DETERMINING THE GENETIC DIVERSITY OF COMMERCIAL SUNFLOWER HYBRIDS BY RAPD ANALYSIS

DETERMINAREA DIVERSITATII GENETICE A UNOR HIBRIZI COMERCIALI DE FLOAREA-SOARELUI PRIN ANALIZA RAPD

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Abstract. *The aim of this study was to determine the genetic similarity among 17 commercial hybrids acquired from Pioneer-Hi-Bred International, Limagrain Romanian and two autentic romanian hybrids Fundulea 225 and Favorit. In order to determine the genetic similarity, these hybrids were analised at the DNA level using RAPD technique. For this purpose an initial screening of 30 decamer primers was made, from which only seven gave polymorphis bands and were used for furhter analisys. Based on the polymorphic band obtained, dendrogram was constructed based on UPGMA cluster analysis according to Lei and Ni similarity index.*
Key words: DNA markers, genetic diversity, RAPD

Rezumat. *Scopul acestui studiu a fost determinarea diversităţii genetice la şaptesprezece hibrizi comerciali de floarea soarelui, procuraţi de la firmele Pioneer-Hi-Bred International, Limagrain România, şi doi hibrizi autohtoni Fundulea 225 şi Favorit. Pentru determinarea similarităţii genetice, s-au efectuat analize la nivel molecular prin tehnica RAPD (Random amplified lengh polymorphism). În acest scop s-a realizat un screening a treizeci de primeri decameri din care, doar şapte au dat benzi polimorfice si au fost utilizate in continuare. Similaritatea genetică a fost determinată cu ajutorul coeficientului Lei si Ni. Pe baza rezultatelor obţinute s-a realizat o dendogramă UPGMA a celor şaptesprezece hibrizi analizaţi.*
Cuvinte cheie: markerii ADN, diversitatea genetică, RAPD

INTRODUCTION

Sunflower (*Heliantus annuus*) is as plant oils of great importance economic and food industry. The sunflower cultivated area in Romania ocupy the third place, after maize and wheat. The seeds fats contents (33-56%), and high quality of oil resulting from the extraction plant, is one the main sources of vegetable fats used in human nutrition, that is the most important source of oil to Romania (Bîlteanu Gh., 1991). Tehnique RAPD was first described by Williams et al. (1990)

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and is based on the allelic polymorphism at the parental forms in the presence or absence of amplifications products. Generally each primer used will determine the amplification of sequences from different loci of the genome, provides an effective method to investigate DNA polymorphisms between individuals (Tingei and del Tufo, 1993). Tehnique RAPD applications are: study of genetic diversity, germplasm characterization, determining the genetic structure of populations, somaclonal variability, identification of cultivars and hybrids purity (Iuoras Monica, Vrânceanu A. V., 1998).

This study shows that RAPD markers can be used successfully to determine the genetic diversity.

MATERIAL AND METHOD

To determine the genetic diversity of 17 commercial sunflower hybrids of different origin were analysed at the DNA level by RAPD technique. Details of hybrids sources can be seen in table 1. The plant material was grown in field crop year 2009-2010 at the Ezăreni farm to multyplied number of seeds.

Table 1

Name and provenience at hybrids

Nr. crt	Hybrid name	Firm
H1	PR 63A50	Pioneer România
H2	PR 63A90	Pioneer România
H3	PR 63A62	Pioneer România
H4	PR 63A15	Pioneer România
H5	PR 64E83	Pioneer România
H6	PR 63A86	Pioneer România
H7	LG 56.58 CL	Limagrain România
H8	PR 64A83	Pioneer România
H9	PR 64A71	Pioneer România
H10	PR 64E71	Pioneer România
H11	PR 64H45	Pioneer România
H12	PR 64G46	Pioneer România
H13	LG 56.65 M	Limagrain România
H14	PR 64F50	Pioneer România
H15	FUNDULEA 225	Comerţ
H16	FAVORIT	Comerţ
H17	PR 64J80	Pioneer România

DNA extraction and analyzing RAPD

CTAB method used for DNA extraction was modified by Doyle & Doyle in 1987. Harvesting of plant material was performed at 6 weeks after emergence. The biological material used for DNA isolation was the young sunflower leaves after harvest which were introduced in tubes printed and then immediately frozen in liquid nitrogen. Preservation of samples was done in a freezer at - 20^0C. DNA content of the solutions resulting from the extraction method following the protocol

described and modified Doyle & Doyle, was determined using a Nano Drop type fluorospectofotometru 3300. Based on reading levels determined to proceed to achieve the necessary dilutions RAPD method, the DNA bringing the solution at a concentration of 5 ng / ml. To obtain PCR reaction was necessary: 5 ng genomic DNA, 10 mm of each dNTP, 25 mM MgCl2, 5pmol / ml decamer primer (Roth), 0.1 units Taq DNA polymerase (Taq polymerase Go - Promega) and 10X buffer. Amplification was performed in Corbett termocycler. The conditions for PCR amplification were: 2 min at 94^0C an initial denaturation, followed a total by 40 cycles, each of the following steps: 1 min denaturation at 94^0C, 1 min at 36^0C attaching primers (annealing), 2 min extension and 7 min at 72^0C final elongation. Amplification products were separated in agarose gel 2% concentration, and were analyzed by staining with ethidium bromide.

Resulting image analysis was performed using 2.1 RFLPscan program based on the assumption that each band has a different length single locus. For analysis of fragments were considered clear only those parts which could not be parsed (Iqbal A., et al., 2010). Following gel analysis was obtained binary matrix was used to calculate genetic distances. To calculate genetic distances and dendogram achievement NTSYS pc 1.7 software was used coefficient application using Nei and Li method UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) for genetic distances and Neighbor Joining Tree method for dendrogram.

RESULTS AND DISCUSSIONS

To determine the genetic diversity of the 17 sunflower hybrids were used a total of seven RAPD decamers primers to observe genetic polymorphism (Karp A., et al., 1997a). The gels obtained by amplifying DNA analysis were taken into account only those bands that were due to polymorphism clear. After analysis of all gels have received a total of 69 fragments between 120 and 956 bp fragments of which 45 were polymorphic.

Table 2

Decamers primers used for analysed RAPD for sunflower genotypes

Nr. Crt.	Name of primer	Sequence (5'-3')
1	ROTH A01	CAG GCC CTT C
2	ROTH A09	GGG TAA CGC C
3	ROTH A13	CAG CAC CCA C
4	ROTH A15	TTC CGA ACC C
5	ROTH A17	GAC CGC TTG T
6	ROTH B04	GGA CTG GAG T
7	ROTH B07	GGT GAC GCA G

To calculate the percentage of polymorphic fragments for each primer in part, and it varied between 44 and 77% as shown in the table 3. The number of amplified fragments ranged from 6 to 17 primer ROTH A15 and ROTH A01. The highest number of polymorphic band generated by primer A01 ROTH and obvious, showed polymorphism.

Table 3

The number and amplified fragmments, the number of polymorphic fragments and percentage with polymorphic fragments amplified with seven primers

Primer name	Amplified fragments	Polimorphic bands	Percentage (%)
ROTH A01	17	12	70%
ROTH A09	9	4	44%
ROTH A13	9	7	77%
ROTH A15	6	3	50%
ROTH A17	8	6	75%
ROTH B04	9	7	77%

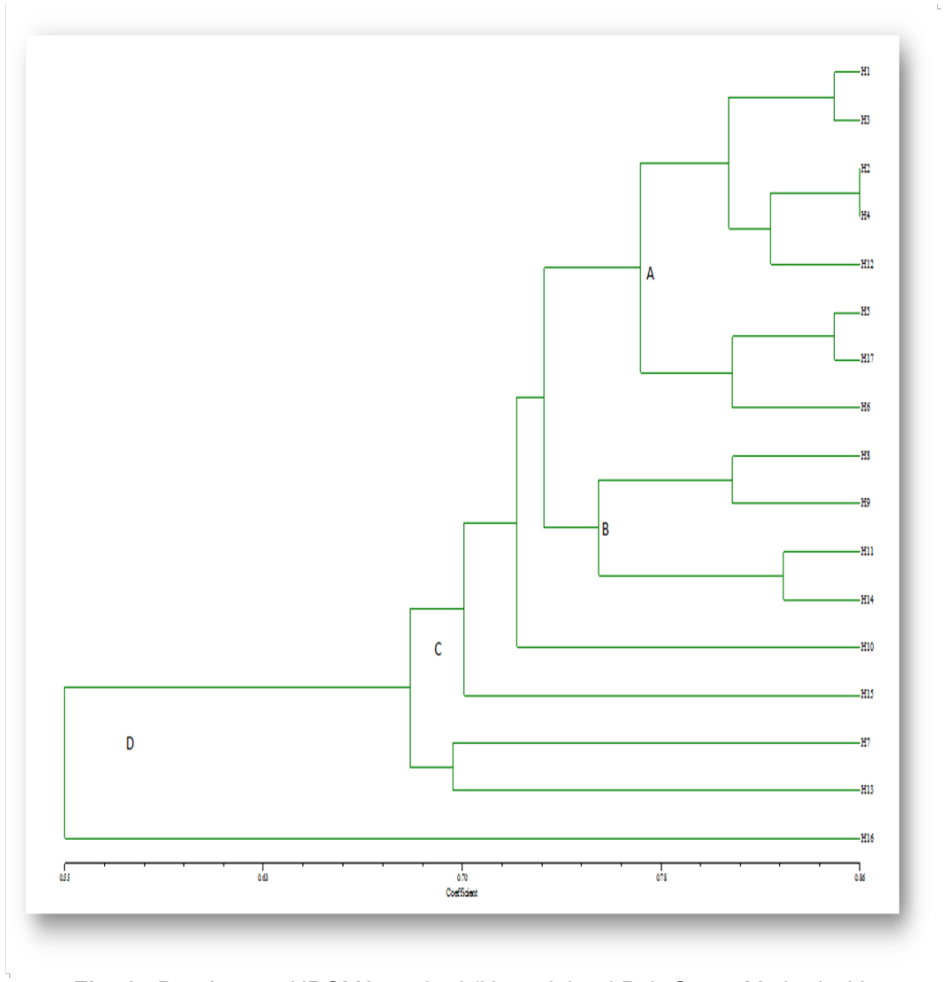

Fig. 1 - Dendogram UPGMA method (Un-weighted Pair Group Method with Arithmetic Mean)

Analyzing data from genetic distances were calculated using the formula for the similarity coefficient of Nei and Li (1979). Based on similarity index was made a dendogram based on UPGMA method (Un-weighted Pair Group Method with Arithmetic Mean) (fig. 1).

Following the results obtained and analyzed dendrogram can be observed that genotypes appear divided into four distinct groups denoted by uppercase ,,A, B, C and D" (Serene Maragatham Isaacs, et al., 2003).

As it was expected, commercial hybrids were grouped in terms of business after producing genetically. So, the Pioneer hybrids appear grouped in the first group, is joining the group the largest number of genotypes in the dendogram. Genotypes closest genetically are: H2 (PR63A90) and H4 (PR64A15) at a genetic distance of 0.86. At the same genetic distance with the two hybrids is the hybrid H12 (PR64G46) which means that probably three hybrids common ancestry. The distance values are smaller, the genotypes are closely related (genetically closer). Most hybrids different from Pioneer hybrids appear H1 (PR63A50) and H14 (PR64F50) who are most genetically removed from the first being in group A (PR63A50) the other being in group C (PR64F50), and also notice that the genotype may form a separate group Favorit to other hybrids, it is found in Group D, one can say that it is genetically different from the other growing at a genetic distance of 0.86.

CONCLUSIONS

1. Clustering analysis allowed to determined genetics similarity and degree relatedness cultivars of sunflower

2. Based on data analysed RAPD method can say ease of use in determining the genetic polymorphism analysis and the detection of hybrids single fragments, which may be included in future studies in the genus *Helianthu*s variability.

3. Our study shows that RAPD analysis revealed a highest level in terms of genetic variability, using a limited number of primers.

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