CLASSIFICATION OF A COLLECTION OF INBRED SUNFLOWER LINES USING MOLECULAR-GENETIC MARKERS

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Abstract

Genetic diversity of 30 inbred sunflower lines was examined by RAPD and isozyme analyses. The inbred lines were shown to be highly polymorphic by RAPD markers. The line distribution on genetic similarity dendrograms based on the RAPD and isozyme data was analyzed. High effectiveness of RAPD analysis for differentiating genotypes of inbred sunflower lines was demonstrated.

Introduction

Classification of original plant material is an important step in breeding. When selecting parental pairs for crossing, it is necessary to take into account the degree of genetic similarity (or genetic diversity) of plant material involved in breeding. At present, some methods based on the application of molecular genetic markers are widely used in order to solve such a problem. These methods of approach to the problem have some advantages including being more informative than those which are based only on estimation of morphological characteristics. Thus, molecular genetic markers were successfully applied to evaluate genetic diversity in such crops as wheat, maize, barley, grape and many others (Sivolap, 1998). Sunflower is not an exception to the rule. In particular, inter- and intraspecific differentiation of the genus *Helianthus* was studied by RAPD-analysis and the degree of genetic similarity at the level of sunflower inbred lines was evaluated (Sivolap and Solodenko, 1998; Sivolap et al., 1998).

The aim of this work was to study the genetic diversity of inbred sunflower lines by means of using molecular markers in the breeding process.

Materials and Methods

Thirty inbred lines of sunflower bred at Yurjev Plant Production Institute were used as plant materials. Out of those, 14 lines; X908, X503, X1002, X1006, X1008, X3848, X4353, X1010, X2111, X2552, X2122, X4021, and X1012 were used as maternal forms. They were produced by means of selection from interspecific hybrids and variety populations. Sixteen lines; X984, X983, X785, X787, X991, X711, X818, X840, X943, X821, X767, X954, X726, X945, X769, and X956 were used as paternal forms (fertility restorers). The given lines mainly originated from American and Yugoslavian varieties. The DNA was isolated from 6-10 mature seeds as described by Plaschke et al, 1995. The DNA member in the samples was determined on agarose gel by means of a calibration curve developed on the basis of measuring samples with known DNA contents.

RAPD-analysis was performed with 12 random primers. Table 1 presents a description of the primers. The PCR was done as described previously by Sivolap et al., 1998. The products of amplification were separated on a 2% agarose gel with subsequent staining by ethidium bromide.

Extraction, electrophoresis, and histochemical staining of isoenzymes were done as described previously by Popov et al. (1999).

The percentage of polymorphism was determined as a ratio of polymorphic loci to a total number of loci revealed. The coefficient of Nei and Li was used to establish genetic distances (Nei and Li, 1979). Genetic distances in inbred lines were determined on TREES program (Kalendar, 1994). The unweighted Pair Group Method analysis (UPGMA) was used.

Results and Discussion

Genetic diversity among 30 inbred lines of sunflower was investigated by RAPD-analysis by using 12 random primers. RAPD bands contained from 14 to 23 DNA fragments. The size of amplified fragments ranged from 250 to 3000 p.n. Each sunflower line differed from the others by the member of DNA fragments by their length and the degree of band staining. Some fragments appeared to be unique, being present in only one genotype. Thus, at amplification with OPW-04 primer in X945 and X1012 lines the fragments of 600 p.n. and 1200 p.n. were present, respectively. The fragment with 900 p.n. was successfully identified using the P38 primer in the X908 line.

Table1. Primers used to investigate genetic variation in 30 inbred lines.

Primer	Sequence 5'-3'	Degree of polymorphism, %
P36	CCGAATTCGC	67
P37	CTGACCAGCC	70
P38	GATACGTTGTC	68
P46	GGTTGGGGAG	78
P53	GTCTAAGTCG	72
T08	AACGGCGACA	75
OPW04	CAGAAGCGGA	62
OPW06	AGGCCCGATG	83
OPW09	GTGACCGAGT	78
OPW10	TCGCATCCCT	77
OPW15	ACACCGGAAC	68
OPX01	CTGGGCACGA	73

221 RAPD loci were tested and 161 loci out of those turned polymorphic. The higher polymorphism, 83%, was found for the OWP-06 primer. The lowest polymorphism was shown for the OWP-04 primer, i.e., 62%. The average polymorphism was 72.6% found for the given primers. A relatively high degree of intraspesific polymorphism was determined by RAPD-analysis in plants. This index depends both on the nucleotide composition of the primers used and on plant species tested. In particular, for RAPD analysis of sunflower inbred lines we used those primers showing a high degree of polymorphism in that species (Sivolap,

1998). On the other hand, sunflower is a cross-pollinated species and it has a high degree of genetic variation.

On the basis of the data resulting from genomic DNA amplification with 12 primers, a dendrogram has been developed that indicates the degree of genetic similarity of 30 inbred sunflower lines (Figure 1A). In the given dendrogram inbred sunflower lines are pooled in 3 clusters.

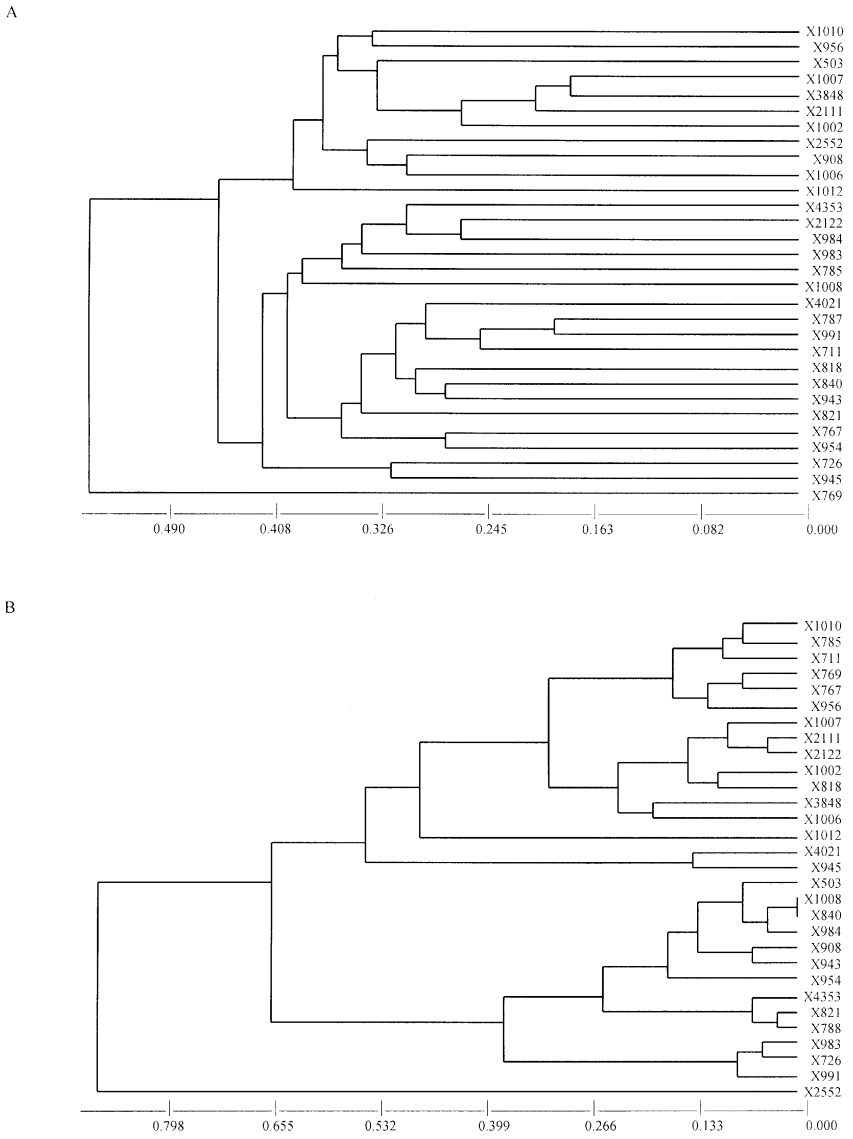

Figure 1. Dendrogram for the degree of genetic identity in 30 inbred sunflower lines constructed on the results of genomic DNA with 12 primers (A) based on isozyme (B) analysis.

The first cluster is represented by the maternal lines X1010, X503, X1007, X3848, X2111, X1002, X2552, X908, X1006, and X1012 and by fertility restorer line X956. This line is selected from a synthetic population, including maternal lines as X1002 and X1006. Genetic similarity of the line to Kh1002 and Kh1006 is shown in the present dendrogram. Maternal lines X4353, 2122, X1008, X4021 and 12 paternal ones, X984, X983, X787, X991, X711, X818, X840, 943, X821, X767, and X954 were included in the second cluster. The third is represented by two lines only: X726 and X945. X769 line was not included in any of those clusters. It is genetically more distant in comparison to the remaining lines. On the whole the genetic distance among 30 lines of sunflower varied from 0.230 to 0.706.

Isoenzymic analysis was also used to estimate a degree of genetic variation between inbred lines. Four isozyme systems were studied: anodal esterase, cathodal esterase, cathodal acid phosphatase and NAD-malatedehydrogenase. The dendrogram designed on the basis of data from the isozyme analysis is given in Figure B. The genetic distance among the lines ranged from 0.000 to 0.726.

As to the results of the isozyme analysis, sunflower inbred lines were classified into three clusters (Figure 1B). The first one is represented by 13 lines, including seven lines used maternally in crosses (X1010, X1007, X2111, X2122, X1002, X3848, X1006) and six lines used as fertility restorers (X785, 711, X769, X767, X956, X818). The second cluster comprises only two lines, one of these maternal X4021, the other paternal, X945. The third cluster is formed by a group of lines comprising 4 maternal lines (X503, X908, X1008, X4353) and nine fertility restorers (X840, X984, X943, X821, X788, X983, X726, and X991). X2552 line stands somewhat aside from the other lines. Larger genetic distances were identified for it. Two lines, X840 and X1008, turned out to be identical according to their isozyme composition. The genetic distance between those lines was 0.000.

It is of interest to compare the two dendrograms designed according to the data, which were obtained by RAPD and isozyme analysis. The coefficient of correlation between genetic distances from RAPD-analysis and isozyme analysis data was 0.07 ($P<0,05$). The dendrogram shown in Figure A differs considerably from the one in Figure B. As to the data of RAPD-analysis, the majority of lines used in breeding as maternal forms construct one cluster and the paternal forms, another cluster. Various lines are heterogenic for represented genotypes.

Genotypes of two lines could not be distinguished, whereas RAPD analysis allowed identification of all the examined genotypes of sunflower inbred lines. The discrepancy of the data shown in the represented dendrograms is connected with the different level of informativeness of two methodical approaches. When using the isozyme analysis, enzyme loci forming two linkage groups were examined. RAPD analysis is a more informative and effective method of estimating the level of genetic diversity of sunflower inbred lines and classifying original breeding material.

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