The economic benefits of the production of double haploid for selection of white cabbage

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Abstract. An important task of any state in the world is to ensure food security. The Russian Federation is the third largest cabbage producer in the world. In the conditions of the modern market, for ensuring successful competitiveness, selection must promptly respond to consumer requests. To reduce some stages of the selection process, we used the modern biotechnological method of culture of isolated microspores in vitro. This method allows one to get homozygous lines in the first generation. In the Federal Scientific Center for Vegetable Growing, the protocol for obtaining doubled haploid cabbage cultures has been optimized and successfully applied in practice. However, for expediency of implementation of the obtained lines in the selection and seed-growing process, it is important for us to know the economic efficiency of production. The paper presents the data on the basic costs of obtaining lines of white cabbage with traditional and modern biotechnological methods of selection. The article provides a comparative assessment of their cost-effectiveness in the selection of white cabbage. It is shown that the use of the biotechnological method in combination with the classical method of selection allows to obtain pure lines with 100% homozygosity for 3 years. At the same time, economic costs of producing lines, when using doubled haploid plants of white cabbage, are reduced in 2 times.

1. Introduction
According to FAO, for 2017, global cabbage production is 71.451 million tons, and the cultivation area is 2.513 million hectares. The areas under cabbage cultivation in the Russian Federation amounted to 105.8 thousand hectares, and the production volume amounted to 3.531 million tons. In comparison to 2016, the area decreased by 6.1%, respectively, the production volume decreased by 2.4%. The same trend is observed in the world. Based on this, there is the task of improving the efficiency of production of cabbage crops in smaller areas, including by reducing production costs. To a greater extent, plant breeding can more effectively increase food production in smaller areas and is often more environmentally friendly today [1].

Among the plants of the Brassica genus, the most common vegetable crop is white cabbage (B. oleracea L. convar capitata (L.) Alef. var. capitata L. f. alba DC). Its popularity is explained by the whole complex of biological and economically useful properties [2]. Selection of white cabbage is mainly focused on the creation of F1 hybrids, differing from the variety populations with high yields, evenness of plants in terms of ripening and quality of productive organs. The most difficult, time consuming and lengthy step in this process is the creation of constant parental lines, which takes from 7 to 14 years to receive [3], [4].
Recent advances in biotechnology are a valuable and powerful tool for improving the efficiency of breeding and reducing the time to create clean lines of agricultural plants. Among biotechnological methods, the culture of isolated microspores in vitro has long been recognized as effective for improving breeding material. The basis of the technology is the ability of microspores to switch from the gametophytic path of development to the sporophytic one under the influence of stress factors such as elevated temperature, high osmotic pressure, etc. Under appropriate conditions, such microspores begin to develop in a sporophytic type, forming embryoids [5]. The first successful microspore culture studies in vegetable crops of the Brassicaceae Burnett family were carried out in the early 1980s [6]. Later, a basic rape seed microspore culture protocol was developed, which serves as the basis of DH-technology for plants of the Brassica L. genus [7]. Then the culture of microspores began to be used for different types of cabbage cultures [8], [9], [10], [11], [12], [13], [14]. At the Federal Scientific Center for Vegetable Growing, the protocol for creating doubled haploid plants in vegetable crops from the Brassicaceae Burnett family is optimized [15].

Despite obvious achievements of scientists in the field of biotechnology, the practical experience of using the obtained doubled haploid plants in domestic breeding is extremely limited [16]. At the Federal Scientific Center for Vegetable Growing, research work on the production of DH-plants of cabbage cultures has been going on for over 20 years, and the following plants have been created and are registered in the State Register of Breeding Achievements: DH-line broccoli “BRI-1” (Breeding achievement patent No. 7144); Dobrynja F1 kohlrabi cabbage hybrid, created on the basis of DH-lines (Breeding achievement patent No. 8866) [18]. A hybrid of white cabbage Natali F1 is undergoing state variety testing.

It should be noted that the feasibility of using modern methods in breeding is determined both by their economic efficiency and time costs. Therefore, an important and crucial step in the introduction of DH-technology in the selection of white cabbage is the determination of the economic benefits of the production of doubled haploid lines.

To solve this goal, the following is necessary:

1. To optimize the classical selection and seed-growing scheme for creating F1 hybrids of white cabbage due to the introduction of DH-lines;
2. To calculate the economic costs of plant production in vitro (cultures of isolated microspores);
3. To conduct a comparative analysis of the economic and time use of the classical and modern biotechnological method in the selection of white cabbage.

2. Method

The work was performed at the Federal Scientific Center for Vegetable Growing using classical and contemporary biotechnological methods for white cabbage breeding from 2014 to 2017. The starting material was the selection of cabbage samples from the white laboratory of selection and seed production of cabbage cultures and regenerated plants obtained in the culture of isolated microspores in vitro in the laboratory of biotechnology.

White cabbage plants were grown according to the method of field experiment according to Dospekhov [17]. Planting was carried out by the seedling method according to the scheme 70x50 cm, the breeding area was 175 m². Plants were selected in the stage of technical maturity of the heading according to the main approbation characteristics. Then, the selected queen cells were planted in 5-liter vegetation vessels with a nutrient mixture consisting of two parts of sod land, one part of humus and peat with the addition of a ammonium nitrate phosphate fertilizer (10 g per vegetal vessel) and dolomite flour. Then, the queen cells were placed on vernalization in a refrigeration chamber with a temperature of 4-6 °C for 60 days. At the end of vernalization (at the stage of acclimatization), the vegetation vessels with plants were placed in the vegetation chamber to receive the peduncle. Within 15-35 days, the temperature was gradually increased from + 8 °C to 16-18 °C, with a mode of 16 hours per day / 8 hours per night and under illumination of 9000 l [4].

Obtaining of regenerated plants by the in vitro culture of isolated microspores was carried out according to the methodological recommendations [15]. Plants, in which the leaves and root system
were normally developed, were transferred to vegetation vessels filled with a mixture of peat and perlite (7:3). To adapt plants to *in vivo* conditions, they were covered with perforated plastic cups. Regenerant plants were grown under the same conditions as donor plants.

The main economic indicators that make up the cost of producing pure lines of white cabbage were determined, guided by the technological map of cultivation of white cabbage in specific conditions of the Moscow region (according to the data of the Economic Department of the Federal Scientific Center for Vegetable Growing), the production of plants doubled haploids was estimated, and highly valuable observations were collected.

3. Results

3.1 Determination of the time spent on creating pure lines of white cabbage

The use of doubled haploids in the selection of white cabbage allows for more efficient selection of the desired genotypes (by expanding the spectrum of genetic recombinant forms). Since the lines of doubled haploids are 100% homozygous, their selection can be carried out in the first generation. In classical breeding, to obtain an aligned homozygous line, 6 generations of inbreeding are required to be carried out, which can take up to 12 years for cabbage with a two-year development cycle. Thus, the time spent on creating a clean line of white cabbage is significantly reduced (Fig. 1).

![Diagram of ways to create a linear material of white cabbage](image)

**Fig. 1.** Diagram of ways to create a linear material of white cabbage.

To speed up individual stages of the selection process, in our work to create homozygous lines, artificial climate chambers with a given light and temperature conditions are used. The use of these climate chambers in breeding work allows us to translate the cultivation of biennial crops into a one-year cycle. The use of artificial climate chambers makes it possible to reduce the selection process by two times under controlled conditions [4].

For the use of white cabbage lines, in breeding, an important step is to determine the ability of the line to produce heterotic offspring in one or another hybrid combination. Thus, taking into account the reduction of individual stages due to the artificial climate chambers, the duration of the selection process for creating and evaluating pure lines of white cabbage will look like this:

1. Sowing of seeds and study of source material, selection of queen cells, vernalization: spring-autumn X - the start of breeding work;
2. Production of doubled haploid lines in an isolated microspore culture (production, adaptation, vernalization at the “stem” stage): winter-autumn X + 1;
3. Duplication of doubled haploid lines, production of hybrid combinations, evaluation of the line combining ability: winter-autumn X + 2.

According to the presented scheme, it takes 3 years to get lines of white cabbage with 100% homozygosity, which can be included in the selection scheme for creating an F1 hybrid.
3.2 Determination of the economic costs of production of white cabbage lines by various methods

To determine the economic benefits of producing white cabbage lines, we compared the traditional method of white cabbage breeding for 12 years in a two-year cycle and for 6 years by converting to a one-year cycle through artificial climate chambers. Also, we compared the traditional method of white cabbage breeding for 12 years in a two-year cycle and for 6 years by shortening the stage of creating a pure homozygous line by introducing DH-technology.

In agriculture, the cost of cultivating any crop, including white cabbage, is determined by drawing up a flow chart, into which major expenses are made necessary during the entire period of crop cultivation. Since white cabbage is a biennial crop, costs must be taken into account over two years of the full cycle of plant development. In the first year, the plants are grown up to the technical ripeness phase of the head and laid for storage and vernalization. In the second year, all necessary testes are grown, and one gets the seeds.

Since we had to compare 3 methods of creating a clean line of white cabbage, we would adhere to the “only difference” principle. At the initial stage of growing the queen cells, conditions were the same for all methods. The collection nursery consisted of 500 plants of white cabbage, covering an area of 175m². The intensity of selection was 10%, so 50 queen cells were deposited. The Federal Scientific Center for Vegetable Growing has a storage facility with a storage space for vegetable crops of 864 m³, while our plants occupied 1.5 m³. After storage, 10% of the plants were culled, so 45 plants were grown in greenhouses on an area of 16m² (Table 1).

Table 1. Costs for cultivation of cabbage plants by the traditional method in a 2-year cycle (direct costs are provided according to the data of the economic department of the Federal Scientific Center for Vegetable Growing).

| No | Expenditure | Unit cost | Amount | Cost, rub. |
|----|-------------|-----------|--------|------------|
| 1  | Growing seedlings | 40 days   | 500 plants (area of 175 m²) | |
| 1.1| Wages | 120.01 | 40 man-hours | 4800.40 |
| 1.2| Cassettes for seedlings | 70.00 | 10 cassettes of 60 cells | 700.00 |
| 1.3| Priming | 3.00 | 10 cassettes (36 l) | 108.00 |
| 1.4| Fertilizers | 250.00 | 1 dressing for the period | 250.00 |
| 1.5| Other | 40 days (depreciation, water, protection, if necessary) | 500.00 |
| 2  | Growing queen cells | 125 days | |
| 2.1| Wages | 120.01 | Total scope of work (tractor drivers and workers) | 38479.60 |
| 2.2| Fuel | 40.00 | When using the MTZ-80 and John Deere tractor (3.8 liters) | 152.43 |
| 2.3| Fertilizers | 155.00 | Minerals - 10.5 kg | 1627.50 |
| 2.4| Means of protection | 1173.00 | 2 treatments of 35 ml | 82.11 |
| 2.5| Watering | 20.00 | 10.5 liters for treatment and watering for 3 months | 4326.00 |
| 2.6| Depreciation | 12618.00 | MTZ-80 and John Deere | 220.82 |
| 2.7| Seeds | 25000 | On the whole area of 2.45 g | 61.25 |
| 3  | Storage of queen cells | 730.46 | From October to April (6 months), 1 container (1.5 m³) | 4382.76 |
| 4  | Total for the year | | | 55690.87 |

| No | Expenditure | Unit cost | Amount | Cost, rub. |
|----|-------------|-----------|--------|------------|
| 1  | Wages | 1713.60 | In the period from April to September (6 months) | 13386.64 |
| 2  | Fuel | 40.00 | When using the Mitsubishi MT-180D mini-tractor (12.6 liters) | 506.25 |
| 3  | Watering | 20.00 | Based on 115.8 liters of water | 2315.25 |
| 4  | Consumables | 100.00 | Insulators, labels, garter rope | 4500.00 |
| 5  | Depreciation | 45.30 | On the occupied area | 724.80 |
6 Fertilizers 155.00 They are worn by hand under each plant (4 times per period) 459.38

| Total: | Per year | One hole cycle | For six cycles |
|--------|----------|----------------|---------------|
|        |          | 21892.31       | 77583.18      | 465499.08    |

Table 2. Estimated costs for the production of doubled haploids of white cabbage by the method of in vitro culture of isolated microspores.

| No | Main expenses | Unit cost | Amount | Amount, rub. |
|----|---------------|-----------|--------|--------------|
| 1  | Climate chamber |           |        |              |
| 1.1 | Light | 4.80 | 120 days (16 hours a day, power 600 W, 6 lamps) | 33177.6 |
| 1.2 | Priming | 3.00 | 50 plants 5l = 250l | 750.00 |
| 1.4 | Fertilizers | 103.00 | 4 kg | 412.00 |
| 1.5 | Vegetation vessels | 60.00 | 50 on 5l | 3000.00 |
| 2  | Laying microspores |           |        |              |
| 2.1 | Nutrient medium | 512.34 | 11 Nitsch | 512.34 |
| 2.2 | Utensils for cooking environment | 240.00 | 2 glasses | 480.00 |
| 2.3 | Cultivation dishes | 660.00 | 2 bottles | 1320.00 |
| 2.4 | Autoclaving | 4.80 | 1 hour (power 3 kW) | 14.40 |
| 2.5 | Electrical costs laminar | 4.80 | 6 hours (power 330 W) | 9.50 |
| 2.6 | Work, man-hours | 960.04 | 2 man-days | 1920.08 |
| 3  | Cultivation of microspores |           |        |              |
| 3.1 | Thermostat, energy costs | 4.80 | 30 days (power 330 W) | 1140.48 |
| 3.2 | Work, man-hours | 120.01 | 4 man-hours | 480.04 |
| 4  | Embryoid cultivation |           |        |              |
| 4.1 | Nutrient medium | 334.75 | MS: 1,5l in cups, 10l * 2 in cans | 7197.13 |
| 4.2 | Utensils for cooking environment | 240.00 | 2 glasses | 480.00 |
| 4.3 | Cultivation dishes | 660.00 | 2 bottles | 1320.00 |
| 4.4 | Autoclaving | 10.00 | 50 Petri dishes, 500 cans with lids | 500.00 |
| 4.5 | Work, man-hours | 26.48 | Other laboratory materials | 1324.00 |
| 4.6 | Autoclaving | 4.80 | 5 hours (power 3 kW) | 2000.00 |
| 4.7 | Electrical costs laminar – Shelving | 4.80 | 40 hours (power 330 W) | 63.36 |
| 4.8 | Work, man-hours | 4.80 | 720 hours (power 36 W, 14 lamps) | 1741.82 |
| 5  | Total | 120.01 | 5 person-days | 4800.16 |

* For genotypes whose embryoids range is from 5 to 20 embryoids per Petri dish (6 mm in diameter).

To determine the cost of creating regenerated plants obtained in an in vitro culture of isolated microspores, the cost estimates for the main expenses were calculated based on obtaining 50 doubled haploid lines of white cabbage, according to the principle of the only difference for all methods (Table
2). At the same time, it is necessary to take into account that the average costs are based on the production of DH-plants in an isolated in vitro microspore culture, since the release of embryoids would strongly depend on the responsiveness of a particular genotype when the technology is used. Further, we carried out vernalization of the obtained plants in the “stem” stage and hybridization in an artificial climate chamber with given light parameters for 5 months (Table 3).

**Table 3.** The costs of hybridization of white cabbage plants in an artificial climate chamber (direct costs according to the Economic Department of the Federal Scientific Center for Vegetable Growing).

| No | Main expenses          | Unit cost | Amount     |
|----|------------------------|-----------|------------|
|    |                        |           | Amount,    |
|    |                        |           | rub.       |
| 1  | Climate chamber        |           |            |
| 1.1| Light                  | 4.80      | 41472.00   |
|    |                        | 150 days (16 hours per day, power 600 W, 6 lamps) |
| 1.2| Plant care, man-hours  | 120.01    | 21001.75   |
|    |                        | 175 man-hours |
| 1.3| Priming                | 3.00      | 750.00     |
|    |                        | 50 plants  |
| 1.4| Fertilizers            | 103.00    | 412.00     |
|    |                        | 4 kg      |
| 1.5| Vegetation vessels     | 60.00     | 3000.00    |
|    |                        | 50 on 5l  |
| 1.6| Total                  |           | 66635.75   |

To determine the economic efficiency of various methods of breeding white cabbage, a comparative assessment of the cost of obtaining 50 plants of white cabbage was carried out according to the principle of the only difference (Table 4).

As a result of the calculation, the obtained data showed that when creating lines for F₁ hybrids of white cabbage, the most cost-effective is the use of contemporary biotechnological method. Since due to the reduction of some stages of the selection process, financial costs are reduced. As well as the use of artificial climate chambers is advisable, since the time to get the lines is reduced in 2 times, which contributes to the rapid entry of hybrids into the market. Consequently, this increases the implementation period and profits.

**Table 4.** Comparative assessment of the cost necessary for creating lines of doubled haploids of white cabbage by various selection methods.

| Indicators                              | Traditional selection method (inbreeding) | Biotechnological method (in vitro culture of isolated microspores 1 year) |
|-----------------------------------------|------------------------------------------|------------------------------------------------------------------------|
|                                        | 12 years                                 | 6 years, sing artificial climate chambers                              |
| Costs of cultivation, rubles / ha       | Open ground                              | 334145.22                                                             |
|                                        | Protected ground                         | 334145.22                                                             |
|                                        | -                                        | 55690.87                                                              |
| Cost of hybridization in an artificial climate chamber, rubles / 50 plants | -                                        | 399814.50                                                             |
|                                        | -                                        | 66635.75                                                              |
| Costs necessary for doubled haploid lines production, rubles / 50 plants | -                                        | 399814.50                                                             |
|                                        | -                                        | 110250.31                                                             |
| Total costs, rub.                       | 465498.86                                | 733959.72                                                             |
| Costs for 1 plant, rub.                 | 9309.98                                  | 14679.19                                                              |
|                                        |                                          | 4651.54                                                              |
4. Conclusion

By calculating the cost of creating pure lines of white cabbage, the economic benefit of using the modern biotechnological method of culture of isolated microspores *in vitro* when creating white cabbage hybrids is proved. At the same time, the production time of hybrids is reduced from 12 to 6 years, and financial costs are reduced in 2 times.

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