The combining ability of self-pollinated lines of the red beet 
\textit{(Beta vulgaris L)}

L N Timakova, A N Khovrin, M A Dolgopolova

All-Russian Horticultural Research Institute – a branch of the Federal Research Center for Horticulture, building 500, Vereya, Rameno district, Moscow Oblast, 140153, Russia

E-mail: ljubovtimakova@rambler.ru

\textbf{Abstract.} The proper choice of starting material determines the results of the selection. If the starting forms of plants are not fit for crossing and do not match the goals set or the soil and climate conditions available, any selection work may prove futile. The creation of new starting material is based on the study of the existing varieties and the selection of the best biotypes, as well as the assessment of a set of biological and economically valuable parameters of breeding. Inbreeding is the most widely used method of obtaining new starting material, especially homozygous lines, and it plays a key role in heterosis selection programs for the majority of agricultural crops. The use of beet heterosis allows for a significant increase in homogeneity, quality, and marketability of the produce. In 2018-2020, nine samples of red beet starting material were assessed. Using the polycross methods, we assessed the general combining ability of self-pollinated red beet lines across the parameters determining the commercial properties of this root crop: the proportion of the root crop top in its diameter, the share of leaves in the plant biomass, and regularity of shape and marketability. The subject matter of this work is the lines of red beet with round root crops. Research material includes the raw data from field measurements and records. We used the Kestrel F1 hybrid variety released for Central Russia. Field tests were carried out using the uniform procedures set out by the All-Russian Horticultural Research Institute - a branch of the Federal Research Center for Horticulture from Moscow Oblast. At the experimental locations, we used the agricultural practices typical of the region. Using the properties - the small proportion of the root crop top (42.1-47%) and the share of leaves in the biomass (24.1-27.5%), high regularity of root crop shape (up to 85.6%), and marketability (88.3-95.9%) - we determined the following lines: 506 I_2, 507 I_2.

1. \textbf{Introduction}

Of all the root crops, the red beet or \textit{Beta vulgaris L} is a traditional vegetable in Russia. It is valued for its taste and great effects on human health. Red beet contains small amounts of organic acids: lactic, tartaric, malic, oxalic, and citric. This plant is rich in carbohydrates, and it contains vitamins C (10-20 mg% in roots and up to mg% in leaves), B1, B2, B6, PP, as well as carotin and pantothenic and folic acids. The crop accumulates the largest amounts of microelements like zinc and iodine compared to other vegetables. With easily digested proteins, carbohydrates, essential amino acids, and microelements, red beetroots are a valuable food for people of all ages [1]. Besides, this root crop may be stored for a long time and preserve its health benefits. This allows for the year-round use of fresh
beet: from young leaves and stalks in early spring to roots up to the spring of the next year. In the summer months, people eat fresh bundles of this root crop.

The current state of the vegetable market set high requirements for the commercial and nutritional properties of the red beet. Modern varieties and hybrids of the red beet must have high marketability, even surface, thin axial root, small top relative to the root diameter, compact leaf rosette, and resistance against cercosporosis.

The production of red beet must be based on comprehensive sets of activities based on new adjusted varieties and hybrids [2]. Their creation requires the availability of diverse, well-studied starting material.

2. Problem Statement
Our red beet selection activities aim at the creation of lines using the inbreeding method. The key advantage of inbreeding is the possibility to obtain high homozygosity in a short time. It helps analyze complicated cross-pollinated populations and provides an opportunity to isolate some homozygous genotypes that have both valuable and negative properties and obtain descendants with equal genetic and morphological properties [3,4]. Self-pollinated lines are valued as the parents of new varieties and hybrids due to their combining ability. The theoretical concept of this parameter and the methods of its calculation for agricultural items have been described in detail in many works by Russian and foreign experts [5-7]. Involving lines with high combining ability in heterosis selection for desired properties may help obtain varieties and hybrids with the set parameters. The selection is only successful if the assessment of the required parameter in lines is sufficiently accurate and performed on time. The duration of the combining ability assessment of lines depends on the stabilization of the required economically valuable properties under self-pollination, and the accuracy of the assessment depends on the method used to determine the combining value [8]. Combining ability is a genetically conditioned property. Johnson I.J., Hayes H.K., Cowan J.R., Green J.M., Zelensky M.A., and Morgun V.V. established that combining ability is inherited by descendants [9-13].

To obtain the required GCA of the bred lines, we relied on crossing with subsequent testing of the hybrids. When determining the combining ability of red beet lines and varieties, the possibility of controlling crossings is a limiting factor because it is difficult to sterilize small bisexual flowers.

Polycross is the simplest type of crossing that does not require significant expenses and is accurate enough for the preliminary selection of breeding materials [14]. The polycross testing is conventionally viewed as a selection method for GCA that helps determine the best genotypes in the analyzed group of specimens. The term “polycross” stands for the descendants of cross-pollinated plants grown from line seeds or clones that can intercross with other lines and clones when planted in the same nursery [15-18].

It is possible to improve the efficiency of selection activities by using parents with high combining ability in crossing. Combining ability is one of the key parameters defining the heterosis of hybrid descendants [19]. The use of heterosis is one of the key aspects of root crop plant selection, including the beet. Heterosis is the first-generation hybrids’ ability to surpass their best parent in some of the properties.

3. Research Questions
The experiments were carried out at the All-Russian Horticultural Research Center - a branch of the Federal Research Center for Horticulture in Moscow Oblast in 2018-2020. The subject matter of the research comprised 9 lines of red beet with round roots. The research material included the raw data from field measurements and records. We used the Kestrel F1 hybrid variety as a reference because it is adapted for Central Russia and widely produced.

4. Purpose of the Study
The goal of the research is to determine the combining ability of inbred lines of the red beet through polycross and to study the competitive heterosis of the key selection parameters.
5. Research Methods
Field tests were carried out using uniform methods [20]. The soil in the experimental field is alluvial meadow, middle-loamy, saturated, and moisture adsorbent. The thickness of topsoil is 27 cm and the groundwater depth is over 2 m. It has a high humus content of 3.5-3.8%, a close to neutral salt extract reaction of 5.5-6.1, the total nitrogen content of 0.19-0.24%, a content of nitrate nitrogen of 2.0-2.8 mg/100g, and the content of liable phosphorus and potassium of 17.6-19.1 mg/100 g and 7.0-8.2 mg/100 g respectively. This set of physical and chemical properties of the soil is especially favorable for growing vegetables. At the experimental locations, we used the agricultural practices typical of the region.

During the selection-genetics studies, we looked into the competitive heterosis (compared the F1 hybrid and the reference) across the key biological and economic properties like marketability and the share of round root crops in the population. In this case, it is determined using the following formula [21]:

\[
G_{con.}(\%) = \frac{F1 - St}{St} \times 100
\]

To assess the combining ability, we studied F1 hybrids obtained as a result of polycrossing of inbred fertile lines of the red beet. The polycross nursery was established in 2018 using wide-row planting (x0.7 m), double replication, and randomized location of components. Seeds were collected separately from each of the mother plants. The obtained hybrid combinations were tested in 2018-2019. The experiment was repeated twice. General combining ability was calculated as the ratio of the quantity of F1 hybrid descendant parameters to the average parameters of all hybrids.

We used the Sprainaitis scale to assess the combining ability [22]:
- Very low <100 % + LSD
- Low <100 % + LSD
- Medium 101-110 % + LSD
- High 111-120 % + LSD

The weather during the experimental years was different. In 2019, precipitation was uneven, which impacted the seed germination time. During the seeding, in the last third of May, there was only 14.7% of the long-time annual average precipitation. The highest precipitation rate was observed in the second-thirds of July and August. The amount of precipitation in the last third of May 2020 exceeded the long-time annual average by almost 3 times. This had a favorable impact on seed germination, and the sprouts appeared evenly on the 12-14th day. In 2020, the amount of precipitation and the average air temperature were close to the long-time annual averages throughout the vegetation period of the red beet.

6. Results
Compact root crop tops are a key factor of consumer appeal of the red beet varieties because it reduces the wastes when the vegetable is processed. Note The top stands for the upper part of the root that has leaves and sprouts. The leaf plays a key role in the life of a plant, as well as the crop formation and its quality. Its life span, age, surface area, and the physiological activity of the leaf tissues and processes occurring in it are important for the proper functioning of the assimilatory mechanism [23]. The share of the leaf rosette in the plant biomass shows how efficient is the work of the photosynthetic mechanism. This parameter impacts the crop’s technological properties. The seeding rate for plants with compact, small-volume leaf rosette is higher than that of the varieties with large leaves. Due to this, we paid special attention to the share of the top in the root diameter and the share of the leaf rosette in the plant's biomass when assessing promising samples.

The share of the top in the maternal line root varied significantly between 48.8 and 65.1%, the variation factor for this parameter was 18.2%. The value of this parameter for lines 511-2, 511-4, and 521 is higher than that of the reference sample and is over 60%.
The results of the two-year-long study of polycross descendants showed that the share of the top in the root diameter decreases compared to the maternal component. Parameter variation also decreased from 42.1 to 52.1% (V-9.8%). This parameter decreased the most in the hybrids whose maternal components had the highest parameter values. The compact top obtained in samples 506F1 and 507 F1 was significantly smaller than the reference, and its source is sample M-12.

### Table 1. The properties of polycross descendants of self-pollinated lines of the red beet for the share of the top in root crop diameter, 2019-2020

| Selection code of descendants | Inbreeding generation of the maternal line | Maternal line source | Share of the top in root crop diameter, % |
|-------------------------------|-------------------------------------------|----------------------|------------------------------------------|
| Kestrel standard              | F1                                        | Sakata               | 52.1                                     |
| 549-2 x mix                   | I₁                                        | Modana               | 51.1                                     |
| 500 x mix                     | I₀                                        | Bohan                | 56.9                                     |
| 511-2 x mix                   | I₂                                        | P-12                 | 50.9                                     |
| 511-4 x mix                   | I₂                                        | P-12                 | 51.3                                     |
| 552 x mix                     | I₀                                        | Modana               | 48.2                                     |
| 553 x mix                     | I₂                                        | Modana               | 55.6                                     |
| 521 x mix                     | I₂                                        | L-12                 | 48.9                                     |
| 506 x mix                     | I₂                                        | M-12                 | 42.1                                     |
| 507 x mix                     | I₂                                        | M-12                 | 46.9                                     |
| Average per experiment        |                                           |                      | 45.2                                     |
| V, %                          |                                           |                      | 9.8                                      |
| LSD₉₅                         |                                           |                      | 3.4                                      |

### Table 2. The properties of polycross descendants of the red beet lines for the share of the leaf rosette in the plant biomass, 2019-2020

| Selection code of descendants | Inbreeding generation of the maternal line | Source | Share of leaves in plant biomass, % |
|-------------------------------|-------------------------------------------|--------|-----------------------------------|
| Kestrel standard              | F1                                        | Sakato | 18.2                              |
| 549-2 x mix                   | I₁                                        | Modana | 28.1                              |
| 500 x mix                     | I₀                                        | Bohan  | 26.3                              |
| 511-2 x mix                   | I₂                                        | P-12   | 27.8                              |
| 511-4 x mix                   | I₂                                        | P-12   | 28.0                              |
| 552 x mix                     | I₀                                        | Modana | 25.2                              |
| 553 x mix                     | I₂                                        | Modana | 31.7                              |
| 521 x mix                     | I₂                                        | L-12   | 28.6                              |
| 506 x mix                     | I₂                                        | M-12   | 27.5                              |
| 507 x mix                     | I₂                                        | M-12   | 24.1                              |
| Average per experiment        |                                           |        | 27.1                              |
| V, %                          |                                           |        | 7.9                               |
| LSD₉₅                         |                                           |        | 1.5                               |

Overall, the average share of leaves in the plant biomass over the years of studies was 27.1% with small parameter variations (variation rate of 7.9%). The average value for maternal forms deviates by just 0.6% but it has a greater variation range between 17 (511-4) and 37% (553) (variation rate of 28.0%).

The key variety value criterion is its yield, which is determined not only by its genetic capacities but also the growing techniques applied. The most important parameter of the red beet, in our opinion,
is its marketability. The experience of advanced farms shows that the yield of market-grade root crops heavily depends on the variety or a hybrid used. The most in-demand varieties and hybrids in the consumer market for the red beet are those with round roots. The share of round roots in the line reflects the line's heterogeneity.

Creating varieties and hybrids with high marketability and root heterogeneity is a key goal of selection work. To this end, we studied the heterosis of polycross descendants across the parameters specified. According to some sources, the heterosis effect can be deemed economically favorable if the hybrid descendants’ yield exceeds the reference by 25% [24]. According to other researchers [25], even the 15% increase can be seen as feasible.

Table 3. The properties of polycross descendants of the red beet for marketability and the share of round roots, 2019-2020

| Selection code of descendants | Inbreeding generation of the line | Source | Share of round-shaped root crops, % | Marketability, % |
|------------------------------|----------------------------------|--------|-------------------------------------|-----------------|
|                              |                                  |        | F1                                  | Competitive heterosis, % |
| 549-2 x mix                  | I₁                               | Modana | 78.1                                | 160.3           |
| 500 x mix                    | I₀                               | Bohan  | 48.2                                | 60.7            |
| 511-2 x mix                  | I₂                               | P-12   | 65.6                                | 118.7           |
| 511-4 x mix                  | I₂                               | P-12   | 62.6                                | 108.7           |
| 552 x mix                    | I₀                               | Modana | 73.1                                | 143.7           |
| 553 x mix                    | I₂                               | Modana | 45.5                                | 51.7            |
| 521 x mix                    | I₂                               | L-12   | 38.6                                | 28.7            |
| 506 x mix                    | I₂                               | M-12   | 66.7                                | 122.3           |
| 507 x mix                    | I₂                               | M-12   | 85.6                                | 185.3           |
| Kestrel (Sakata)             | F₁ standard                      |        | 78.1                                | 118.7           |
| Average per experiment       |                                  |        | 59.4                                | 88.6            |
| LSD₀.₀₅                      |                                  |        | 12.9                                | 3.8             |

A total of 67% of polycross descendants feature high competitive heterosis (over 100%) for the share of round roots. This is because we used second and third-generation self-pollinated lines leveled in this parameter in the crossing. As for root crop marketability, polycross descendants on average comply with the reference Kestrel F₁ variety. The highest marketability (over 90%) was observed in polycross hybrids F₁ 500, 511-2, and 507.

The lines’ GCA level reflects the possibility of their use in heterosis selection. Creating inbreeding lines with high combining ability is a promising method of heterosis hybrid selection. Polycross descendants with low GCA have the highest value according to the share of the top and the share of leaves in the plant biomass parameters because the red beet selection activities aim to reduce these parameters. Taking into account the parameters in question, polycross descendants of lines 506 I₂ and 507 I₂ and the Modana variety can be deemed the most interesting. High GCA in root shape regularity was observed in the Modana variety lines (starting and I₀) and selection samples 506 and 507. The lines covered did not demonstrate high GCA in marketability. Thus, lines 506 I₂ and 507 I₂ featuring very low GCA in the share of the top and the share of leaves in the plant biomass, as well as high GCA in root regularity and marketability can be recommended for use in the selection works on the red beet to obtain synthetic varieties and hybrid selection.

As a result of polycrossing, 67% of hybrid samples have a reduced share of the top in the root diameter. The lowest values of this parameter (42.1 and 46.9%) were observed in polycross descendants of lines 506 and 507.
The share of leaves in the plant biomass in F1 hybrids is slightly different from that of their maternal forms. This parameter reduces by 1.2-6% in 44% of hybrid descendants and increases in the rest of them.

As for the share of round roots, high competitive heterosis (over 100%) was observed in 67% of polycross descendants.

**Table 4.** The general combining ability of lines across the key selection-relevant parameters, % (2019-2020).

| Selection code of descendants | Inbreeding generation | Share of the top in root crop diameter | Share of leaves in biomass | Share of round-shaped root crops | Marketability |
|------------------------------|-----------------------|---------------------------------------|---------------------------|----------------------------------|--------------|
| 549-2                        | I₀                    | 88.6                                  | 88.1                      | 160.2                            | 98.3         |
| 553                          | I₂                    | 98.4                                  | 102.7                     | 132.1                            | 90.9         |
| 500                          | starting              | 101.5                                 | 113.0                     | 85.9                             | 92.9         |
| 511-2 P-12                   | I₂                    | 117.5                                 | 97.0                      | 75.2                             | 104.7        |
| 511-4 P-12                   | I₂                    | 93.0                                  | 101.0                     | 118.1                            | 98.8         |
| 521 L-12                     | I₂                    | 111.4                                 | 99.9                      | 140.9                            | 105.5        |
| 506M-12                      | I₂                    | 102.5                                 | 100.2                     | 97.5                             | 96.8         |
| 507 M-12                     | I₂                    | 85.9                                  | 99.4                      | 101.9                            | 98.9         |

**7. Conclusion**

We obtained lines 506I₂, 507I₂ that have a set of selection-relevant parameters, including the small proportion of the root crop top (42.10%) and the share of leaves in the biomass (24.1-27.5%), high regularity of root crop shape (up to 85.6%) and marketability (88.3-95.9%) The obtained lines can be used as the starting material for producing new varieties and hybrid selection.

**References**

[1] Borisov V A, Litvinov S S, Romanova A V 2003 *Quality and storability of vegetables* (Moscow) pp 210-212
[2] Zhuchenko A A 1995 Problems of adaptation in the selection, seed farming, and crop variety testing for agricultural crops *Genetical bases of selection* (Moscow) pp 3-9
[3] Oldemeyer R K, Davis W, Bush H, Erichsen A 1968 The evolution of and the use of the top-cross test as a method of selecting inbred lines of sugar-beets for general combining ability *Journ. Amer. Soc. Sugarbeet Techn.* 15(1) 49-60
[4] Shevtsov I A 1983 *Using plant inbreeding* (Kiev: Naukova dumka) 199 p
[5] Rokitsky P F 1978 *Introduction to statistical genetics* (Minsk: Vysheynaya shkola) 448 p
[6] Ayala F J, Kiger J A 1988 *Modern genetics* Translated from English: Vol. 2 (Moscow: Mir) 368 p
[7] Falconer D S 1985 *Introduction to Quantitative Genetics* (Moscow: Agropromizdat) 486 p
[8] Doloty L A 1984 *The study of general combining ability of self-pollinated red beet lines*, Dissertation for candidate degree (Kiev) 171 p
[9] Johnson I J, Hayes H K 1940 The value in hybrid combinations of inbred lines of corn selected from single crosses by the pedigree method of breeding *J. Amer. Soc. Agron.* 32 479-485
[10] Cowan J R 1945 The value of double-cross hybrids involving in-breeds of similar and diverse genetic origin *Sci. Agr.* 25 287-296
[11] Green J M 1948 Inheritance of combining in maize hybrids *J. Amer. Soc. Agron.* 40(1) 58-63
[12] Hayes H 1955 Development of the Heterosis Concept, in *Hybrid corn* (Moscow: Izdatelstvo Inostrannoy Literatury) pp 73-93
[13] Zelensky M A, Morgen V V 1969 Genetic bases and method of corn selection for combining ability *Cytology and Genetics* 3(2) 150-157
[14] Turbin N V, Kedrov-Zikhman O O 1971 The polycross method in plant selection and its theoretical justification Genetic bases of heterosis population selection (Minsk) pp 3-20
[15] Kedrov-Zikhman O O 1974 Polycross testing in plant selection Nauka i Tekhnika (Minsk) 128 p
[16] Wright J W 1978 Introduction to Forest Genetics (Moscow: Lesnaya Promyshlennost) 470 p
[17] Kotov M M 1997 Genetics and selection Pt. 1 (Yoshkar Ola: MarSTU) 280 p
[18] Guzhov Yu L, Fuks A, Valichek P 1991 Selection and seed farming of cultivated plants (Moscow: Agropromizdat) 463 p
[19] Turbin N V, Khotyleva L V, Kaminskaya L N, Polonetskaya L M 1977 Improving the combining ability of the starting material through periodic selection IX meeting of EUCARPIA. Abstract (Krasnodar) 158 p
[20] Burenin V I 1989 Guidelines for studying and maintaining the world root-crop collection (Leningrad) 165 p
[21] Omarov D S 1975 On the methods of plant heterosis recording and assessment Agricultural Biology 10(1) (Moscow) 123-127
[22] Sprainaitis A P, Svirskis A A 1985 The efficiency of polycross in white clover selection Breeding and seed production 3 (Moscow) 19-21
[23] Khitryuk L A 1985 The formation of the assimilation mechanism in carrots and beets in different growing spaces Advanced agricultural practices for vegetable and fruit crops: a collection of research works (Gorki: BSAA) pp 10-12
[24] Kobylyansky V D, Lapikov N S 1982 The heterosis effect in line-strain hybrids of winter rye Proceedings on Applied Botany, Genetics, and Breeding (All-Russian Institute of Plant Genetic Resources, St. Petersburg) 73(1) 41-49
[25] Derevyanko V P, Litun P P, Azamchuk G K, Zdrilko A F 1990 The genetic aspects of heterosis selection of winter rye Proceedings of EUCARPIA Symposium (Leningrad) pp 37-39