Factors of the haploproducer method in the F1 hybrids system
*T. aestivum L.* - *Z. mays L.* optimization

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**Abstract.** Preliminary results of optimization of the method of haploproducer *Zea mays* L. for hybrid genotypes of spring common wheat *Triticum aestivum* L. in order to create diploidized haploid lines and their integration into practical breeding are presented. Regularities of the regeneration of normal chlorophyll-bearing haploid plants and the dependence of the morphogenesis of haplo-embryoids in vitro on optimization factors of various genesis and their effectiveness on hybrids created on the basis of varieties of different ecological and geographical origin were revealed. It was found that the manifestation of the effect of heterosis contributes to an increase in the yield of haploid chlorophyll-bearing regenerants when receiving hybrids from parental forms with different responsiveness to androgenesis and haploproducing. The advantage of the ecological-geographical principle of selection of parental pairs in increasing the degree of responsiveness of hybrid genotypes of spring wheat to in vitro androgenesis and haploproducing is shown. The practical advantage of the haploproducer method in the F1 system of *T. aestivum* - *Z. mays* in comparison with the androgenesis method in the anther culture in vitro is a higher optimization potential in summa. In order to intensify the biotechnological breeding process, it is recommended to use the optimized method of the haploproducer - *Z. mays*, which provides for the cumulative twelvehaplo- method of pollination with a mixture of pollen from maize lines with different haplo-producing properties (increasing the quantitative yield of haplo-embryos) and a hormone stimulation scheme with pretreatment of the spike material of the hybrids with epibrassinolide (increasing the degree of their differentiation), which in total makes it possible to significantly accelerate the creation of diploidized haploid lines, regardless of the influence of the genotype of the original hybrids.

1. Introduction

The production of soft wheat grain in the Russian Federation is one of the largest branches of agriculture; along with its social significance as an everyday food product, it has an important economic value. The producers in all grain-producing regions require spring wheat varieties with consistently high yields and high grain quality, adaptability, environmental sustainability, including resistance to biotic and abiotic stressors. The problem of selecting the starting material for the creation of new commercial varieties is central, in particular, the successful combination of the newly created genotypes of a
complex of limiting traits for the conditions of the Center of the Non-Black Earth Zone of the Russian Federation [1-4].

Intensification of the breeding process of *T. aestivum* is possible with biotechnology methods in combination with hybridization and selection. Gamete selection in combination with haploidy is of practical interest. Diploidization of haploids created on the basis of hybrid genotypes, along with a high morphogenetic process, ensures the fixation of homozygotes and increases the selection efficiency. Realization of the potential of haploidy in F1 hybrids of *T. aestivum* the absence of simple and reliable methods of mass production of haploids, regardless of the influence of the genotype, limits the practice of haplo-biotechnology in breeding, and puts it on the empirical path [5-7].

The aim was to study and to give a comparative assessment of the factors of optimization of the method of haploproducer - maize lines (*Z. mays*) in vitro, using the example of intersvarietal hybrids F1 *T. aestivum*, and to create diploidized haploid lines on their basis.

2. Materials and methods

The research was performed in the laboratory of biotechnology, selection and primary seed production of spring wheat of the Federal Research Center "Nemchinovka". The varieties of spring bread wheat: Amir, Ester, Lada, Vizit, Tasos, Saratovskaya 68, Tulaykovskaya 10 and reciprocal hybrids obtained on their basis were the objects of the research. The maize lines SM-7 and KR 935/86 (*Z. mays*) were haploproducers. Pollination was made with an accumulative twell method. In vitro cultivation of haploproduced embryos was performed on R-8 medium (Lukyanyuk and Ignatova, 1983) without vitamin E, modified with wheat starch. Normal regenerants were transferred to a hormone-free MS medium, after the establishment of the haploid status by microscopy of temporal acetocarmine preparations of roots, they were diploidized with colchicine [6; 8-10].

3. Results and discussion

The regularities of the processes of regeneration of haploid plants, the dependence of the morphogenesis of microspores in the culture of anthers and haplo-embryogenesis in vitro on the optimization factors of various genesis and their effectiveness on hybrids created on the basis of lines of various ecological and geographical origin were revealed as a result of the developed optimizational additions. It was found that the effect of heterosis contributes to an increase in the yield of haploid chlorophyll-bearing regenerants when obtaining hybrids from parental forms with different responsiveness to androgenesis and haploproducing. The practical advantage of the haploproducer method in the system F1 *T. aestivum* - *Z. mays* in comparison with the method of androgenesis in in vitro anther culture is a higher optimization potential in summa.

The use of mixed haploproducing systems made it possible to determine the frequency of manifestation of reciprocal effects and to distribute hybrid genotypes into 3 groups according to the prevailing effects (synergistic, additive and antagonistic) on incongruent pollination, the use of a mixture of 2,4-D (100 mg / L) and GK (75 mg / l) (table 1).

Evaluation of the manifestation of the frequencies of positive types of reactions (synergistic and additive) of haplo-embryogenesis to the degree of differentiation made it possible to identify several valuable groups. Group I (morpholax type) is characterized by a high frequency of manifestation of positive types of interactions of parental genomes in hybrid genotypes, which leads to an increase in the total number of haploid embryos and the degree of their differentiation (Amir and Vizit varieties). Group IV (qualitative type) is characterized by the optional manifestation of positive effects on increasing the degree of differentiation (Tasos variety). The total efficiency of optimization for the haploproducer method (epibrassinolide (25 ∙ 10-5%) prestimulation 3-5 days before pollination, cumulative twell-method of pollination with a mixture of pollen from *Z. mays* lines, subsequent stimulation with 2,4-D (100 mg/l) and GK (75 mg/l) in the first three days and 5-6 days after pollination) comparing to the initial procedure (forced pollination method - *Z. mays* (KR 935/86) and stimulation with a mixture of 2,4-D (100 mg/l) and GK (75 mg/l) in the first three days after
pollination) for haplo-embryogenesis was 327.0% and for the degree of differentiation was 161.4% [6].

Table 1. Efficiency of haplo-embryogenesis in the F1 system T. aestivum - Z. mays.

| Hybrids F1                   | Z. mays       | Mix (SM-7: KR 935/86) | 1:1 | 1:2 | 1:4 | P, %** |
|-----------------------------|---------------|-----------------------|-----|-----|-----|--------|
|                            | SM-7          | KR 935/86             | x±Sx | x±Sx | x±Sx | x±Sx  |
| Tulaykovskaya 10 x Vizit (A)| 2.0±0.5       | 25.5±1.1              | 29.4±0.9 | 33.4±1.6 | 39.8±1.4 |
| Vizit x Tulaykovskaya 10 (B)| 3.3±0.4       | 23.6±0.7              | 27.6±0.8 | 30.2±1.2 | 35.3±1.2 | 2.3*   |
| (B - A)                     | 1.3*          | 1.9*                  | 1.8*  | 3.2*  | 4.5*  |
| Ester x Vizit (C)           | 14.3±1.6      | 20.1±0.5              | 23.5±1.2 | 22.7±1.0 | 28.2±1.4 |
| Vizit x Ester (D)           | 14.4±1.6      | 21.2±0.4              | 22.0±1.0 | 25.2±1.1 | 29.5±1.2 | 0.9    |
| (D - C)                     | 0.1           | 1.1                   | 1.5*  | 2.5*  | 1.3   |
| Saratovskaya 68 x Tasos (E) | 1.4±0.5       | 26.9±0.3              | 25.5±0.7 | 35.6±1.3 | 34.5±1.6 |
| Tasos x Saratovskaya 68 (F) | 1.1±0.4       | 26.6±0.2              | 26.1±0.9 | 31.8±1.4 | 31.3±1.3 | 0.9    |
| (E - F)                     | 0.3           | 0.3                   | 0.6   | 3.8*  | 3.2*  |
| P**(hybrids)                | 3.6±0.7       | 23.8±0.5              | 25.6±0.9 | 29.5±1.3 | 30.9±1.3 | -      |
| General (χ²)                | 2.1           | 2.6                   | 2.8   | 3.1   | 3.5   | -      |
| Reciprocal (χ²)             | 1.2           | 1.4                   | 1.4   | 1.8   | 2.2   | 1.5    |

Note: HCP05 = 0.5 - factor A (hybrid genotype), HCP95 = 2.6 - factor B (haploproducer genotype), HCP05 = 3.90 - factor C (mixture of haploproducer pollen); * - reciprocal differences are significant at 5% significance level (n> 150); ** - geometric mean, *** - power value of reciprocal effects.

In turn, the use of mathematical analysis with the method of orthogonal regression of the degree of responsiveness to optimization made it possible to determine the groups of released hybrids according to a complex of biotechnological indicators, suggesting the use of various methods of haplo-biotechnology. Group I is the most valuable (high yield of haploproduced and androclinic regenerants), includes F1 hybrids: (Vizit x Amir), (Thassos x Lada), (Tulaykovskaya 10 x Vizit) and (Esther x Vizit). Group II (high efficiency of the androgenesis method) is represented by F1 hybrids: (Saratovskaya 68 x Amir), (Vizit x Lada) and (Tulaykovskaya 10 x Thassos). Group III (low efficiency of androgenesis and haploproducer methods) included a hybrid (Tulaykovskaya 10 x Ester) F1. Group IV is valuable (high efficiency of the haploproducer method) and is represented by F1 hybrids: (Ester x Thassos) and (Amir x Esther) (figure 1).

Multivariate analysis of the induction of haploid regenerants for the optimized haploproducer method in the system F1 T. aestivum – Z. mays made it possible to establish that in 20.4% of all cases the result was influenced by the combined use of phytohormonal stimulating complexes, in 36.0% - the genotype of donor plants, in 43.6% - the genotypes of haploproducers and their pollen mixture. This allows us to recommend the method of the haploproducer Z. mays as the most effective for the creation of diploidized haploid T. aestivum lines, regardless of the influences of the initial genotype.

4. Conclusion

As the main methodological method for increasing the efficiency of haploproduction processes in hybrid T. aestivum genotypes, when creating diploidized haploid lines, the ecological-geographical principle of selection of parental pairs should be applied. The varieties (Amir, Vizit, Tasos) should be used that contribute to an increase in the degree of responsiveness to androgenesis and haploproduction in in vitro systems and with high recombination ability. In order to intensify the biotechnological breeding process of T. aestivum, it is recommended to use the optimized method of the haploproducer Z. mays, which provides the cumulative twel-method of pollination with a mixture of pollen from maize lines with different haplo-producing properties (increasing the quantitative yield of haplo-embryos) and a hormone stimulation scheme with epibrassinolide pre-processing of the spike
material of hybrids (increasing the degree of their differentiation), which in total makes it possible to significantly accelerate the creation of diploidized haploid lines, regardless of the influence of the genotype of the original hybrids.

\[ y = -0.1884x + 15.538 \]

**Figure 1.** The level of haploproduction F1 of *T. aestivum* for the optimized method of androgenesis relative to the method of haploproducer *Z. mays* in vitro. Group I is especially valuable (high yield of haploproduced and androclinic chlorophyll-containing regenerants): 1 - F1 (Vizit x Amir); 2 - F1 (Tasos x Lada); 3 - F1 (Tulaykovskaya 10 x Vizit); 4 - F1 (Esther x Vizit).

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