RAPD MARKERS FOR POLYMORPHISM IDENTIFICATION IN PARASITIC WEED *OROBANCHE CUMANA* Wallr.

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ABSTRACT

Random Amplified Polymorphic DNA (RAPD), a technique, was used to evaluate the genetic variability of 10 broomrape populations. Fifteen primers were used in this study. Different DNA fingerprints have been produced by 4 primers. Two primers have clearly been differentiated the source Romania-Calarasi, compared with all other sources. Only one primer differentiated the source Baraganu, compared with all other sources and one primer showed similarity of the source Spain and Romania – Baraganu source and similarity of the Romania - Constanta 3 and Yugoslavia sources. Genetic distance for RAPD polymorphism was not correlated with geographic distance.

Key words: broomrape, genetic variability, RAPD markers

INTRODUCTION

The parasitic plant broomrape (*Orobanche cumana* Wallr., belongs to *Orobanche* genus – *Orobanche* (*Ospreleon*) section, *Orobanchaceae* family, *Tubiflorales* order, *Dicotyledoneae* class.

Sunflower broomrape (*Orobanche cumana* Wallr.) is a parasitic weed, completely without chlorophyll, that infests the roots of sunflower plants. This parasitic plant establishes a connection with the vascular system of the host-sunflower, via a specialized organ-haustorium and draws water and nutrients from them.

Broomrape is one of the most important constraints on sunflower production in areas of Eastern and Southern Europe. Yield losses resulting from a severe attack of the parasite can reach 50-90%. Breeding sunflower hybrids resistant to *O. cumana* is the most effective method to control this parasitic weed. To identify *Orobanche* species and variety accessions, low morphological variability, pollen morphology, seed micromorphology and chemotaxonomy have not been successful differentiating *Orobanche* sub-sections (Fernandez-Martinez et al., 2000; Joel et al., 1998). Different ecotypes of *O. cumana* designated as races (Vranceanu, 1977) or physiological races have shown a different racial composition (Melero-Vara et al., 1989). The characterization of *Orobanche* is also necessary for population studies, but the pattern and distribution of genetic variation within the frame of this important genus is virtually unknown (Katzir et al., 1996).

In the recent years, DNA - based markers have been successfully applied to discriminate between taxa and between individual genotypes in a wide range of plant and animal species. DNA - based markers are not dependent on environmental and developmental factors. Random Amplified Polymorphic DNA (RAPD) is a technique used to detect polymorphisms in a DNA sequence. The method relies on amplification of polymorphic DNA fragments by the Polymerase Chain Reaction (PCR) using a single oligonucleotide primer of arbitrary sequence.

RAPD markers have already been used to differentiate the important species of the genus *Orobanche* in Israel (Katzir et al., 1996) and Egypt (Zeid et al., 1997) and to evaluate the magnitude of intra-specific genetic variability within *Orobanche* species (Joel et al., 2004).

The study of population genetics of *Orobanche* is of a great importance since the understanding of the variability within and between pathogenic populations is essential if selection programmes are to develop sources of resistance.

The aim of this study is to understand the genetic variability of the parasite, in the frame of the parasitic system *Helianthus annuus* L. – *Orobanche cumana* Wallr.

MATERIAL AND METHODS

Plant material

Ten populations of *O. cumana* have been analysed using RAPD markers. The seeds harvested on plants obtained from Spain, Yugoslavia and Romania (Calarasi, Constanta 1, 2, 3; Tulcea

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1, 2; Bucharest and Baraganu) were co-cultivated with a susceptible sunflower inbred line.

DNA analysis

1. *Isolation of plant DNA*

Total DNA was isolated from floral buds following the protocol proposed by Doyle & Doyle (1991), with modifications, optimized for parasitic plants.

2. *RAPD analysis*

The RAPD method was optimized to generate reproducible fingerprints from genomic DNA. All primers were synthesized by University British Columbia (UBC), Canada. Amplification reactions were carried out in a volumes of 25 µl, containing: 1x buffer, Taq polymerase (Stoffel Fragments)-2U/25 µl, dNTP - 1,25 mM, $MgCl_2$ - 2,5 mM , primer – 0,2 µM. The template genomic DNA 20-25 ng was determined by fluorometric quantification (DyNA 200-Hoefer). The reaction mixture was supplemented with sterile Milli Q water to final volume of 25 µl. A few drops of sterile mineral oil were pipetted on top of the reaction mixture to prevent evaporation. The amplification was performed using a M. J. Research thermal cycler, programmed for 45 cycles, each consisting of: 1 min. at 94°C, 1 min at 36°C, 2 min. at 72ºC. RAPD amplification products were evaluated by electrophoresis on 1,2 % agarose gels in 0.5x TBE buffer, stained with ethidium bromide and BioPrint images were obtained.

3. *Statistical analysis*

Genetic distance was computed after Nei & Li (1979) formula, using TREECON 1.3b software package. A cluster analysis was performed using the unweighted pair group method with arithmetical averages (UPGMA) and the dendrogram was obtained in order to visualize the relationship among broomrape populations.

RESULTS AND DISCUSSION

In order to obtain sunflower cultivars resistant to *Orobanche*, it is important to know the amount of genetic variability of the parasite.

Broomrape populations are commonly used for virulence studies, but more than one race can occur in a population and this can lead to confusing results.

Ten *O. cumana* genotypes with different geographic origins have been chosen for this study. The 15 primers were used for genetic variability evaluation of the populations. From these primers only a few (7) have produced polymorphic bands. Although the number of score polymorphisms yielded by the 7 primers was small, only the most consistently reproducible bands from repeated PCR amplification were considered in the study. DNA profiles showed relatively low polymorphism between these populations. However, the RAPD technique reveals much more variation than the traditional morphological markers.

The RAPD pattern with UBC 8 primer (Figure 1) revealed that the source Romania – Calarasi is different compared with all other sources.

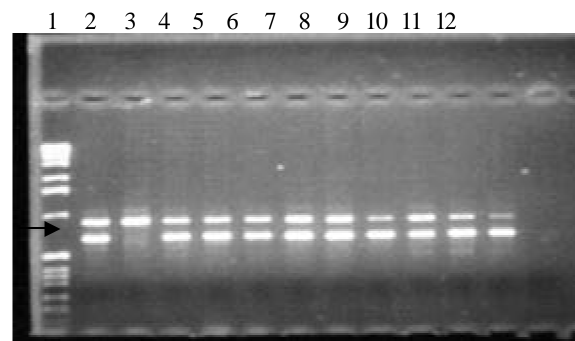

Figure 1 . RAPD pattern obtained with UBC 8.

Lanes: 1-Ladder 1 kb; 2-Spain; 3-Calarasi; 4-Yugoslavia; 5-unknown; 6-Constanta 1; 7-Bucharest; 8-Tulcea 1; 9-Tulcea 2; 10-Constanta 2; 11-Constanta 3; 12-Baraganu sources.

A similar result has been obtained with primer UBC 21 (Figure 2).

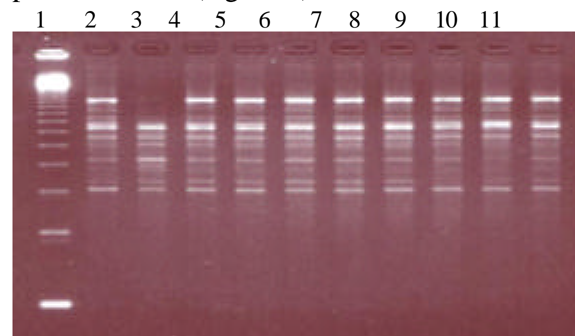

Figure 2. RAPD pattern obtained with UBC 21.

Lanes: 1-Ladder 123 pb; 2-Spain; 3- Calarasi; 4-Yugoslavia; 5-Constanta 1; 6-Bucharest;

7-Tulcea 1; 8-Tulcea 2; 9-Constanta 2;
10-Constanta 3; 11-Baraganu sources

On the other hand, the RAPD pattern with UBC 225 primer (Figure 3) revealed that the source Romania - Baraganu is different compared with all the other sources.

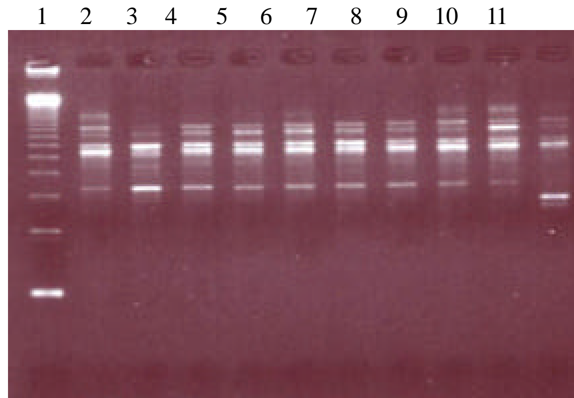

Figure 3. RAPD pattern obtained with UBC 225.

Lanes: 1-Ladder 123 pb; 2-Spain; 3-Calarasi;
4-Yugoslavia; 5- Constanta 1; 6-Bucharest;
7-Tulcea 1; 8-Tulcea 2; 9-Constanta 2;
10-Constanta 3; 11-Baraganu sources

The UBC 250 primer showed different patterns, such as: similarity of the source Spain and Romania - Baraganu and, on the other hand, similarity of the sources Romania - Constanta 3 and Yugoslavia, also, a different pattern for Tulcea 2, that presented all polymorphic bands existent at the sources of Spain and Calarasi. The other sources were different (Figure 4).

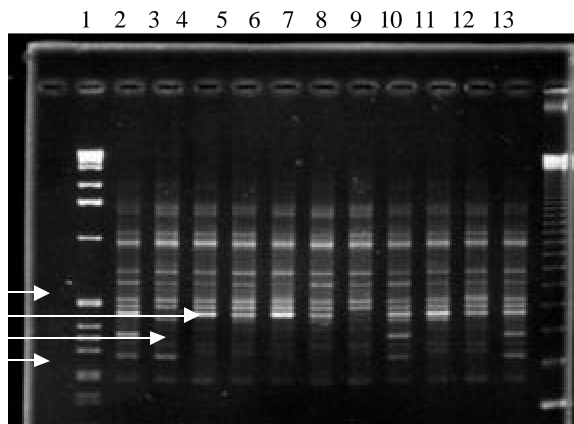

Figure 4. RAPD pattern obtained with UBC 250.

Lanes: 1-Ladder 1 kb; 2-Spain; 3-Calarasi;
4-Yugoslavia; 5- unknown; 6-Constanta 1;
7-Bucharest ; 8-Tulcea 1; 9-Tulcea 2 ;
10-Constanta 2; 11-Constanta 3; 12-Baraganu sources; 13-Ladder 123 pb

According to our results there is a clear difference between the source Calarasi (primer UBC 8, 21) and Baraganu (primer UBC 225), both from Romania, compared with all the other sources.

The cluster analysis based on RAPD amplification products of *O. cumana* populations is given in figure 5. The dendrogram divided the ten *O. cumana* populations in two groups: one group for Calarasi source and the second group for all the other sources.

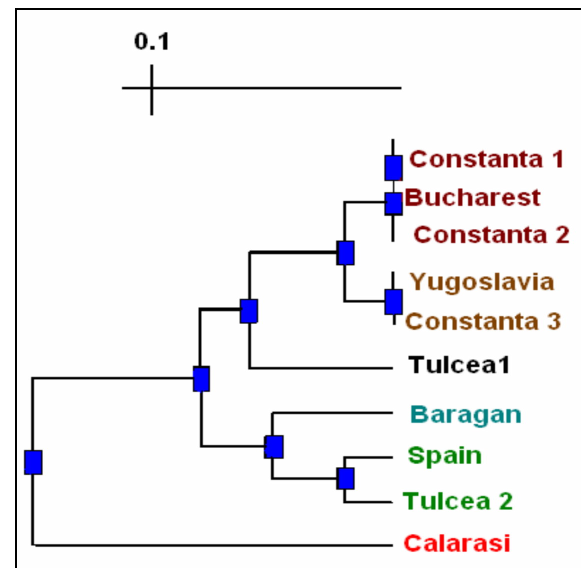

Figure 5. UPGMA dendrogram using Nei & Li (1979) genetic distance among *Orobanche cumana* populations

In fact, the second group comprises other two categories, grouping the sources Baraganu, Spain, Tulcea 2 in one group, the sources of Spain and Tulcea 2 being similar and Tulcea 1, Constanta 3, Yugoslavia, Constanta 2, Bucharest, Constanta 1 sources in another group. In the frame of this group there is a similarity between Constanta 1, Bucharest, Constanta 2, on one hand, and between Yugoslavia, Constanta 3 sources, on the after hand. The Tulcea 1 source is separate from all other sources as part of the group.

The genetic distance is not correlated with the geographic distance.

CONCLUSION

The study revealed a low polymorphism in the frame of ten *O. cumana* populations.

The most different source according to our molecular data is Calarasi-Romania source. There is a considerable similarity between the sources Spain, Tulcea 2 and between Yugoslavia, Constanta 3. There is no correlation between geographic origin and genetic distance.

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