Variability of the effects of combining ability in the breeding of F1 radish hybrids

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Abstract. Based on homozygous lines, three sterile and seven fertile, radishes were crossed by the method of two groups of genotypes. The resulting offspring and parental lines were tested when growing in soil, in a film greenhouse, using traditional techniques. The experiment was repeated twice to identify hybrid combinations showing consistent performance. The grown plants were evaluated according to economically valuable traits: the weight of the root crop and the aboveground vegetative part, the length, and diameter of the root crop. In the method proposed by V.K. Savchenko, statistical indicators of the combining ability of lines were calculated, including the effects of GCA. When tested at different times, we identified lines that changed the direction of the action of genes on the manifestation of quantitative traits. For the selection of lines for future breeding programs, lines were selected that showed stable performance. The test took the indicators of the inbred lines themselves, which made it possible to assess the relationship of the phenotypic manifestation of the trait with the effects of GCA of the same lines. The presence of close relationships allows simplifying the work of breeders, namely, to assess the manifestation of a trait in plants, without evaluating the offspring. For the same purpose, the presence of relationships between quantitative signs was assessed; during the test, close correlations were found in the first period, while in the second period they were absent. The data obtained indicate the unreliability of even close correlations obtained from the results of single tests. The indicators of the GCA effects of the studied lines are applicable for the selection of only a specific breeding institution, while the comparison of indicators for two or more test periods helps to identify patterns in the control of the inheritance of the studied quantitative traits.

1. Introduction

The main selection method for the creation of F1 hybrids in cabbage crops is the use of self-incompatibility or nuclear-cytoplasmic male sterility [1–3]. Nuclear cytoplasmic male sterility allows reducing labor costs when breeding parental lines, compared to the costs of manually propagating self-incompatible lines. Simultaneously with this advantage, the use of NCMS makes it possible to obtain 100% hybrid seeds. In addition, a plus for breeders is the possibility of breeding a male-sterile F1 hybrid, provided that a sterility fixer is used as a paternal component, which protects intellectual rights to the created selection achievement to a greater extent than the legislation of individual countries [4, 5]. Based on the above, the enthusiasm for the F1 assortment of cabbage hybrids, including radish, is an urgent problem that requires the creation of sterile lines, sterility fixing lines, paternal pollinator lines, and a constant assessment of the combinational ability of new lines in combination with those already tested earlier. Assessment of the combinational ability is a laborious and financially costly exercise. Therefore,
approaches are needed to reduce the cost of the breeding process. Separate approaches are knowledge of correlations and effects of ACS of previously tested lines based on offspring.

To determine the combining ability, the following crossing methods are used: polycross, topcross, diallele [6–10]. The use of this or that method depends on the number of lines and on the ability to carry out reciprocal crosses. When working with sterile lines, the most used system is topcross. However, there is an analog of this system in the world, when using more than one sterile line, makes it possible to assess the combinational ability of both pollinator lines and sterile lines, the name of such a crossing system is crossing of two groups of genotypes [11].

Correlation is a statistical relationship between two or more random variables, in which a change in one of the values will lead to a change in another or other values. The use of relationships between quantitative and qualitative traits can reduce the time spent on selection by breeders and, thus, reduce the cost of breeding hybrids [12].

2. Material and methods

The studies were carried out when grown in the ground, in a film greenhouse without heating and additional lighting. The experiment was carried out in two periods: I in the period from 21.04.2020 to 29.05.2020 and II from 06.08.2020 to 10.09.2020.

The material was hybrid combinations obtained since sterile lines MS1, MS5, MSKBK radish, and fertile lines Solito, Printo, 5, Diego, KBK, ChB, Zha9, as well as sterile lines MS1, MS5, KBK.

Sowing of hybrid seeds in the ground was carried out on 21.04.2020 and 06.08.2020. The experiment was set by the method of randomization. Sowing scheme 8x8 cm to a depth of 1-2 cm, 256 seeds per variant, in triplicate. For counting, we took 50 plants located in the middle of the block. Plant care is traditional. Mass seedlings were received in 4 days. The plants were harvested, and their subsequent assessment was carried out on May 29, 2020, and September 10, 2020. The obtained hybrid combinations were evaluated according to the following parameters: root crop weight, leaf rosette weight, root crop diameter, and length.

Combining ability was assessed by crossing two groups of genotypes. This method was proposed by V.K. Savchenko in 1973. The method of crossing two groups of genotypes allows you to quickly assess the combinational ability of parental forms. Its essence lies in the crossing of genetically different lines (the system was originally developed to assess the combining ability of tomato lines with normal and functional male sterility), including sterile and fertile lines. The reliability of the method depends on the number of lines used for the assessment (at least two in each group). To obtain more accurate results, it is necessary to increase the number of replicates when testing hybrid combinations and to assess the combinational ability at least twice. When crossing genetically different sets of parental lines, the statistical analysis of combining ability makes it possible to estimate the same parameters as in diallel crosses, but significantly reduce the amount of time and work for crosses and field trials.

Statistical data processing: analysis of variance and correlation, indicators of GCA effects were calculated according to Savchenko [10].

3. Results and Discussions

The experiment was set up with the most similar conditions, but with a slight difference in the microclimate of the greenhouse, due to the external air temperature and the amount of solar radiation. This led to a significant change in the quantitative parameters of plants, including the average weight of the radish root crop (Tables 1 and 2). On average, in the second period, the roots were 1.5 times smaller. These changes are very useful, as they allow you to assess the breeding value of lines, namely the stability of the manifestation of traits; and to identify hybrid combinations that consistently exhibit indicators of commodity bodies.

When tested at different times, hybrid combinations with the participation of Diego and ChB lines showed the most differing data. And the greatest stability, in terms of hybrid combinations, was noted with the participation of lines MS5, KBK, and Solito (Tables 1 and 2). Stability among hybrid
combinations was noted for MS5xPrinto and MSKBKxSolito, and among standards, F1 Mars confirmed its qualities.

Table 1. Average weight of F1 hybrids and ACS effects of parental radish lines (gr.), I term

|       | Printo | Solito | 5   | Zha9 | Diego | KBK  | ChB  | Gi  |
|-------|--------|--------|-----|------|-------|------|------|-----|
| MS1   | 21.6   | 24     | 31  | 30.6 | 26.1  | 32.4 | 33.2 | 1.7 |
| MS5   | 21.8   | 22.2   | 23.5| 28.9 | 33.2  | 32.1 | 28.6 | 0.5 |
| MSKBK | 32.9   | 26     | 20  | 23.6 | 33.7  | 8.7  | 26.7 | -2.2|
| Gj    | -1.3   | -2.6   | -1.9| 1.0  | 4.3   | -2.3 | 2.8  | Ě=26.7|

LSD05=14.5 LSD05gj=2.96  LSD05 gj=1.9  Standards: Vena = 30.1 Mars = 28.8

Table 2. Average weight of F1 hybrids and ACS effects of parental radish lines (gr.), II term

|       | Printo | Solito | 5   | Zha9 | Diego | KBK  | ChB  | Gi  |
|-------|--------|--------|-----|------|-------|------|------|-----|
| MS1   | 13.4   | 16.4   | 16.1| 15.7 | 12.8  | 18.8 | 14.6 | -1.07|
| MS5   | 23.7   | 16     | 21.2| 16   | 17.8  | 19.4 | 16.3 | 2.16 |
| MSKBK | 14.6   | 23.6   | 12.8| 15.4 | 13.9  | 16.2 | 11.2 | -1.09|
| Gj    | 0.74   | 2.18   | 0.24| -0.76| -1.62 | 1.64 | -2.42| Ě=16.5|

LSD05=7.85 LSD05Gj=4.90  LSD05 Gj=3.53  Standards: Vena = 43.5 Mars = 33.2

When testing hybrid combinations in the first and the second period, no combinations exceeding the most yielding standard were found. However, some combinations were on this level. So, in the first term there were 7 such combinations, and in the second - 2 pcs. Moreover, these were different combinations. The best hybrid combination in the first term MsKBKxDiego (33.7 g) showed significantly lower results in the second term (13.9 g).

When selecting lines for high yields, according to the results of testing hybrid combinations in the first period, Diego lines (GCA equal to 4.3), Zha9 (GCA equal to 1.0), and Ms1 (GCA equal to 1.7) should be used. However, when re-evaluated in the second term, the indicators of GCA effects of the same lines changed sign from positive to negative.

Indicators of GCA effects indicate the influence of genes that control a trait on the phenotypic manifestation of a trait. The GCA effect is not a stable value, but it has acceptable stability when evaluating hybrid combinations at different times and years, even when using the line in a cross-system with a completely different set of lines. Thus, the GCA effect is a mathematically calculated value that makes it possible to predict the manifestation of a trait in hybrids with the participation of a particular line. And with the known values of the GCA effects of two lines, it is possible to predict the manifestation of a trait in a hybrid, with the participation of these lines.

It is not worth the unambiguous task of always increasing the indicators of the manifestation of a trait in breeding achievements. It all depends on the needs of the market and, accordingly, on the tasks for the breeders. So, if it is necessary to create a highly productive hybrid, then it should be used among sterile lines - the Ms5 line (on average for both periods, the GCA effect on the weight of the root crop is 0.36), and among fertile lines - Diego (on average for both periods, the GCA effect is 1.35) (Table 3). When cultivating radish hybrids in greenhouses, a minimum volume of aboveground vegetative mass is required; accordingly, Ms1 lines will be promising among sterile lines (on average for both periods, the GCA effect on leaf weight is minus 1.7), and among fertile lines - Solito (on average, the GCA effect is equal to minus 3.05) and Diego (on average for both periods, the GCA effect is equal to minus 1.75). The most popular is the rounded shape of the root crop. This is achievable through the selection of high GCA values of both parental lines in terms of the diameter and length of the root crop (line Ms5 (the GCA effect in diameter on average for both periods is 2.3, and in length is equal to 1.45) and Diego's line (GCA effect in diameter on average for both periods is equal to 1.55, and in length is equal to 2.75)).
Through the selection of negative indicators of both lines (the MsKBK line (the GCA effect in diameter on average for both periods is equal to minus 2.4, and in length is equal to minus 1.45) and the KBK line (the GCA effect in diameter on average for both periods is minus 2.9, and in length is equal to minus 2.45)). The indicators could also differ insignificantly from zero.

Table 3. Indices of ACS effects of parental lines of radish hybrids

| Lines    | Rootmass | Leafmass | Rootdiameter | Rootlength |
|----------|----------|----------|--------------|------------|
|          | I term   | II term  | I term       | II term    |
| MS1      | 1.7      | -11      | -17          | -17        |
| MS5      | 0.5      | 2.2      | -0.2         | 0.2        |
| MSkBK    | -2.2     | -1.1     | 1.9          | 1.5        |
| LSD05    | 1.9      | 4.9      | 1.68         | 2.5        |
| Printo   | -1.3     | 0.7      | -1.2         | -1.7       |
| Solito   | -2.6     | 2.2      | -5.4         | -0.7       |
| 5        | -1.9     | 0.2      | 2.51         | -0.6       |
| Zha9     | 1.0      | -0.8     | 0.1          | -0.7       |
| Diego    | 4.3      | -1.6     | -1.8         | -1.7       |
| KBK      | -2.3     | 1.6      | 6.4          | 5.7        |
| ChB      | 2.8      | -2.4     | -0.6         | -0.3       |
| LSD05    | 2.96     | 3.53     | 2.57         | 2.44       |

In addition to directing the action of genes to increase or decrease a trait, the stability of these indicators is important. The most stable (according to the results of the analysis of the data of two terms) in our study were indicators of GCA effects on the mass of leaves (the correlation coefficient is 0.8 ± 0.26). The rest of the indicators behaved changeably (the correlation of GCA effects on the weight of the root crop is -0.7 ± 0.17, along the diameter of the root crop is equal to 0.1 ± 0.17, along the length of the root crop is equal to 0.5 ± 0.22). The data obtained indicate the need to increase the number of trials of the same hybrid combinations to obtain stable indicators of GCA effects and selective selection of lines for this indicator.

Table 4. Correlation links between the studied features

|          | Root length | Root diameter | Leaf mass |
|----------|-------------|---------------|-----------|
|          | I term      | II term       | I term    | II term |
| Root mass| 0.7±0.12    | -0.2±0.34     | 0.8±0.22  | 0.4±0.09 |
| Root length| 1           | 1             | 0.7±0.18  | 0.5±0.22 |
| Root diameter| 1       | 1             | 1          | 0.1±0.20 |

Inbred sterile lines were added to the study, which made it possible to carry out a correlation analysis and reveal very valuable data for selection on the dependence of line indices on the effects of GCA of the same lines. In our experiment, close relationships were revealed between the phenotypic manifestation of the trait and the GCA effects of the same sterile lines in terms of the average root weight, length, and diameter of the root crop (the correlation coefficients were 0.9 in all cases). This makes it possible to predict the manifestation of the trait in hybrid combinations according to the indicators of the parental lines.

When creating breeding achievements, it is necessary to evaluate many diverse traits. Therefore, to simplify the selection process, it is necessary to know the presence or absence of correlations between the studied characters. For these purposes, close correlations are most valuable. In our study (Table 4), close correlations were found only in the first period (between the weight of the root crop and its length, between the weight of the root crop and its diameter, between the length and diameter of the root crop), however, they did not repeat themselves in a similar study of hybrid combinations in the second period.
There was only one correlation of the average strength between the diameters of the root crop and its weight (the first term $r = 0.8 \pm 0.22$ and the second term $r = 0.4 \pm 0.09$). As a result, in this study, there were no correlations between the studied traits. This fact allows breeders to create hybrid combinations with any manifestation of signs of root mass, its length, and diameter, as well as the mass of leaves.

4. Conclusion
When assessing the combinational ability by crossing two groups of genotypes, the effects of GCA on the mass of leaves ($r = 0.8 \pm 0.26$) are most stable. Close correlations ($r = 0.9$) between the indices of sterile inbred lines and their GCA effects in terms of root mass, length, and diameter of root crops, allow predicting the manifestation of a trait in hybrid combinations in terms of parental lines. There are no correlations between the traits of the mass of the root crop and leaves, the length and diameter of the root crop, there are no correlations between the indicators of hybrid combinations, except for the relationship between the average strength between the mass and the diameter of the root crop.

References
[1] Ogura H 1968 Studies on the new male sterility in Japanese radish, with special references to utilization of this sterility towards the practical raising of hybrid seeds Mem. Fac. Agric. Kagoshima Univ. 6 39-78
[2] Brown G G, Domaj M, DuPauw M, Jean M, Li X-Q, Landry B S 1998 Molecular analysis of brassica cms and its application to hybrid seed production Acta Horticulturae 459 265-274
[3] Nieuwhof M 1990 Cytoplasmic-genetic male sterility in radish (Raphanus sativus L.). Identification of maintainers, inheritance of male sterility and effect of environmental factors Euphytica 47(2) 171-177
[4] Thakur H, Vidyasagar 2016 Comparing of self-incompatibility and cytoplasmic male sterility systems for the performance of F1 hybrids in cabbage (brassica oleracea var. capitata L.) Electronic Journal of Plant Breeding 7(3) 602-610. doi:10.5958/0975-928X.2016.00077.6
[5] Singh P K, Tripathi S K, Somani K V 2001 Hybrid seed production of radish (raphanus sativus L.) Journal of New Seeds 3(4) 51-58. doi:10.1300/J153v03n04_05
[6] Mather K, Jinks J L 1972 Biometrical genetics Heredity 29(1) 101-102. doi:10.1038/hdy.1972.69
[7] Griffing B 1956 Concept of general and specific combining ability in relation to diallele crossing systems Australian Journal of Biology 463
[8] Hayman B I 1954 The analysis of variance of diallel cross Biometrics 235
[9] Jinks J L, Hayman B I 1953 Analysis of diallel crosses Maize Genetics Cooperation Hews Letter 27 48
[10] Aastveit A H, Aastveit K 1990 Theory and application of open-pollination and polycross in forage grass breeding Theoretical and Applied Genetics 79(5) 618-624. doi:10.1007/BF00226874
[11] Savchenko V K 1978 Many target method of quantitative estimation of combining ability in breeding for heterosis Genetics 14(5) 793-804
[12] Young H J, Stanton M L, Ellstrand N C, Clegg J M 1994 Temporal and spatial variation in heritability and genetic correlations among floral traits in raphanus sativus, wild radish Heredity 73(3) 298-308. doi:10.1038/hdy.1994.137