Economic efficiency of obtaining carrot lines using classical and biotechnological methods

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Abstract. Garden carrot is a traditional food in Russia. Based on the consumption rate of 10 kg per year per person, 1,469 thousand tons of carrots per year must be produced for the population of the Russian Federation. To meet the needs of the population in fresh and processed carrot products, the development of new highly productive and resistant varieties and hybrids is necessary. The traditional production of linear material and hybrids in the breeding of garden carrots is a laborious, lengthy process due to the 2-year cycle of plant development, self-incompatibility, and inbreeding depression. On average, 16-20 years can be spent on creating a hybrid of carrots with the successful course of the selection process. Using biotechnological methods, completely homozygous lines can be obtained in 1 year and a wide variety of starting material can be created for inclusion in the breeding process. The article presents an analysis of economic efficiency and a comparative assessment of the production of garden carrot lines using classical breeding and biotechnology methods. Calculations are based on the rates being used at the Federal Scientific Center for Vegetable Growing. It is shown that the economic costs of obtaining lines when using doubled haploid plants of the garden carrot are reduced in almost five times. To increase the efficiency of the breeding process, biotechnological methods need to be embedded in classical breeding schemes.

1. Introduction

Garden carrot is a valuable, indispensable for rational human nutrition vegetable culture, characterized by a high content of vitamins, biologically active substances, mineral salts, phytoncides, essential oils and dietary fibers necessary for the normal functioning of the human body. Also, it is used for medicinal purposes and in the perfume industry [1]. To meet the needs of the population in fresh vegetables, it is necessary to produce 17.9 million tons of vegetables. In the Russian Federation, 66.3 thousand hectares are occupied under crops of carrots and turnips (according to the FAOSTAT statistics database, 2018). The difference between demand and production is made up for by imported products from the countries of near and far abroad. In comparison to 2017, carrot imports decreased by 10.6% in 2018. The main exporters to Russia are Israel, China, Belarus, Egypt.

150 varieties and 150 hybrids of garden carrots are in the State Register of Breeding Achievements for 2019. Most of them are represented by foreign companies. Manufacturers prefer F₁ hybrids, which are most aligned, plastic and, as a result, technologically advanced compared to varieties. Their cultivation to the maximum eliminates manual labor, in connection with which, the time and cost are reduced.
Table carrot is a cross-pollinated plant, its varieties represent a population of biotypes that are close to each other in morphological and economic characteristics. The traditional production of linear material and hybrids in the breeding of garden carrots is a laborious, lengthy process due to the 2-year cycle of plant development, self-incompatibility, and inbreeding depression. Carrot plants bloom and produce seed offspring only in the second year of life \textit{(in vivo)}. Root crops need to go through the vernalization stage for 3-5 months at a temperature of 2-3 °C during their winter storage.

Currently, one of the main ways to obtain carrot hybrids is the use of forms with cytoplasmic male sterility (CMS). Three types of lines are necessary to create a hybrid \textit{ms line A}, which has a CMS, line \textit{B} - a fixer of a CMS at line \textit{A}, and line \textit{C}, which is a pollinator of line \textit{A}. These lines are created by the classical selection methods by inbreeding (forced self-pollination) and selection for 6-7 generations. This is a laborious and complex breeding work that requires a sterility control in the interaction of nucleus genes with cytoplasmic sterility genes. On average, 16-20 years can be spent on creating a carrot hybrid with a successful selection process [2]. Over this long period of time, the renewal of the fleet of agricultural machinery occurs, phytopathogens overcome the resistance of plants and consumer demands are changing. Thus, traditional breeding lags behind the technical development of the agro-industrial complex, does not keep pace with changes in consumer preferences and the evolution of harmful organisms. In addition, it is very important that the lines that make up the basis of the hybrid are aligned, have high combining ability in terms of yield, quality and adaptability, which cannot be achieved with 100% probability using only classical methods of creating linear material.

The key stage, which takes the longest time in the breeding scheme for creating a hybrid of canteen carrots, is to obtain clean lines, then the inclusion of doubled haploids in the breeding scheme (DH-technology) can reduce the time for creating a hybrid to 4-6 years. Obtaining doubled haploids \textit{in vitro} significantly accelerates the creation of completely homozygous lines in one generation; while increasing the accuracy and efficiency of the selection process, allows you to quickly find the desired combination, reduces the time to create lines and hybrids. The development and implementation of doubled haploid technology has significantly changed the plant breeding process in the world. Nowadays, this innovative and effective technology is included in the breeding programs of many globally important crops such as corn, wheat, barley, rape, sugar beets, peppers, and cucumbers [3]. Every year, in the world, a significant number of new plant varieties obtained using haploid technologies are recorded. Today, the leaders in the field of haploid technologies are the EU countries, Australia, Canada, the USA and China [4]. We are aware of a registered patent for the production of doubled haploids of agricultural crops of the Apiaceae family in November 2006 (WO 2006/125310). In Russia, such studies are conducted only in a small number of scientific institutions. We are aware of only one registered variety of garden carrots, namely the Sonata, created with the participation of VNIISSOK (now Federal Scientific Center for Vegetable Growing) on the basis of DH-lines (Copyright certificate No. 34702 of 11.29.2000).

Doubled garden carrot haploids can be obtained using the method of gynogenesis (culture of dustless ovules) and androgenesis (anther culture, culture of isolated microspores). The complexity of the application of the anther culture method is associated with the uncertainty of the result, since embryoids and callus can develop both from microspore cells and from anther somatic tissues. The same problem exists when using the method of cultivating unfertilized ovules (gynogenesis) of carrots. However, this method is the only way to obtain doubled haploids from sterile plants. The most promising is the production of DH-plants in a culture of isolated microspores. This excludes the presence in the in vitro culture of somatic tissues, which cast doubt on the haploid nature of the resulting plants. Success in using the method of culture of microspores garden carrots was achieved only in recent years. The authors of [5] first reported the formation of multinucleated structures and callus in a culture of isolated microspores. The authors first received DH-carrot plants and presented their protocol [6], [7]. At the Federal Scientific Center for Vegetable Growing, we have created an effective technology for producing doubled haploids in the culture of isolated microspores \textit{in vitro} [8]. The use of this technology and \textit{in vitro} dustless ovules culture (for sterile samples) makes it possible to significantly reduce the time required to obtain homozygous lines. In this regard, we conducted a study comparing the economic
efficiency of obtaining clean garden carrot lines using classical and biotechnological methods in 2017-2018.

2. Materials and Method
The studies were carried out on the basis of the Federal Scientific Center for Vegetable Growing in 2017-2018. Calculating the costs of creating a line of garden carrots was carried out taking into account labor resources, as well as the cost of materials and fixed assets on average for 2017-2018. We collected and analyzed experimental data on labor and material resources at all stages of the selection process when creating lines.

We compared the following two methods of creating clean lines of garden carrots in the conditions of the Moscow region: (1) in the open ground and using unheated greenhouse; (2) using a biotechnological method of an in vitro culture of isolated microspores.

We used samples of carrots from the collection of the laboratory of genetics and cytology and the laboratory of table root vegetables of the Federal Scientific Center for Vegetable Growing. Donor plants were grown in open ground conditions with the observance of agrotechnical measures for the production of testes. Root crops, after vernalization in a refrigerating chamber at a temperature of + 4 ... 6 °C for 3-3.5 months, were planted in a climate chamber at around-the-clock temperature of 19 °C, illumination of 9000 lux, and a light mode of 16 hours a day / 8 hours a night.

For the introduction into culture, the selection of buds was performed using cytological study of the stages of development of microspores. A differential staining technique [9] and an Axio Imager A2 microscope (Zeiss, Germany) were used to determine the stage of development of microspores. The buds collected from plants were sterilized for 30 seconds in 96% ethanol, then for 5 minutes in a 50% aqueous solution of the Belizna preparation, with adding 1 drop per 100 ml of Tvin-20. Then, the buds were washed three times in sterile distilled water. The cultivation of microspores was carried out on ½ NLN medium with a sucrose concentration of 13% and MS in Petri dishes 6 cm in diameter according to the developed procedure [8].

3. Results
The cost of breeding one variety or hybrid varies significantly and depends on the methods and technological receptions, technical equipment and skills of workers. The high cost of foreign seeds (France, Holland, China) is associated with high costs for the creation of hybrids. They include the cost of selected varietal samples, which is about 70% of the total costs.

The criterion for the effectiveness of methods for obtaining clean lines is the acceleration of the process with minimal expenditure of material and labor resources. Accounting for specific indicators reflecting the impact of various factors on this process is necessary. When evaluating performance, attention should be paid to the features that influence the final results.

To identify the economic effect, knowledge of total labor costs, which provided a specific result, is necessary. The same result can be achieved with different costs and time. For a comparative assessment of economic efficiency, we considered direct variable costs. They include the following: material costs for seeds, fertilizers, plant protection products, combustible and lubricating materials, electricity, maintenance of machinery and other material costs.

In the collection of variety samples of the source material, we studied the economically valuable traits and carried out the search and selection of plants of interest for inclusion in the breeding process (August - September). When calculating, we took into account the costs of growing 1000 plants in a collection nursery (open ground), occupying an area of 30 m². The selection efficiency for further work is 25% (i.e. 250 plants). Losses during storage (October - April) amounted to 10%, and we planted 225 root crops in the greenhouse in April. When using the trilinear hybrid creation scheme, for 6 cycles (seed-roots-self-pollination / inbreeding-seeds), at least three aligned lines (line A, B, C) should be provided after evaluation by economically valuable attributes.

Economic evaluation for the year was carried out by comparing the cost of the occupied area and was calculated for one unit. To determine the costs, flow charts on the culture of garden carrots were
made on the basis of prices prevailing in 2017 - 2018. Analysis of the impact of costs on the cultivation of plants in open ground shows that the main part of them falls on the share of wages, namely 37,453 rubles (Table 1).

Table 1. Costs for obtaining lines in open ground and unheated greenhouse (6 generations of inbreeding).

| No | Main expenses                                      | Unit cost, rub | Amount, pieces | Sum, rub |
|----|---------------------------------------------------|---------------|----------------|---------|
| 1  | Wages, rub.                                       | 37453.00      |                | 37453.00|
| 2  | The cost of fuels and lubricants                  | 40.00         | MTZ-80, John Deere | 26.13   |
| 3  | The cost of mineral fertilizers, rub.             | 155.00        | Mineral fertilizers - 1.8 kg | 279.00 |
| 4  | Plant protection products                         | 1227.00       | 3 treatments of 0.6 ml | 56.10   |
| 5  | Depreciation                                      | 12618.00      | MTZ-80, John Deere | 37.90   |
| 6  | Seeds                                             | 25000.00      | On the whole area, 6 g | 150.00  |
| 7  | Storage costs                                     | 730.46        | Within 6 months (October-April) 1.5 m³ (container) | 4382.76 |

Total for 1 year: 42384.89 rubles

Protected ground, area 40 m², 225 plants*

| No | Main expenses                                      | Unit cost, rub | Amount, pieces | Sum, rub |
|----|---------------------------------------------------|---------------|----------------|---------|
| 1  | Wages, rub.                                       | 4284.00       | In the period from April to September (6 months) | 27935.00 |
| 2  | The cost of fuels and lubricants                  | 40.00         | When using a mini-tractor Mitsubishi MT-180 D | 1265.00 |
| 3  | Watering                                          | 20.00         | As needed      | 5788.00 |
| 4  | Consumables                                       | 100.00        | Insulators, labels, twine | 22500.00 |
| 5  | Depreciation                                      | 45.30         | On the occupied area | 1812.00 |
| 6  | Fertilizers                                       | 155.00        | On the 40 m² (4 feeding) | 1488.00 |

Total for the second year: 60788.00 rubles

Total for 2 years: 103172.89 rubles

Only 6 cycles: 619037.30 rubles

* A sample of 225 plants was taken to simplify calculations; it also allows one to evaluate and maintain the necessary (at least three) to create a hybrid lines.

Since carrots are a biennial crop, the total costs consist of the costs of growing plants in open and protected ground. Using classical breeding methods, at least 6 generations of inbreeding are required to create a line for biennial cultures, and this is at least 12 years. For 6 cycles, the economic costs of carrying out inbreeding on the minimum number of samples (to create a subsequent hybrid) amount to 619,037.30 rubles. At the same time, it is necessary to take into account that even after 6 generations of inbreeding, 100% evenness of lines cannot be guaranteed.

One solution to this problem is to use the biotechnological method of in vitro culture of isolated microspores, which allows one to reduce the time to create clean lines compared to traditional methods. To determine the necessary costs for the creation of homozygous carrot lines in an in vitro culture of isolated microspores, an estimate of labor costs and material resources at current prices on the basis of 50 doubled haploid carrot lines were obtained. According to the results of our research, costs amounted to 129,063.60 rubles (Table 2).

In addition, there are differences in the responsiveness of a particular genotype, which affect the final yield of doubled haploids. The calculations made are given for samples whose responsiveness in a microspore culture is up to 10 embryos per 1 cultured Petri dish 6 mm in diameter. For poorly responsive genotypes, larger studies need to be carried out, which would entail additional costs for modifying the
standard technique. However, a significant reduction in the time interval for creating a fully homozygous line to one year increases the economic benefit. The important point is that in doubled haploids, all recessive signs are visible and not disguised under the influence of dominant genes, in connection with which selection is greatly simplified and its accuracy increases.

Table 2. Costs for obtaining a line of garden carrots by using a microspore culture in vitro.

| No | Main expenses                          | Unit cost, rub | Amount | Amount, rub |
|----|----------------------------------------|----------------|--------|-------------|
| 1  | Laying microspores                     |                |        |             |
| 1.1| Nutrient medium                        | 850.00         | 1 NLN  | 850.00      |
| 1.2| Utensils for cooking medium            | 240.00         | 2 glasses | 480.00   |
|     |                                        | 660.00         | 2 bottles | 1320.00  |
| 1.3| Cultivation dishes                    | 10.00          | Petri dishes - 50 pieces | 500.00 |
| 1.4| Autoclaving                            | 4.80           | 1 hour (power 3 kW) | 14.40 |
| 1.5| Electric costs - Laminar               | 4.80           | 6 hours (power 330 W) | 9.50  |
| 1.6| Work, man-hours                        | 120.01         | 4 man-hours | 480.04  |
| 2  | Microspores cultivation                |                |        |             |
| 2.1| Energy costs - thermostat              | 4.80           | 60 days (330 W) | 2280.96 |
| 2.2| Work, man-hours                        | 120.01         | 4 man-hours | 480.04  |
| 3  | Embryoid cultivation                   |                |        |             |
| 3.1| Nutrient medium                        | 1000.00        | MSm - 2 l (with hormones) | 2000.00 |
|     |                                        | 850.00         | NLN - 2 l | 1700.00  |
| 3.2| Utensils for cooking medium*           | 240.00         | 2 glasses | 480.00   |
|     |                                        | 660.00         | 2 bottles | 1320.00  |
| 3.3| Cultivation dishes                    | 8.00           | Petri dishes - 50 pieces | 400.00 |
|     |                                        | 12.00          | Test tubes * PB2-16x150 - 500 pieces | 6000.00 |
| 3.4| Autoclaving                            | 4.80           | 5 hours (power 3 kW) | 72.00  |
| 3.5| Electric costs - Laminar               | 4.80           | 40 hours (power 330 W) | 63.36  |
| 3.6| Electric costs - racks                 | 4.80           | 1800 hours (power 36 W, 14 lamps) | 3110.40 |
| 3.7| Work, man-hours                        | 120.01         | 5 man-days | 4800.16  |
| 4  | Cultivation of regenerated plants      |                |        |             |
| 4.1| The soil                               | 5.00           | 50 plants / 5 l = 250 l | 1250.00 |
| 4.2| Light                                  | 4.80           | 90 days (16 hours / day, power 600 W 6 lamps) | 24883.20 |
| 4.3| Plant care, man-hours                  | 120.01         | 110 man-hours | 13201.10 |
| 4.4| Fertilizers                            | 250.00         | Top dressing 4-5 times for the period | 500.00  |
| 4.5| Vegetation vessels                     | 60.00          | 50 pieces on 5 l | 3000.00 |
| 4.6| Vernalization                          | 4.80           | 90 days - 2160 hours Refrigerator (power 105 W) | 45360.00 |
| 6  | Other expenses                         | 1000.00        | 1 laying | 1000.00  |
| 7  | Growing plants in the greenhouse to seed| 50.00         | The complex of necessary expenses | 13508.44 |
|    | Total                                  |                |        | 129063.60  |

4. Conclusion
As a result of calculations, it is clearly shown that, in terms of the cost of production and a significant reduction in the time to create lines, biotechnological methods are the most cost-effective. They allow
to significantly reduce the duration of the selection process for 8 - 12 years. To create competitive new
domestic hybrids of garden carrots, biotechnology methods need to be embedded in traditional breeding
schemes.

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