Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Estimation of genetic diversity among sunflower genotypes through random amplified polymorphic DNA analysis

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Genet. Mol. Res. 7 (4): 1408-1413 (2008)
Received October 13, 2008
Accepted October 23, 2008
Published December 23, 2008

ABSTRACT. The genetic diversity among eight sunflower lines was determined through the estimation of the random amplified polymorphic DNA method. One hundred and fifty-six DNA fragments were generated by 20 random primers, for an average of about 7.8 bands per primer. Of these amplified DNA fragments, 104 were polymorphic among the eight sunflower lines. Nei and Li's similarity matrix gave values from 51.59 to 77.78%, which indicated a broad genetic base. The maximum similarity, 77.78%, was observed between R-SIN-82 and RN-46. The lowest similarity, 51.59%, was observed between the exotic lines CM-612 and HA-27. After knowing the knowledge of genetic diversity based on these random amplified polymorphic DNA markers, highly diverse lines can be used for further breeding programs to develop an ideal local hybrid of sunflower.

Key words: Genetic diversity; Random amplified polymorphic DNA; Sunflower diversity; DNA markers

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INTRODUCTION

The shortage of edible oil is still persisting in Pakistan. Although the country has made an impressive improvement in agriculture, only 30% of the edible oil requirement of Pakistan is met through local production, and the rest 70% of the country's requirement is met through importation, costing a deal of foreign exchange. The imported edible oils are mainly palm oil and soybean oil. For the last few years, the situation with oil production in Pakistan has improved, but on account of its increasing demand by the ever-increasing population, continuous improvement in its productivity is highly desirable. However, for the last few years, the yield improvement in sunflower varieties has not been substantial, and the narrow genetic base of the germplasm used has been considered the major reason impediment in the development of an ideal high yielding local hybrid. Sunflower is of great importance also because its seed has high oil contents ranging 40-50% (Skoric and Marinkovic, 1986). Knowledge of diversity patterns (Liu et al., 2003) allows the plant breeders to better understand the evolutionary relationships among accessions. Information about genetic diversity and relatedness in the available germplasm and among elite breeding material is of fundamental importance in plant breeding (Mosges and Friedt, 1994). Future breeding programs depend on the availability of genetic variability to increase productivity. Traditionally, assessment of genetic diversity has been based on the differences in morphological and agronomic traits or pedigree information in different crops. More lately, restriction fragment length polymorphism and isozyme markers have been used for diversity studies and gene mapping of different crops. But, their use remains limited as they reveal a low level of polymorphism and isozyme expression is highly influenced by the environmental conditions (Hernández et al., 2001). However, polymerase chain reaction (PCR)-based DNA marker techniques seem to provide the means for generating useful information on polymorphism, genetic relatedness and diversity. PCR-based random amplified polymorphic DNA (RAPD) markers are dominant markers and extensively used in genetic mapping (Chalmers et al., 2001) and identification of markers linked with different traits (Bai et al., 2003). Due to technical simplicity and speed, the RAPD method has been used for diversity analyses in several crops (Li and Nelson, 2001). Sunflower is characterized by a small genome size, and little or no sequence information is available for sunflower genomes. In the study reported herein, RAPD analysis was carried out in eight exotic genotypes of sunflower to estimate genetic diversity and genetic relatedness among them, and to compare the exotic genotypes with the local variety. This information will help the breeder to develop a high yielding and disease-resistance local hybrid.

MATERIAL AND METHODS

Plant material

The plant material used in the study consisted of eight exotic genotypes of sunflower, namely CM-612, HA-27, B-SIN-82, HA-314, RL-54, RL-51, R-SIN-82, and RL-46. All genotypes were planted in pots in a greenhouse.

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DNA extraction

The sunflower genotypes were grown in plastic containers (250 mL) and 0.2-0.3 g of leaf tissue was obtained from 6-day-old leaves of the sunflower genotypes. The weighed leaf tissue was then transferred immediately to zip-lock plastic bags containing 1.5 mL CTAB. Leaf material was completely homogenized with a hand roller. After incubation at 65°C for 30 min, the homogenized leaf tissue was transferred to two 1.5-mL Eppendorf tubes. An equal volume (0.75 mL) of chloroform-isoamyl alcohol was added, and the tubes were inverted 5-10 times followed by centrifugation at 13,000 rpm for 10 min. After centrifugation, 800 µL supernatant was transferred from both tubes to another 1.5-mL Eppendorf tube. Next, approximately 700 µL (0.9 volume) isopropanol was added to the supernatant and mixed by inverting the tube about 10 times. The DNA was pelleted and washed and resuspended in 150 µL 0.1X TE. Finally, the concentration of DNA was measured at 260 nm in a spectrophotometer (CECIL CE 2021 2000 Series). The quality of DNA was checked by running 5 µL DNA on 0.8% agarose gel prepared in 0.5X TBE buffer. The DNA samples giving a smear in the gel were rejected.

RAPD analyses

DNA concentration in the working solution of approximately 15 ng/µL in deionized double distilled water was confirmed by spectrophotometer. For RAPD analysis, the concentration of genomic DNA, 10X PCR buffer with $(NH_4)_2SO_4$, $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), 10 mer random primer and Taq DNA polymerase were optimized. The 10-base oligonucleotide primers obtained from GeneLink (USA) were used for the amplification of the genomic DNA. Taq polymerase together with buffer, $MgCl_2$, dNTPs, and gelatin were purchased from Fermentas.

DNA amplification reactions were performed in a thermal cycler (Eppendorf AG No. 5333 00839). The PCR profile was as follows: one cycle of 94°C for 5 min, 40 cycles of 94°C for 1 min, 36°C for 1 min, 72°C for 2 min, and a final extension for 10 min at 72°C.

Analysis of RAPD data

The RAPD fragments were analyzed by electrophoresis on 1.2% agarose gels with ethidium bromide (10 ng/100 mL agarose solution in TBE). The bands were counted by starting from the top of the lanes. All visible and unambiguously scorable fragments amplified by the primers were scored under the heading of total scorable fragments. Amplification profiles of the eight exotic genotypes were compared with each other and bands of DNA fragments were scored as present (1) or absent (0).

The data of the primers were used to estimate genetic similarity on the basis of number of shared amplification products (Nei and Li, 1979) (Table 1). The equation used was: No. of shared amplification products = 2 x (No. of common bands between any two lanes) / (Total No. of bands in the same two lanes). Genetic relationship among the genotypes was estimated with a dendrogram (Figure 1) constructed using unweighted pair group of arithmetic means (UPGMA) (Sneath and Sokal, 1973).

Table1. Similarity matrix of eight sunflower genotypes obtained from random amplified polymorphic DNA markers.

	CM-612	HA-27	B-SIN-82	HA-314	RL-54	RL-51	R-SIN-82	RL-46
CM-612	1	0.6349	0.6032	0.6349	0.5952	0.6349	0.6429	0.5635
HA-27		1	0.6349	0.5238	0.5159	0.5238	0.5794	0.5952
B-SIN-82			1	0.7143	0.6270	0.5397	0.5159	0.5159
HA-314				1	0.6587	0.6667	0.5952	0.5476
RL-54					1	0.7540	0.6032	0.6190
RL-51						1	0.7540	0.6429
R-SIN-82							1	0.7778
RL-46								1

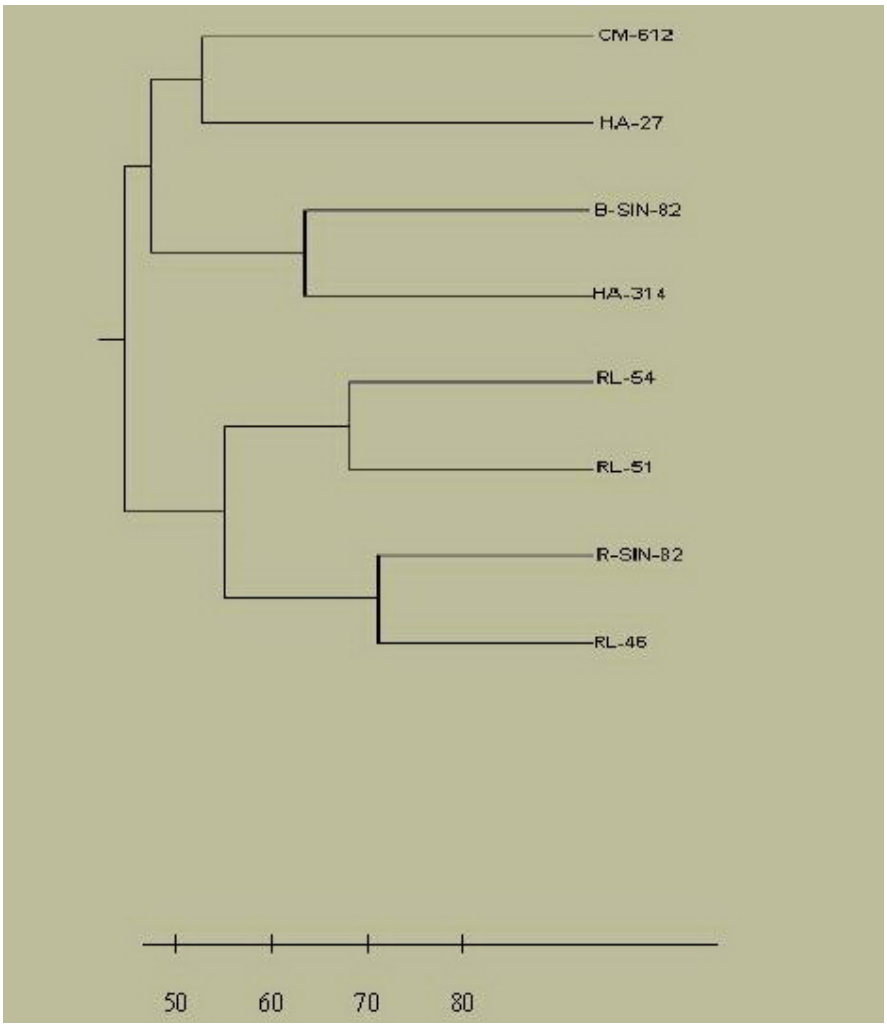

Figure 1. Dendrogram of eight exotic sunflower genotypes developed from random amplified polymorphic DNA data using unweighted pair group of arithmetic means (UPGMA).

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RESULTS

DNA of eight exotic lines of sunflower was amplified with 20 different random primers, i.e., A1, A2, A4, A9, A13, A15, B5, B7, B11, B12, B17, C2, C13, C15, C18, C19, D8, D12, D13, and D16. A total of 156 DNA fragments were generated by the 20 random primers with an average of 7.8 bands per primer. Of these 156 amplified DNA fragments, 104 fragments showed polymorphism among the eight sunflower exotic lines. The number and size of the DNA fragments were strictly dependent upon the sequence of the primer. Reactions were repeated two to three times to check the consistency of the amplified products, and only easily resolved and bright DNA bands were counted. All genotypes showed diversity in their amplification profile based on these 156 DNA bands amplified by 20 primers.

DISCUSSION

These results suggest that RAPD markers provide information for the identification of sunflower genotypes (Lawson et al., 1994). The maximum similarity, 77.78%, was observed between R-SIN-82 and RL-46. The lowest similarity, 51.59%, was observed between the exotic lines CM-612 and HA-27. The reproducibility of the RAPD technique can be influenced by various factors, such as the sequence of the primer, template quality and quantity, the type of thermal cycler, and polymerase concentration (Hernández et al., 1999). However, the use of a standardized RAPD protocol can ensure a reproducible RAPD pattern.

Different concentrations of $MgCl_2$, Taq DNA polymerase and concentration of template DNA were optimized for PCR conditions. DNA concentrations of 5, 10, 15, 20, and 25 ng/25 µL in each reaction were studied. The concentration of 10 ng/25 µL was found to produce the most consistent and reproducible banding patterns. Nabulsi et al. (2001) used the RAPD technique to evaluate some garlic mutants and found that 3 mM $MgCl_2$ was optimal for better amplification. In this study, 3 mM concentration of $MgCl_2$ was found to be optimal for consistent results. More than 3 mM $MgCl_2$ produced nonspecific amplification. Similarly, one unit concentration of Taq DNA polymerase was found to be optimal for better amplification of genomic DNA. Other reaction conditions were also kept constant, and results were found to be consistent and reproducible. All amplified bands were identical in each repetition. CM-612 was 63.49% similar to HA-27 while B-SIN-82 was 71.43% similar to HA-314. Both these groups showed similarity to each other at 63.49%. This group of four genotypes has a very close resemblance. On the other hand, the genotype RL-54 is 75.40% similar to RL-51 and the line R-SIN-82 showed maximal similarity to RL-46, i.e., 77.78%, and both these groups showed 65.87% similarity to each other. Forapani et al. (2001) compared hemp varieties using RAPD and found an average of 97.1% polymorphism over all varieties and loci. Mukhtar et al. (2002) observed 445 DNA fragments that were amplified with 50 random primers for 20 varieties and revealed 64.38% polymorphism. In this study, 112 DNA fragments were amplified with 15 random primers with an average of 7.4 bands/primer. The RAPD technique used here was found to be quite effective in determining the genetic variation among sunflower exotic genotypes. By knowing about the diversity of exotic sunflower lines, a plant breeder can use the highly diverse lines in further breeding programs (Quagliaro et al., 2001).

ACKNOWLEDGMENTS

Special thanks to Director/Dean Professor Dr. Iftikhar Ahmad Khan, Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad, Pakistan.

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