MAINSTREAM TECHNOLOGIES
IN PLANT GENETICS AND BREEDING
Original article

Вавиловский журнал генетики и селекции. 2023;27(8):1022-1030
DOI 10.18699/VJGB-23-117

Original Russian text https://vavilovj-icg.ru/

Study of wheat (*Triticum aestivum* L.) breeding material potential for *in vitro* androgenesis

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Abstract. Doubled haploid technology is a valuable biotechnological approach in plant breeding that enables one to quickly create new varieties through the single-stage production of homozygous lines. The aim of this study was to assess the indicators of *in vitro* androgenesis in the anther culture of the initial breeding material of varieties and combinations of F_1 and F_2 and to identify promising accessions with good responsiveness. For that purpose, the plant material that proved promising for the breeding programs of Siberian Research Institute of Plant Production and Breeding (SibRIPP&B) was used. Ten cultivars of common wheat and the F_1 and F_2 hybrids of nine combinations were evaluated for the main parameters of *in vitro* androgenesis such as the number of new formations, albino, green and all regenerated plants. Induction of androgenesis *in vitro* was carried out in anther culture in growth medium Chu (N6) containing 1 mg/l of growth regulator 2,4-D. The studied samples showed different responses to induction. The maximum level of new formations was found in F_2 hybrids Novosibirskaya 15 × Lutescens ShT-335. The largest number of green plants was found in F_1 Novosibirskaya 15 × Lutescens ShT-335. According to the results of variance analysis, a significant ($p < 0.01$) influence of genotype on the studied traits was established. Varieties with good responsiveness to anther culture (Novosibirskaya 15) and lack of responsiveness to *in vitro* androgenesis (Novosibirskaya 31) were identified. Novosibirskaya 16 was characterized by a low regeneration capacity of new formations. A significant heterotic effect was revealed considering the number of new formations per 100 anthers among the hybrids of such combinations as Novosibirskaya 15 × Lutescens ShT-335, Novosibirskaya 15 × Lutescens 111/09, and Zagora Novosibirskaya × Obskaya 2. Novosibirskaya 15 was recommended for inclusion in crossings as a parental form that provides high hybrid responsiveness during *in vitro* androgenesis. The use of doubled haploid technology made it possible to quickly create DH-lines based on the breeding material.
Key words: doubled haploids; *in vitro* androgenesis; anther culture; *Triticum aestivum* L.; heterosis.

For citation: Petrash N.V., Kapko T.N., Sovetov V.V. Study of wheat (*Triticum aestivum* L.) breeding material potential for *in vitro* androgenesis. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding*. 2023;27(8): 1022-1030. DOI 10.18699/VJGB-23-117

Изучение потенциала исходного селекционного материала пшеницы (*Triticum aestivum* L.) в андрогенезе *in vitro*

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Аннотация. Создание удвоенных гаплоидов – ценный биотехнологический подход в селекции растений, позволяющий ускоренно создавать новые сорта за счет одноэтапного получения гомозиготных линий. Целью настоящего исследования было проведение оценки показателей андрогенеза *in vitro* в культуре пыльников исходного селекционного материала сортов и комбинаций F_1 и F_2 и выявление перспективных образцов с хорошей отзывчивостью. В работе использован растительный материал, перспективный для селекционных программ Сибирского научно-исследовательского института растениеводства и селекции – филиала ИЦиГ СО РАН. Десять сортов мягкой пшеницы и гибриды F_1 и F_2 девяти комбинаций скрещивания оценивали по основным параметрам андрогенеза *in vitro*: числу новообразований, числу альбиносов и зеленых растений-регенерантов и всех регенерировавших растений. Индукцию андрогенеза *in vitro* проводили в культуре пыльников на питательной среде Chu (N6), в качестве регулятора роста использовали 1 мг/л 2.4-Д. У изучаемых образцов обнаружен различный ответ на индукцию андрогенеза *in vitro*. Отмечен максимальный выход новообразований у гибридов F_2 Новосибирская 15 × Лютесценс ШТ-335. Наибольшее количество зеленых растений-регенерантов обнаружено у F_1 Новосибирская 15 × Лютесценс ШТ-335. По результатам дисперсионного анализа установлено достоверное ($p < 0.01$) влияние генотипа на изучаемые признаки. Выявлены сорта с хорошей отзывчивостью

в культуре пыльников (Новосибирская 15) и с отсутствием отзывчивости к андрогенезу *in vitro* (Новосибирская 31). Сорт Новосибирская 16 характеризовался низкой регенерационной способностью новообразований. Среди гибридов значительный гетерозисный эффект отмечен по признаку «число новообразований на 100 пыльников» в комбинациях Новосибирская 15 × Лютесценс ШТ-335, Новосибирская 15 × Лютесценс 111/09, Загора Новосибирская × Обская 2. Сорт Новосибирская 15 рекомендован к включению в скрещивания как сорт, обеспечивающий высокую отзывчивость в андрогенезе *in vitro* гибридов. Применение технологии удвоенных гаплоидов позволило быстро создать DH-линии на основе изучаемого материала.
Ключевые слова: удвоенные гаплоиды; андрогенез *in vitro*; культура пыльников; *Triticum aestivum* L.; гетерозисный эффект.

Introduction

Common wheat (*Triticum aestivum* L.) is a critical cereal crop and the main source of vegetable protein for humans. According to the Food and Agriculture Organization of the United Nations (FAO), over 760 million tons of wheat was annually produced around the world in 2019–2021, with Russia having harvested around 78.8 million tons[1]. As the world's population grows, increasing cereal production becomes a necessity. According to projections, the world cereal production is expected to reach 840 million tons by 2030 thanks to, among other things, higher wheat yields[2].

As for breeding efforts, their main goal of is to develop new varieties combining high productivity, environmental plasticity, and resistance to diseases and other environmental stresses. Reaching this goal requires the use of new breeding material and advanced biotechnological methods.

In addition to conventional wheat breeding methods including hybridization and multistage selection followed by a series of self-pollinations to achieve homogeneity and persistence, various optimization approaches have been widely used in recent years, such as production of DH (doubled haploids) lines. The latter are completely homozygous lines obtained by doubling the number of chromosomes in haploid plants. Their use accelerates the breeding process and makes it less laborious, while also providing unique genetic material for mapping populations, phenotyping, and genotyping (Hao et al., 2013; Hale et al., 2022).

DH make it possible to obtain homozygous lines from hybrid material in one generation, while conventional methods take five-six self-pollination generations. This allows plant breeders to produce a new variety in five-seven years and respond quickly to the needs of the grain market.

In recent years, researchers have focused on improving DH production protocols, which has allowed DH technology to become a fast and accurate tool for achieving homozygosity of the original breeding material (Maluszynski et al., 2003; Wędzony et al., 2009; Seguí-Simarro et al., 2021b). The research received a boost with the discovery of *Datura* anther culture's ability to form haploid embryos and seedlings (Guha, Maheshvari, 1964). At present, DH production protocols are available for almost 400 species (Seguí-Simarro et al., 2021a). According to some authors, over 300 varieties have been produced using DH technologies in 12 plant species around the world (Forster, Thomas, 2005).

Doubled haploids may be obtained *in vivo* and *in vitro*. The use of *in vivo* systems implies obtaining a haploid embryo by parthenogenesis, pseudogamy, distant hybridization with subsequent elimination of alien pollinator chromosomes or as a result of intraspecific crosses (pollination by pretreated pollen, crosses with haploid induction lines). Chromosome doubling is a required step in all these DH production techniques. *In vitro* methods are based on obtaining plants from gametophyte cells by gynogenesis (cultivation of ovaries and flowers on nutrient media) or androgenesis (cultivation of anthers and isolated microspores) (Forster, Thomas, 2005; Seguí-Simarro et al., 2021b).

The isolated microspore culture and anther culture are widely used for production of haploids and DH plants in wheat breeding programs (Dunwell, 2010; Lantos et al., 2013; Seguí-Simarro et al., 2021a). DH production by *in vitro* androgenesis in anther culture (AC) is a simple and efficient method of obtaining pure lines (Castillo et al., 2015; Urazaliyev, 2015; Lantos, Pauk, 2016; Kolesnikova et al., 2021). The process is based on changing microspore development program from gametophyte way (pollen grain formation) to sporophyte, and the obtained embryo-like structures (ELS) and calluses are then used to grow regenerated plants (Embryological Foundations..., 2005). These plants are of significant breeding value because they develop from cells following the meiotic division, and thus have unique gene combinations. Haploid cells on the nutrient medium may undergo genome doubling and produce spontaneous DH plants with 100 % homozygosity as a result. In homozygous organisms, the effect of recessive genes can be seen along with that of dominant genes, which significantly accelerates genotype selection (Kasha, Maluszynski, 2003).

The efficiency of androgenesis in AC is affected by many factors, including donor growth conditions, microspore development stage, pretreatment conditions, nutrient medium composition, but genotype is what affects it the most (Tuvesson et al., 2000; Lantos, Pauk, 2020; Seguí-Simarro et al., 2021b; Hale, 2022). The success in obtaining androgenic regenerant plants is limited due to albinism and significant genotypic dependency (Li et al., 2013; Zhao L. et al., 2015). Genotype-dependent variation in responsiveness can be seen both at intraspecific and interspecific levels. For example, hexaploid winter wheats show better *in vitro* androgenic responsiveness than the spring ones (Sharma et al., 2005; Lazaridou et al., 2016). A wheat-rye 1RS.1BL translocation has a positive effect on plant regeneration in *in vitro* androgenesis (Agache et al., 1989; Pershina et al., 2013; Timonova et al., 2022).

[1] Crops and livestock products. https://www.fao.org/faostat/en/#data/QCL
[2] OECD/FAO (2021), OECD-FAO Agricultural Outlook 2021–2030, OECD Publishing, Paris. https://doi.org/10.1787/19428846-en

Table 1. F_1–F_2 combinations and their parent varieties assessed with respect to *in vitro* androgenic responsiveness in the anther culture

No.	Varieties	No.	Combinations, generation F_1, F_2	No. combination
1	Novosibirskaya 15	11	Novosibirskaya 15 × Lutescens ShT-335	No. 3
2	Novosibirskaya 16	12	Novosibirskaya 15 × Lutescens 111/09	No. 2
3	Novosibirskaya 18	13	Novosibirskaya 16 × Lutescens 111/09	No. 7
4	Novosibirskaya 31	14	Novosibirskaya 18 × Lutescens 111/09	No. 9
5	Novosibirskaya 75	15	Novosibirskaya 18 × Sigma	No. 61
6	Zagora Novosibirskaya	16	Novosibirskaya 75 × Lutescens 111/09	No. 23
7	Obskaya 2	17	Novosibirskaya 31 × Lutescens 111/09	No. 14
8	Sigma	18	Zagora Novosibirskaya × Lutescens 111/09	No. 26
9	Lutescens 111/09	19	Zagora Novosibirskaya × Obskaya 2	No. 24
10	Lutescens ShT-335			

Additive, dominant, and epistatic relationships between genes responsible for inheritance of androgenic traits in AC were observed (Chaudhary et al., 2003; Dagüstü, 2008; Grauda et al., 2016). At the same time, some authors showed that androgenic responsiveness in AC followed a simple inheritance scheme and was controlled by dominant genes (El-Hennawy et al., 2011). B.E.S. Abd El-Fatah et al. (2020) demonstrated that additive effects prevailed over dominance effects in terms of genetic control of *in vitro* androgenic traits.

A viable strategy of overcoming genotypic dependency is to use breeding material with high *in vitro* androgenic responsiveness, i. e. one of the parents in the cross should induce the development of green regenerants in hybrids (Tuvesson et al., 2003; Kondic-Špika et al., 2011; Lantos, Pauk, 2020). Thus, it seems reasonable to assess the initial breeding samples and use the ones with good *in vitro* androgenic responsiveness in crosses.

The goal of the present study was to assess *in vitro* androgenic indicators in the anther culture of the initial breeding material from spring varieties of common wheat and combinations of F_1 and F_2, as well as identify promising accessions with good responsiveness.

Materials and methods

Spring common wheat samples showing promise under the breeding program of Siberian Research Institute of Plant Production and Breeding (SibRIPP&B) – Branch of ICG SB RAS were used as breeding material. Nine combinations of F_1 and F_2 and ten parent varieties were selected for the assessment of *in vitro* androgenic responsiveness (Table 1).

Anther donor plants were grown on the field of Siberian Research Institute of Plant Production and Breeding in 2022. The spikes were harvested from leading shoots while most of the microspores were at the mid to late uninucleate stage. In terms of visual evidence, it meant that the middle of the spike was at the same height as the second top leaf sheath. Microspore development stage was identified using a Leica CME microscope (Leica Microsistems, Russia) in acetocarmine-stained cytological squash preparations.

The harvested spikes were stored in a temperature controlled container with cooling agents, transported to the laboratory, placed in test tubes with distilled water, and kept in a refrigerated thermostat TVL-K at +4 °C for seven days. After the cold pretreatment, the spikes were sterilized with wipes soaked in 70 % and then 96 % alcohol and transported to a biosafety box. The anthers were obtained from lateral flowers from the middle of the spike, with the average of about 50 anthers per spike. The experiments were performed in triplicate with one Petri dish for each measurement and with at least 100 anthers obtained for each accession.

The anthers from two spikes with the same genotype were inoculated in 100 mm Ø Petri dish with 15–20 ml of Chu's N6 induction medium (Chu, 1978), 90 g/l sugars (sucrose: maltose in the ratio of 2:1); 100 mg/l myo-inositol; 1 mg/l 2,4-D, 0.5 mg/l kinetin, and 6 g/l plant agar. Petri dishes with anthers were incubated in the dark at 28 °C until the emergence of the first microspore-derived structures, and then at 25 °C for the further growth of the obtained structures. Following the incubation period of 30–40 days, the ELSs and calluses reaching 1.5–2 mm in diameter were placed in quantities of 3 to 5 in 28 mm Ø test tubes with Gamborg's B5 medium (Gamborg et al., 1968), 30 g/l sucrose, 5 g/l plant agar without growth regulators. Plantlets regenerated under LED lights with photosynthetic photon flux density (PPFD) of 751.6 μmol/m^2/s at 18–20 °C for 20–30 days with photoperiod of 16 hours.

Green plantlets with well-developed roots and leaves were taken out from the test tubes, with the remains of the nutrient medium thoroughly washed away from the roots, and planted into separate pots (0.8 l) with a mixture of coconut substrate, all-purpose soil, and vermiculite in the ratio of 3:1:1. The rooted plants were grown under the same LED lights at temperatures of 19–21 °C and humidity of about 50–60 %. The plants were grown to full maturity. Only the fertile plants (spontaneous DH) were selected for further study, while partially fertile or sterile plants were discarded.

The responsiveness of the AC was assessed using the following indicators: number of neoplasms (ELSs and calluses) per 100 isolated anthers (N/100A); number of albino plantlets

per 100 isolated anthers (AP/100A); number of green plantlets per 100 isolated anthers (GP/100A); total plantlets per 100 neoplasms (TP/100N).

Statistical processing of the data was performed using Microsoft Excel 2010. Analysis of variance was carried out using SNEDECOR software (Sorokin, 2004). True (H_{tr}, %) and hypothetical (H_{hyp}, %) heterosis were calculated using Eqs. (1) and (2) based on (Omarov, 1975):

$$H_{tr} = F_1 - P_{best} / P_{best} \times 100\ \%, \quad (1)$$

$$H_{hyp} = F_1 - P_{av} / P_{av} \times 100\ \%, \quad (2)$$

where F_1 is the value of interest in the hybrid; P_{best} is the same value in the best parent; P_{av} is the average value between parents (P1 + P2)/2.

The degree of phenotypic dominance (Hp) acting as an inheritance indicator in the controlled crosses was calculated using Eq. (3) based on (Griffing, 1956):

$$Hp = F_1 - MF/HF - MF, \quad (3)$$

where Hp is the dominance value; F_1 is the observed mean of F_1; MF is the average attribute value between parents; and HF is the attribute value in the best parent. The interpretation was as follows: Hp > 1 was recognized as positive heterosis, Hp = 0.5–1.0 as positive dominance, Hp from +0.5 to –0.5 as intermediate inheritance, Hp = –0.5 to –1.0 as negative dominance, and Hp < –1.0 as negative heterosis. Inbreeding depression (ID %) was calculated using Eq. (4) based on (Pederson, 1971):

$$ID = (F_2 - F_1/F_1) \times 100\ \%, \quad (4)$$

where ID is the inbreeding depression, F_1 is the average attribute value in the first-generation hybrid family, F_2 is the average attribute value in the second-generation hybrid family.

Results and discussion

The success of DH technology in breeding programs depends on the genotype's ability to regenerate green plants in *in vitro* androgenesis.

In the present paper, the assessment of *in vitro* androgenic responsiveness is presented for 10 varieties and 9 combinations, generations F_1 and F_2. A total of 16,598 anthers have been isolated and placed in induction medium, with at least 100 anthers analyzed in triplicate for each accession. The single-factor analysis of variance showed a significant effect of genotype on all *in vitro* androgenic indicators of interest (Table 2).

The studied samples showed a variety of *in vitro* androgenic responses (Table 3). The number of neoplasms per 100 isolated anthers (N/100A) indicates the quantity of structures (ELSs and calluses) developing from microspores. This attribute varied from 0 to 17.20 (Novosibirskaya 15 × Lutescens ShT-335, F_2), the average being 3.74. The average number of green regenerants per 100 anthers (GP/100A) was 1.45. Maximum values were observed in F_1 Novosibirskaya 15 × Lutescens ShT-335 and Novosibirskaya 15 × Lutescens 111/09, at 12.15 and 12.50, respectively. The average number of albino plantlets per 100 anthers (AP/100A) was 0.63. Maximum values were observed in Novosibirskaya 15 (2.67), F_2 Novosibirskaya 15 × Lutescens ShT-335 (2.40), and F_1 Zagora Novosibirskaya × Obskaya 2 (2.92). The average total number of regenerants per 100 anthers was 2.08. Maximum values with prevalence of green regenerants were observed in F_1 Novosibirskaya 15 × Lutescens ShT-335 and Novosibirskaya 15 × Lutescens 111/09. A total of 150 green plantlets were obtained in the experiment.

The analysis showed that high neoplasm production was not directly associated with a high number of regenerants. For instance, varieties Novosibirskaya 15 ($p < 0.10$) and Novosibirskaya 16 ($p < 0.05$) both showed above average neoplasm production, but Novosibirskaya 15 also showed higher regeneration ability (TP/100A = 4.33, $p < 0.05$). Novosibirskaya 16 produced 12.40 neoplasms per 100 anthers with 1.80 regenerated plantlets per 100 anthers (see Table 3). This observation confirms the literature data that *in vitro* androgenic indicators are polygenically controlled and independently inherited (Ekiz, Konzak, 1994; Nielsen et al., 2015; Abd El-Fatah et al., 2020). Novosibirskaya 31 and, notably, its combinations in the first and second generations did not produce any structures, allowing us to assume that a non-responsive genotype worthy of further research has been discovered.

The ability of calli and embryo structures to regenerate into plantlets is reflected in the number of green regenerants per 100 neoplasms and the number of albino plantlets per 100 neoplasms (see the Figure). The experiment showed that the average number of regenerated green plantlets per 100 neoplasms was higher than the number of albino plantlets, the respective values being 26.41 and 18.74. The highest regeneration ability, with more than half of neoplasms regenerating into plants, was observed in hybrids F_1 No. 3 (Novosibirskaya 15 × Lutescens ShT-335), No. 2 (Novosibirskaya 15 × Lutescens 111/09), No. 7 (Novosibirskaya 16 × Lutescens 111/09), No. 61 (Novosibirskaya 18 × Sigma), No. 26 (Zagora Novosibirskaya × Lutescens 111/09), and F_2 No. 26 (Zagora Novosibirskaya × Lutescens 111/09) (see the Figure). Notably, the number of green regenerants per 100 neoplasms was above 100 for hybrid F_1 No. 3 (Novosibirskaya 15 × Lutescens ShT-335), possibly

Table 2. Single-factor analysis of variance for *in vitro* androgenic responsiveness indicators in the anther culture of wheat varieties and F_1–F_2 hybrids

Source of variation	*df*	N/100A		GP/100A		AP/100A	
		Effect, %	F_{fact}	Effect, %	F_{fact}	Effect, %	F_{fact}
Genotype	27	73.44	9.30*	78.57	12.00*	51.82	4.23*
Random factors	56	26.56	–	21.43	–	48.18	–

*$p < 0.01$ ($F_{tab.\ 0.99} = 2.18$); *df* is the number of degrees of freedom; F_{fact} is the calculated Fisher test value; N/100A is the number of neoplasms per 100 anthers; GP/100A is the number of green plantlets per 100 anthers; AP/100A is the number of albino plantlets per 100 anthers.

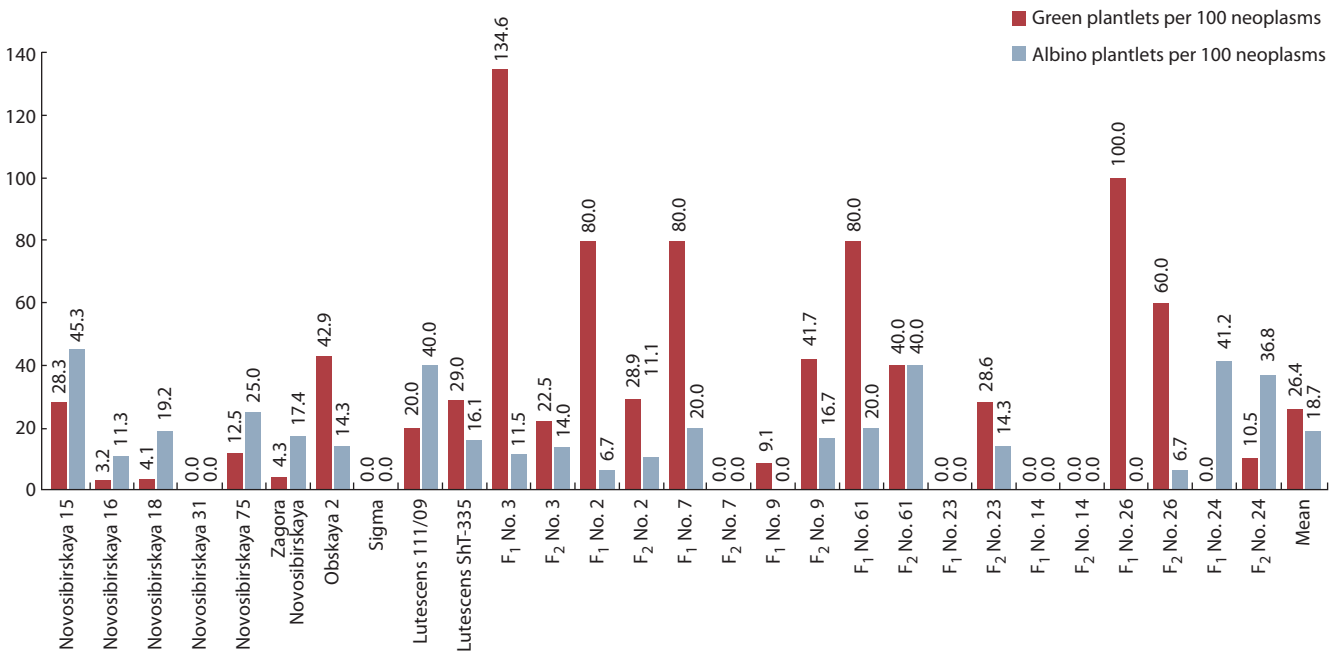
Table 3. *In vitro* androgenic responsiveness indicators in the anther culture of wheat varieties and F_1–F_2 hybrids

Genotype	N/100A	GP/100A	AP/100A	TP/100A
Novosibirskaya 15	5.89[2] ± 1.26	1.67 ± 0.33	2.67[1] ± 0.33	4.33[1] ± 0.58
Novosibirskaya 16	12.40[1] ± 2.67	0.40 ± 0.40	1.40[1] ± 0.36	1.80 ± 0.73
Novosibirskaya 18	5.21 ± 0.70	0.21 ± 0.22	1.00 ± 0.55	1.21 ± 0.70
Novosibirskaya 31	0.00	0.00	0.00	0.00
Novosibirskaya 75	2.13 ± 0.46	0.27 ± 0.46	0.53 ± 0.32	0.80 ± 0.78
Zagora Novosibirskaya	2.71 ± 0.66	0.12 ± 0.21	0.47 ± 0.41	0.59 ± 0.21
Obskaya 2	0.47 ± 0.31	0.20 ± 0.35	0.07 ± 0.12	0.27 ± 0.46
Sigma	0.40 ± 0.29	0.00	0.00	0.00
Lutescens 111/09	1.43 ± 0.73	0.29 ± 0.08	0.57 ± 0.31	0.86 ± 0.37
Lutescens ShT-335	2.58 ± 0.25	0.75 ± 0.25	0.42 ± 0.52	1.17 ± 0.52
F_1 (Novosib. 15 × Lut. ShT-335)	9.03[1] ± 1.35	12.15[1] ± 4.51	1.04[2] ± 0.00	13.19[1] ± 4.51
F_2 (Novosib. 15 × Lut. ShT-335)	17.20[1] ± 2.95	3.87[1] ± 0.61	2.40[1] ± 1.06	6.27[1] ± 1.40
F_1 (Novosib. 15 × Lut. 111/09)	15.63[1] ± 2.76	12.50[1] ± 2.08	1.04[2] ± 0.00	13.54[1] ± 2.08
F_2 (Novosib. 15 × Lut. 111/09)	4.50 ± 0.81	1.30 ± 0.63	0.50 ± 0.17	1.80 ± 0.61
F_1 (Novosib. 16 × Lut. 111/09)	1.74 ± 1.54	1.39 ± 0.68	0.35 ± 0.60	1.74 ± 1.28
F_2 (Novosib. 16 × Lut. 111/09)	0.40 ± 0.23	0.00	0.00	0.00
F_1 (Novosib. 18 × Lut. 111/09)	3.27 ± 1.68	0.30 ± 0.26	0.00	0.30 ± 0.26
F_2 (Novosib. 18 × Lut. 111/09)	1.33 ± 1.04	0.56 ± 0.39	0.22 ± 0.20	0.78 ± 0.49
F_1 (Novosib. 18 × Sigma)	1.74 ± 0.31	1.39 ± 0.68	0.35 ± 0.30	1.74 ± 0.88
F_2 (Novosib. 18 × Sigma)	1.00 ± 0.2	0.40 ± 0.36	0.40 ± 0.40	0.80 ± 0.70
F_1 (Novosib. 75 × Lut. 111/09)	0.69 ± 0.60	0.00	0.00	0.00
F_2 (Novosib. 75 × Lut. 111/09)	1.17 ± 0.38	0.33 ± 0.29	0.17 ± 0.29	0.50 ± 0.00
F_1 (Novosib. 31 × Lut. 111/09)	0.00	0.00	0.00	0.00
F_2 (Novosib. 31 × Lut. 111/09)	0.00	0.00	0.00	0.00
F_1 (Zagora Novosib. × Lut. 111/09)	0.35 ± 0.34	0.35 ± 0.34	0.00	0.35 ± 0.34
F_2 (Zagora Novosib. × Lut. 111/09)	3.00 ± 1.05	1.80 ± 0.35	0.20 ± 0.35	2.00 ± 0.35
F_1 (Zagora Novosib. × Obskaya 2)	7.08[1] ± 3.94	0.00	2.92[1] ± 1.13	2.92 ± 1.13
F_2 (Zagora Novosib. × Obskaya 2)	3.80 ± 1.28	0.40	1.40[1] ± 1.08	1.80 ± 1.24
Mean	3.74	1.45	0.63	2.08
$LSD_{0.05}$	2.50	1.72	0.42	1.88
$LSD_{0.10}$	2.11	1.45	0.36	1.58

Note. N – neoplasms; GP – green plantlets; AP – albino plantlets; A – anthers; TP – total plantlets; Novosib. – Novosibirskaya; Lut. – Lutescens.
[1] Differences from the mean are significant at $p = 0.05$; [2] differences from the mean are significant at $p = 0.10$.

due to secondary embryogenesis or an ELS developing into polyembryoids (structures with several shoot growth points (Seldimirova, 2009; Pershina et al., 2020)). Both mechanisms produce clones or sister plants.

Albinism acts as a limitation for DH production in *in vitro* androgenesis. Our experiment showed the prevalence of green plantlets in the total number of plantlets in varieties as follows: Obskaya 2, Lutescens ShT-335, F_1 hybrids Novosibirskaya 15 × Lutescens ShT-335, Novosibirskaya 15 × Lutescens 111/09, Novosibirskaya 16 × Lutescens 111/09, Novosibirskaya 18 × Sigma, Zagora Novosibirskaya × Lutescens 111/09, and F_2 hybrids Novosibirskaya 18 × Lutescens 111/09, Novosibirskaya 75 × Lutescens 111/09, Zagora Novosibirskaya × Lutescens 111/09 (see the Figure). It follows



Percentage of green and albino plantlets per 100 neoplasms in *in vitro* androgenesis of wheat varieties and F_1–F_2 hybrids.

No. 3 – (Novosibirskaya 15 × Lutescens ShT-335); No. 2 – (Novosibirskaya 15 × Lutescens 111/09); No. 7 – (Novosibirskaya 16 × Lutescens 111/09); No. 9 – (Novosibirskaya 18 × Lutescens 111/09); No. 61 – (Novosibirskaya 18 × Sigma); No. 23 – (Novosibirskaya 75 × Lutescens 111/09); No. 14 – (Novosibirskaya 31 × Lutescens 111/09), No. 26 – (Zagora Novosibirskaya × Lutescens 111/09); No. 24 – (Zagora Novosibirskaya × Obskaya 2); $LSD_{0.05}$ (GP/100N) = 19.51; $LSD_{0.05}$ (AP/100N) = 7.81.

from the analysis of variance that around 50 % of albinism cases are genotype-related (see Table 2).

There are several factors increasing the chance of albinism, including genotype, donor growth conditions, cultivation conditions, medium composition, incompatibility of nuclear and plastid genomes, and plastid DNA deletions or mutations (Nielsen et al., 2015; Zhao P. et al., 2017). The high significance of the genotype's effect on the number of albino plantlets is demonstrated in a number of papers (Lantos, Pauk, 2016; Castillo et al., 2019; Abd El-Fatah et al., 2020; Kanbar et al., 2020).

Genotypic dependency of albinism is associated with transcription activation of specific genes involved in chloroplast biogenesis at early stages (Mozgova et al., 2006; Canonge et al., 2021). Chloroplast DNA deletions were observed in albino plants, along with inhibited transcription of the nuclear genes coding for chloroplast-localized proteins, while levels of transcripts coding for proteins not present in chloroplasts were identical to those in green plants (Dunford, Walden, 1991).

To evaluate the prospects of using the studied varieties in further crosses, the heterosis effect in their hybrids was analyzed. Heterosis effect of *in vitro* androgenic responsiveness was described earlier, and its degree was shown to vary between genotypes (Ouyang et al., 1973; Ekiz, Konzak, 1994).

True (H_{tr}) and hypothetical (H_{hyp}) heterosis, inheritance indicator (Hp), and inbreeding depression (ID %) were calculated based on the number of neoplasms per 100 anthers, since, according to the analysis of variance, genotype significantly contributes to this value (73.44 %, see Table 2) and directly affects the subsequent *in vitro* androgenic responsiveness indicators. Maximum hypothetical heterosis was observed in Zagora Novosibirskaya × Obskaya 2, and minimum, in Novosibirskaya 31 × Lutescens 111/09 (Table 4). True heterosis characterizes stronger manifestation of the trait in F_1 compared to the best parent.

Maximum true heterosis of 100 % was observed in Novosibirskaya 15 × Lutescens 111/09, negative heterosis was observed in hybrids with Novosibirskaya 31 demonstrating androgenic non-responsiveness. Significant negative H_{tr} was also observed in Novosibirskaya 16 × Lutescens 111/09 and Zagora Novosibirskaya × Lutescens 111/09.

Analysis of the inheritance indicator showed positive heterosis for Novosibirskaya 15 × Lutescens ShT-335, Novosibirskaya 15 × Lutescens 111/09, Zagora Novosibirskaya × Obskaya 2. Intermediate inheritance was observed in combinations with Novosibirskaya 18. Negative dominance was observed in Novosibirskaya 16 × Lutescens 111/09, and negative heterosis, in Novosibirskaya 75 × Lutescens 111/09 and Zagora Novosibirskaya × Lutescens 111/09.

The degree of manifestation of *in vitro* androgenic attributes varies between F_1 and F_2 hybrids. The first generation outperformed the second one in neoplasms per 100 anthers in Novosibirskaya 15 × Lutescens 111/09, Novosibirskaya 16 × Lutescens 111/09, Novosibirskaya 18 × Lutescens 111/09, Novosibirskaya 18 × Sigma, Zagora Novosibirskaya × Obskaya 2. Inbreeding depression was observed in Novosibirskaya 15 × Lutescens ShT-335, Novosibirskaya 75 × Lutescens 111/09, Zagora Novosibirskaya × Lutescens 111/09 (see Table 4). Negative ID % value shows that F_1 hybrids outperform F_2 in terms of manifestation of the attribute.

To summarize the analysis of the inherited ability to produce structures from microspores in various combinations, it is

Table 4. Heterosis effect and inheritance indicator for the number of neoplasms per 100 anthers in nine common wheat combinations

Combination	Indicator			
	H_{hyp}, %	H_{tr},%	Hp	ID %
Novosibirskaya 15 × Lutescens ShT-335	113.22	53.31	2.90[1]	90.48
Novosibirskaya 15 × Lutescens 111/09	327.05	165.37	5.37[1]	–71.21
Novosibirskaya 16 × Lutescens 111/09	–74.84	–85.97	–0.94[3]	–77.01
Novosibirskaya 18 × Lutescens 111/09	–1.51	–37.24	–0.03[2]	–59.33
Novosibirskaya 18 × Sigma	–37.97	–66.60	–0.44[2]	–42.53
Novosibirskaya 75 × Lutescens 111/09	–61.24	–67.61	–3.11[4]	69.57
Novosibirskaya 31 × Lutescens 111/09	–100	–100	–*	–*
Zagora Novosibirskaya × Lutescens 111/09	–83.09	–87.08	–2.69[4]	757.14
Zagora Novosibirskaya × Obskaya 2	345.28	161.25	4.90[1]	–46.33

Note. H_{hyp}, % is the hypothetical heterosis; H_{tr},% is the true heterosis; ID % is the inbreeding depression; Hp is the degree of dominance; [1] positive heterosis; [2] intermediate inheritance; [3] negative dominance; [4] negative heterosis; * not available due to absence of neoplasms.

worth focusing on positive values observed for combinations with Novosibirskaya 15. These results agree with the previously obtained data on the responsiveness of F_1 and F_2 hybrids Obskaya 2 × Novosibirskaya 15 compared to parent varieties (Petrash et al., 2022). Studying the inheritance patterns in multiple combinations makes it possible to estimate positive *in vitro* androgenic responsiveness in hybrids to ensure effective pair selection for crosses under future breeding programs using doubled haploid technology.

Conclusion

The goal of the paper was to study the potential of the initial breeding material from the perspective of *in vitro* androgenesis in 10 different common wheat varieties and 9 combinations of F_1 and F_2, with a total of 28 genotypes analyzed. The androgenic indicators analyzed included the number of neoplasms (ELSs and calluses), green plantlets, albino plantlets, and the total number of regenerated plants.

As a result, the varieties showing *in vitro* androgenic responsiveness (Novosibirskaya 15) and non-responsiveness (Novosibirskaya 31) in the anther culture have been identified. Novosibirskaya 16 was characterized by low neoplasm regeneration ability. A significant heterosis effect was observed in hybrids Novosibirskaya 15 × Lutescens ShT-335, Novosibirskaya 15 × Lutescens 111/09, Zagora Novosibirskaya × Obskaya 2. Positive heterosis in terms of neoplasms per 100 anthers was observed in combinations with Novosibirskaya 15, and intermediate inheritance, in combinations with Novosibirskaya 18. Novosibirskaya 15 is recommended for inclusion into crosses as a variety ensuring high *in vitro* androgenic responsiveness in hybrids compared to the second parent. Doubled haploid technology made it possible to use the discussed hybrid material to produce DH lines, which are now being tested in the field.

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Acknowledgements. This work was supported by Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences budget project No. FWNR-2022-0017.

Conflict of interest. The authors declare no conflict of interest.

Received August 1, 2023. Revised September 29, 2023. Accepted October 5, 2023.