploids: Limitations and opportunities in plant breeding. *Biotechnology Advances*, *33*, 812–829.
14. Belicuas, P. R., Guimarães, C. T., Paiva, L. V., Duarte, J. M., Maluf, W. R., & Paiva, E. (2007). Androgenetic haploids and SSR markers as tools for the

development of tropical maize hybrids. *Euphytica*, *156*, 95–102. https://doi.org/10.1007/s10681-007-9356-z. EDN: https://elibrary.ru/XPVIZH
15. Germana, M. A. (2011). Anther culture for haploid and doubled haploid production. *Plant Cell, Tissue and Organ Culture*, *104*, 283–300. https://doi.org/10.1007/s11240-010-9852-z. EDN: https://elibrary.ru/VXVTHW
16. Бутенко, Р. Г., & Тихонович, Г. И. (1990). Культивирование изолированных клеток и тканей в селекции растений. В кн.: *Основы сельскохозяйственной биотехнологии* (с. 162–165).
17. Blaydes, D. F. (1966). Interaction of kinetin and various inhibitors in the growth of soybean tissue. *Physiologia Plantarum*, *19*, 748–753.
18. Murashige, T. A., & Skoog, F. (1962). Revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, *15*, 473–497.

AUTHOR CONTRIBUTIONS

The authors contributed equally to this article.

ВКЛАД АВТОРОВ

Все авторы сделали эквивалентный вклад в подготовку статьи для публикации.

DATA ABOUT THE AUTHORS

Elena G. Savenko, Candidate of Biological Sciences, Head of the Laboratory of Biotechnology and Molecular Biology, Leading Researcher
Federal State Budgetary Scientific Institution "Federal Scientific Rice Centre"
3, Belozerny, Krasnodar, 350921, Russian Federation
avena5@rambler.ru
SPIN-code: 1555-6845
ORCID: https://orcid.org/0000-0001-9110-032X
ResearcherID: ABA-3492-2021
Scopus Author ID: 57207914936

Valentina A. Glazyrina, Senior Researcher of the Laboratory of Biotechnology and Molecular Biology
Federal State Budgetary Scientific Institution "Federal Scientific Rice Centre"
3, Belozerny, Krasnodar, 350921, Russian Federation
valentinaglazyrina@rambler.ru
SPIN-code: 5516-8009

Ludmila A. Shundrina, Scientist of Laboratory of the Laboratory of Biotechnology and Molecular
Federal State Budgetary Scientific Institution "Federal Scientific Rice Centre"
3, Belozerny, Krasnodar, 350921, Russian Federation
ljuda-shundrina@rambler.ru
SPIN-code: 8524-7373

Zhanna M. Mukhina, Doctor of Biological Sciences, Dr Chief Scientist of the Laboratory of Biotechnology and Molecular Biology
Federal State Budgetary Scientific Institution "Federal Scientific Rice Centre"
3, Belozerny, Krasnodar, 350921, Russian Federation
agroplazma@gmail.com
SPIN- code:3701-4655
ORCID: https://orcid.org/0000-0003-3557-1615
Scopus Author ID: 22941688700

Lyubov V. Esaulova, Candidate of Biological Sciences, Deputy Director of Science
Federal State Budgetary Scientific Institution "Federal Scientific Rice Centre"
3, Belozerny, Krasnodar, 350921, Russian Federation
l.esaulova@mail.ru
SPIN-code: 9335-1160
ORCID: https://orcid.org/0000-0002-0907-2524
ResearcherID: AAG-2469-2020
Scopus Author ID: 56803891700

ДАННЫЕ ОБ АВТОРАХ

Савенко Елена Георгиевна, канд. биол. наук, заведующая лабораторией биотехнологии и молекулярной биологии, ведущий научный сотрудник
Федеральное государственное бюджетное научное учреждение «Федеральный научный центр риса»
пос. Белозерный, 3, г. Краснодар, 350921, Российская Федерация
avena5@rambler.ru

Глазырина Валентина Александровна, ст. научн. сотрудник лаборатории биотехнологии и молекулярной биологии

Федеральное государственное бюджетное научное учреждение «Федеральный научный центр риса»
пос. Белозерный, 3, г. Краснодар, 350921, Российская Федерация
valentinaglazyrina@rambler.ru

Шундрина Людмила Анатольевна, научный сотрудник лаборатории биотехнологии и молекулярной биологии
Федеральное государственное бюджетное научное учреждение «Федеральный научный центр риса»
пос. Белозерный, 3, г. Краснодар, 350921, Российская Федерация
ljuda-shundrina@rambler.ru

Мухина Жанна Михайловна, д-р биол. наук, главный научный сотрудник лаборатории биотехнологии и молекулярной биологии
Федеральное государственное бюджетное научное учреждение «Федеральный научный центр риса»
пос. Белозерный, 3, г. Краснодар, 350921, Российская Федерация
agroplazma@gmail.com

Есаулова Любовь Владимировна, кандидат биологических наук, зам. директора по науке
Федеральное государственное бюджетное научное учреждение «Федеральный научный центр риса»
пос. Белозерный, 3, г. Краснодар, 350921, Российская Федерация
L.esaulova@mail.ru

Поступила 17.11.2025 Received 17.11.2025
После рецензирования 02.12.2025 Revised 02.12.2025
Принята 19.12.2025 Accepted 19.12.2025