Molecular genetic study of Beta vulgaris L

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Abstract. The results of molecular genetic studies are considered as a basis for the accelerated breeding of sugar beet. By PCR analysis (SSR, IRAP, RAPD), sugar beet parental lines were identified. Polymorphisms of DNA loci for each line were genotyped. The research was demonstrate a number of informative molecular markers as the most informative to reveal heterogeneity of starting material and effective to forecast heterosis.

1. Introduction

Studying the morphological characteristics of plants does not provide the opportunity to obtain in-depth information about the genotype. Currently, the development and use of molecular markers is of great importance for solving breeding problems [1]. Their introduction into the breeding process allows quick and efficient selection the desired plant genotypes. At the present stage, one of the possibilities of using molecular markers in practical selection is the selection and identification of parental components of crosses, and the prediction of the effect of heterosis. To solve the above most important breeding problems, it is necessary to obtain information about the genetic diversity of breeding materials, select effective molecular markers, establish the relationship between molecular divergence and heterosis, use the combined molecular-genetic and statistical methods of analysis to apply the data in the breeding process to create new hybrids. The considered methodological approaches for the most part are already universally used in various breeding programs abroad. In Russia, interest in them is also increasing [2]. The creation of reliable methods for the accelerated selection of parental pairs with high specific combining ability based on molecular marking methods will significantly save time and material costs when creating sugar beet hybrids. IRAP-, RAPD and SSR-PCR methods are widely used to assess the DNA heterogeneity of selection material.

RAPDs (Random Amplified Polymorphic DNAs) are DNA fragments amplified by PCR using short single primers of random sequence.

IRAP (Inter-Retrotransposon Amplified Polymorphism) is a method for amplifying genomic DNA between closely spaced retrotransposon sequences. Genomic DNA PCR amplification product is a stable genetic IRAP marker [3].

Microsatellites or simple sequence repeats (SSRs) are very effective molecular markers in population genetics, genome mapping, taxonomic study and other large-scale studies [4]. Some authors have studied the genetic diversity of wild, cultivated, transgenic and weed forms of Beta vulgaris using microsatellite markers [5,6,7,8]. Belarusian researchers using 15 pairs of microsatellite primers were identified lines and hybrids of sugar beet, compiled standard formulas [9].
2. Materials and methods

Diploid male-sterile lines and multigerm sugar beet pollinators (MS 1101, MS 1126, MS 2093, MS 1134, MS 1141, MS 1117, MS 1113, MS 1131, MS 1137, OP 1122, OP 1207, OP 1211, were used as materials for this research (a total of 120 individual samples). Genomic DNA was isolated from plant tissue by the phenol-chloroform method [10,11]. The DNA was dissolved in 10 mM Tris-HCl buffer, pH 8.0, containing 0.1 mM EDTA and used for PCR analysis.

Genues thermocycler was used for DNA amplification. We were used microsatellite, IRAP and RAPD primers: Bvv15, Bvv 17 Bvv21, Bvv 23, Bvv 30, Bvv 32, Bvv 43, Bvv 51, Bvv 53, Bvv 60, Bvv 64, Bvv 61, PAWS 5, PAWS 6,PAWS 16, PAWS 17, UBC278, AB-9-3, AB-6-15, AB-2-2, AB 3-3. The PCR program is described in [12,13,14,15]. Cluster analysis was performed using the Statistica 6.0 program.

3. Genetic diversity in sugar beet parental lines based on SSR, IRAP and RAPD markers

The results of molecular evaluation of sugar beet lines using molecular markers are obtained. Molecular-genetic selection of sugar beet parental lines for hybridization has been performed. In this study diploid CMS lines and lines multigerm pollinators were used. The genetic diversity of 12 sugar beet breeding lines were assessed using 12 microsatellite (SSR), 4 IRAP and 5 RAPD markers. Genetic distances were determined, and the cluster analysis was performed allowing differentiation of the studied varieties between clusters depending on genetic relationship.

In total, using four IRAP primers, 208 amplicons were obtained, of which 136 were polymorphic. The length range of the obtained DNA fragments using four primers is from 150 to 1100 bp. Figure 1 show that the greatest polymorphism of sugar beet materials was identified with single primer PAWS 16.

![Figure 1. Products of PCR with primers PAWS 16](image)

The results of PCR analysis of 12 lines of sugar beet (MS, multigerm pollinators) with five RAPD markers (AB 6-15, AB 9-3, AB2-2, AB 3-3, UBC 278) was found amplicons ranged from 100 to 1600 bp.

PCR analysis with primer AB 3-3 revealed from 0 to 1 DNA short fragment (500 bp) depending on genotype. PCR analysis with primer AB 6-15 made it possible to differentiate between lines of OP 1165 and OP 1187, which have the identical DNA fingerprints (3 bands from 100 to 800 bp) from another materials, for which 5 amplification products were detected, lengths from 100 to 1200 bp.
Amplification of the genomic DNA of initial materials with the UBC 278 primer made it possible to obtain from 3 to 4 DNA fragments from 100 to 1200 bp. for different genotypes.

The greatest polymorphism was found for loci identified using primers AB 9-3 and AB-2-2, which allows differentiating the selection material. Primer AB 9-3 were produced 3 polymorphic bands for genotypes (100, 500 and 800 bp), with a total number of amplicons of 24. Using primer AB 2-2 a 5 amplicons for each genotype were identified, of which 3 (60%) polymorphic fragments were found to be polymorphic for the studied selection materials. The total number of DNA fragments for all materials are 41. Sugar beet lines MS 1137, MS 1131 and OP 1180 have identical fingerprint of amplification products with primer AB 2-2 (5 amplicons from 100 to 1600 bp), which indicates their genetic proximity.

Results-based PCR analysis with 5 RAPD primers (UBC278, AB-9-3, AB-6-15, AB-2-2, AB 3-) and 4 IRAP primers (PAWS 5, PAWS 6, PAWS 16, PAWS 17 ) were compiled matrices for the presence / absence of amplicons. Genetic distances between pollinators and male-sterile lines were detected in the range of 3.16-4.8.

The dendrogram on figure 2 illustrates the relationship between breeding materials. Cluster analysis made it possible to divide experimental samples of sugar beet into 3 divergent classes. Lines № 1203 and № 1117 were not included in any of the clusters.

![Tree Diagram for 12 Variables](image)

**Figure 2.** Dendrogram of genetic distances between the initial lines of sugar beet, based on the data of RAPD- and IRAP analysis

1 - MS 1134, 2 - MS 1141, 3 - MS 1117, 4 - MS1113, 5 - MS 1131, 6 - OP 1165, 7 - OP 1197, 8 - OP 1180, 9 - OP 1203, 10 – OP 1137, 11 - OP 1172, 12 - OP 1195.

The results of DNA amplification with the IRAP and RAPD primers revealed genotype-specific bands characterizing the initial parental forms. Based on the data obtained, molecular genetic formulas are compiled that reflect the genetic structure of individual sugar beet materials. The relationship of these markers with the manifestation of heterosis was not identified.
Figure 3 show that the greatest polymorphism of sugar beet materials with 12 microsatellite markers was identified with pairs of microsatellite primers Bvv 30 + Bvv 64.

**Figure 3.** Products of PCR with primers Bvv 30 + Bvv 64

1 - MC 1134, 2 - MC 1141, 3 - MC 1117, 4 - MC 1131, 5 - MC 1113, 6 - ОП 1165, 7 - ОП 1197, 8 - ОП 1180, 9 - ОП 1203, 10 – MC 1137, 11 - ОП 1172, 12 - ОП 1195, М – DNA length marker.

**Figure 4.** Dendrogram of genetic distances between the initial lines of sugar beet, based on the data of SSR-analysis with primers Bvv 23, Bvv 30, Bvv 32, Bvv 64
The investigations of polymorphism with 21 markers resulted in selection of 2 pairs of molecular markers – Bvv 30 + Bvv 64 and Bvv 23 + Bvv 32 – as the most informative to reveal heterogeneity of starting material and effective to forecast heterosis.

The PCR-analysis of sugar beet initial lines with these pairs of microsatellite primers allowed determination of specific DNA-profiles for breeding material.

The results of PCR analysis with primers Bvv 23, Bvv 30, Bvv 32, Bvv 64 were used to determine the level of divergence between the studied lines by the clustering method. Based on the data obtained, genetic distances (Euclidean) between samples were calculated that varied from 0.0 to 2.24. The smallest genetic distances (D = 0.0-1.0) were found for crossing combinations MS 1117 x OP 1180, MS 1117 x OP 1197, MS 1131 x OP 1203, MS 1134 x OP 1203, MS 1113 x OD 1203, MS 1137 x OP 1165 and MS 1117 x OP 1203. The greatest genetic distances (D = 1.73-2.0) were found for the parental pairs MS 1117 X OP 1195, MS 1134 X OP 1195, MS 1131 X OP 1195, MS 1137 X OP 1197, MS 1137 X OP 1195, MS 1141 X OP 1195 and others. The result of clustering the data is shown in Figure 4. The success rate of the prediction of obtaining heterosis hybrids at maximum genetic distances (D = 1.73-2.0) based on data obtained by SSR analysis with these 4 primers is 42.86%.

4. Conclusion

The investigations of polymorphism of microsatellite and single (IRAP, RAPD) markers resulted in selection of 2 pairs of molecular markers – Bvv 30 + Bvv 64 and Bvv 23 + Bvv 32 – as the most informative to reveal heterogeneity of parental forms and effective to forecast heterosis.

It is advisable to exclude hybrid combinations with genetic distance less than 1.41 from further studying in connection with low efficiency. The research findings are important for hybrid sugar beet breeding.

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