
CHARACTERIZATION OF SUNFLOWER LINES WITH DIFFERENT RESISTANCE TO BROOMRAPE

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ABSTRACT

The sunflower is one of the most important oil crops. Orobanche cumana is a holoparasite, that infests sunflower plants. We have provided RAPD analysis of six sunflower lines, two of which are susceptible, and four are resistant to broomrape. Our results showed a polymorphism between susceptible and resistant sunflower lines in RAPD patterns generated with eight of totally seventy primers used.

Introduction

The cultivated sunflower (*Helianthus annuus* L.) is one of the four major annual crops in the world grown for edible oils. Sunflower broomrape (*Orobanche cernua* Loefl. syn. *O. cumana* Wallr.) is an important angiosperm holoparasite, totally devoid of chlorophyll, that infests the roots of sunflower plants. All over the South-Eastern and Mediterranean parts of Europe sunflower crop is strongly attacked by the broomrape *Orobanche cumana* Wallr. The infestation is frequently severe and yield losses can reach 50% (10). The problem with *Orobanche* is difficult to control. The broomrape produces an extraordinary high number of tiny seeds, which remain viable in soil for longer than 10 years. Hand weeding or the use of mechanical or chemical tools for *Orobanche* control is laborious and time consuming. Chemical control by herbicides is limited due to a high phytotoxicity on crop plant and yield reduction (20). Success could be expected by selecting or breeding crops which are tolerant or resistant to *Orobanche*

spp. Breeding was started early last century and resulted in a range of resistant sunflower cultivars.

The first references of broomrape attacking sunflower appeared in the thirties, last century and the term "race" appeared for the first time. The broomrape attacking the old Russian sunflower cultivars was considered as belonging to race A (16). From this time until now successively new *O. cumana* races have been evolved. The evolution of *Orobanche* species is still in progress. Soon after a new resistant line of sunflower was found, a new, more virulent race of *Orobanche* appears. The reactions of resistance in sunflower go from R₁ up to R₅, paralleling the name of the simple major genes (Or₁ to Or₅) conferring the resistance to races A through E. Effectively, each of these genes conferred resistance not only to the corresponding race, but also to the older ones (1). In our study we performed RAPD analyses of six sunflower lines, two of which are susceptible to *O. cumana* races C to E, and the other four are resistant to the same races.

Breeding of resistant crops is a relatively long process, so our aim is to find molecular markers for rapid screening and selection of resistant sunflower lines.

Materials and Methods

Plant material:

The number of induced mutations derived cultivars is rapidly increasing. Many induced sunflower mutants with improved agronomical traits were obtained in the Institute of Wheat and Sunflower, near Gen. Toshevo Bulgaria.

Six sunflower lines and cultivars were used in this study:

Cv. Peredovik – a susceptible control (resistant to races A and B of *O. cumana*); cv. VNIIMK 8931 – a susceptible control (resistant to races A and B of *O. cumana*). Several sunflower mutant lines were obtained after treatment with γ -rays (Co^{60}) at doses 150 and 200 Gy from cultivar VNIIMK 8931: № 6116A and № MX655 – which are 100% resistant to race E of *O. cumana*; cv. Vega – 100% resistant to race E of *O. cumana*; and line 7009R, derived from interspecific hybridization *H. annuus* x *H. tuberosus* (F12) – 90% resistant to race E of *O. cumana* (Batchvarova, 2001).

Preparation of genomic DNA:

The isolation of genomic DNA was performed from young and fresh leaves of sunflower plants according to Dellaporta (1983), with some modifications.

RAPD analysis:

Genomic DNA from each genotype was amplified by using seventy decamer primers (Operon Technologies Inc. – USA, Amersham Biosciences – USA, the University of British Columbia – Canada). Amplification reaction was performed in conformity with the requirement of the kit “Ready-To-Go RAPD Beads” – Amersham Biosciences (USA). For RAPD analyses samples were

predenatured for 5 min at 95°C, followed by 45 cycles of polymerization reaction each consisting of a denaturation step for 1 min, an annealing step for 1 min at 36°C and an extension step for 2 min at 72°C. The last cycle was followed by a pause of 5 min at 72°C. The amplification program was performed on a thermocycler model “MiniCycler™”, MJ Research (USA). Seventy ten mer arbitrary primers were used to amplify sunflower samples of genomic DNA. Amplified DNA fragment were separated in 2% agarose gels (1xTAE) stained with ethidium bromide, visualized under UV light and photographed.

Results and Discussion

RAPD analysis of mutant lines and interspecific hybrids was performed using a set of seventy, ten mer primers with arbitrary sequence and 60-70% G+C content.

The RAPD banding patterns obtained are characterized with reproducibility under the established from us PCR conditions.

Most of the tested primers did not show polymorphism between the included in this study susceptible and resistant lines and hybrids.

Seven out of totally seventy used primers (OPA-02, OPA-11, OPB-01, OPB-10, OPB-13, OPB-18 and OPK-17 – Operon Technologies Inc., USA) (**Table 1**) amplified polymorphic bands which were not observed in susceptible lines – cv. Peredovik and VNIIMK 8931 (**Fig. 1, 2, 3, 4, 5, 6, 7, 8**).

Four of these primers (OPA-11, OPB-01, OPB-18 and OPK-18) amplified polymorphic fragments in all studied resistant lines (MX655, 6116A, cv. Vega and 7009R) (**Fig. 2, 3, 4, 5**). The sizes of the specific for resistant lines and cultivars amplified polymorphic bands are as follows:

- 690 bp fragment in RAPD pattern generated by primer OPK-17 (**Fig. 2**).
- 290 bp fragment in RAPD pattern generated by primer OPA-11 (**Fig. 3**).

- over 1 kb fragment in RAPD pattern generated by primer OPB-01 (Fig.4).
- 820 bp fragment in RAPD pattern generated by primer OPB-18 (Fig.5).

The remaining 3 primers (OPA-02, OPB-10, OPB-13) generated polymorphic products only in 3 of all included in this study resistant lines.

Primers OPA-02 and OPA-13 amplified polymorphic bands of about 590 bp and 850 bp respectively in RAPD patterns of mutant lines MX655, 6116A and cv.Vega, which were not observed in 7009R line.

In contra version primer OPB-10 produced 520 bp polymorphic bands in mutant lines and line 7009R derived from interspecific hybridization *H. annuus* x *H. tuberosus* (F12) but not in cv.Vega.

We can speculate that the obtained by primers OPA-02, OPA-13 and OPB-10 polymorphisms probably correspond to different type of resistance to race E of *O. cumana*. More detailed studies are necessary to confirm this supposition.

This study showed that only one of all tested seventy primers (RAPD analysis primer 1 – Amersham Biosciences, USA) amplified 600 bp polymorphic fragment in the susceptible lines – cv.Peredovik and cv.VNIIMK 8931 (Fig. 1).

The mutant lines with complete resistance to races A to E of *O. cumana* included in this study derived from a susceptible cv.VNIIMK 8931. They are appropriate material for further molecular analyses in order to find marker(s) closely linked to the gene(s) for resistance to broomrape. Polymorphic DNA products obtained in RAPD patterns of cv.VNIIMK 8931 and mutant lines MX655 and 6116A were isolated and will be cloned and sequenced.

There are many investigations on the genetics of the resistance in sunflower (1, 16) All these results indicate that although major

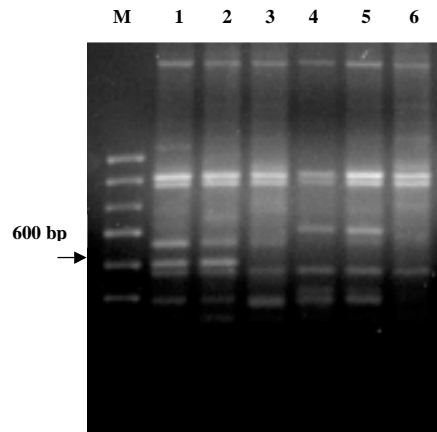


Fig. 1. RAPD banding patterns of different lines and cultivars *Helianthus annuus* (1 – 6) showing specific amplification product (indicated by arrow) in susceptible cultivars generated by RAPD analysis primer 1. M – 100 bp DNA size marker (Amersham Biosciences, USA); 1 – cv.Peredovik; 2 – cv.VNIIMK 8931; 3 – MX 655; 4 – 6116A; 5 – cv.Vega; 6 – 7009R.

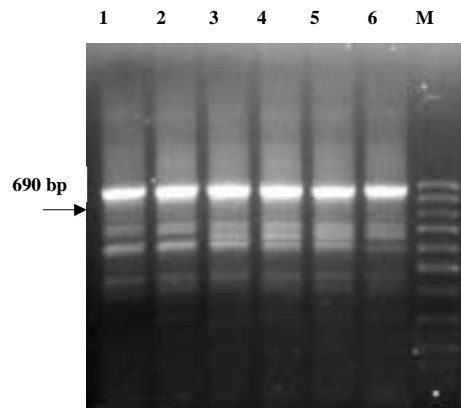


Fig. 2. RAPD banding patterns of different lines and cultivars *Helianthus annuus* (1 – 6) showing specific amplification product (indicated by arrow) in resistant lines and cultivars, generated by primer OPK-17. M- 100 bp DNA size marker (Amersham Biosciences, USA); 1 – cv.Peredovik; 2 – cv.VNIIMK 8931; 3 – MX655; 4 – 6116A; 5 – cv.Vega; 6 – 7009R.

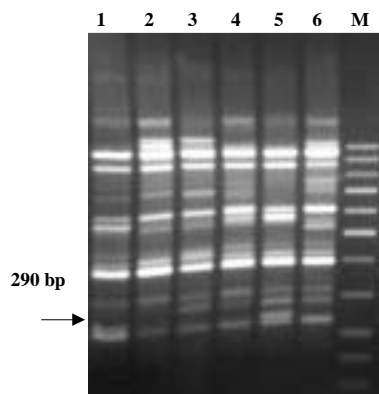


Fig. 3. RAPD banding patterns of different lines and cultivars *Helianthus annuus* (1 – 6) showing specific amplification product (indicated by arrow) in resistant lines and cultivars, generated by primer OPA-11. M - 100 bp DNA size marker (Amersham Biosciences, USA); 1 – cv.Peredovik; 2 – cv.VNIIMK 8931; 3 – MX655; 4 – 6116A; 5 – cv.Vega; 6 – 7009R.

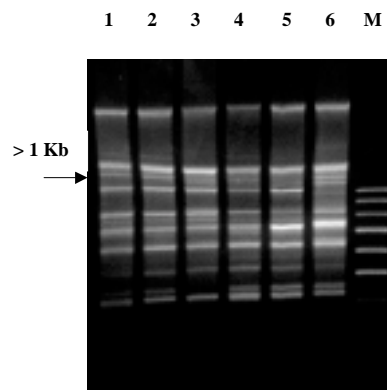


Fig. 4. RAPD banding patterns of different lines and cultivars *Helianthus annuus* (1 – 6) showing specific amplification product (indicated by arrow) in resistant lines and cultivars, generated by primer OPB-01. M - 100 bp DNA size marker (Amersham Biosciences, USA); 1 – cv.Peredovik; 2 – cv.VNIIMK 8931; 3 – MX655; 4 – 6116A; 5 – cv.Vega; 6 – 7009R.

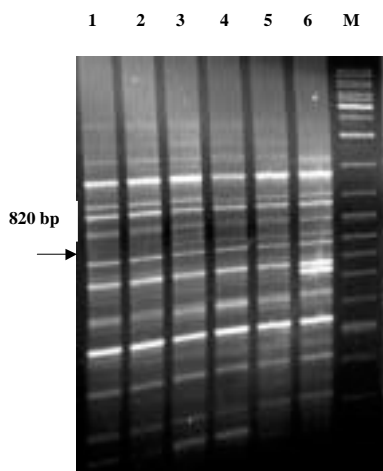


Fig. 5. RAPD banding patterns of different lines and cultivars *Helianthus annuus* (1 – 6) showing specific amplification product (indicated by arrow) in resistant lines and cultivars, generated by primer OPB-18. M - Gene Ruler DNA Ladder Mix (MBI Fermentas, USA); 1 – cv.Peredovik; 2 – cv.VNIIMK 8931; 3 – MX655; 4 – 6116A; 5 – cv.Vega; 6 – 7009R.

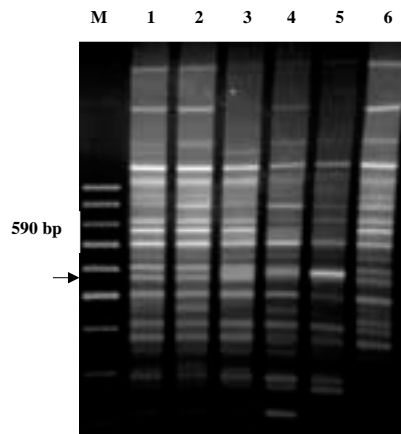


Fig. 6. RAPD banding patterns of different lines and cultivars *Helianthus annuus* (1 – 6) showing specific amplification product (indicated by arrow) in three of resistant lines (3, 4, 5), generated by primer OPA-02. M - 100 bp DNA size marker (Amersham Biosciences, USA); 1 – cv.Peredovik; 2 – cv.VNIIMK 8931; 3 – MX655; 4 – 6116A; 5 – cv.Vega; 6 – 7009R.

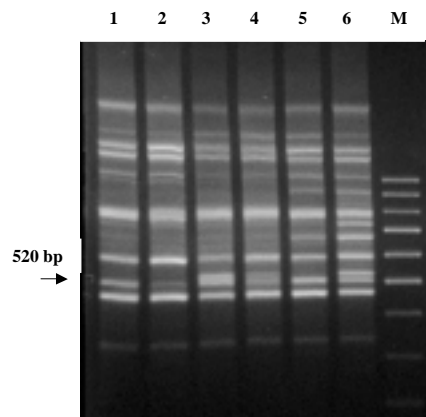


Fig. 7. RAPD banding patterns of different lines and cultivars *Helianthus annuus* (1 – 6) showing specific amplification product (indicated by arrow) in three of the resistant lines (3, 4, 6), generated by primer OPB-10. M – 100 bp DNA size marker (Amersham Biosciences, USA); 1 – cv.Peredovik; 2 – cv.VNIIMK 8931; 3 – MX655; 4 – 6116A; 5 – cv.Vega; 6 – 7009R.

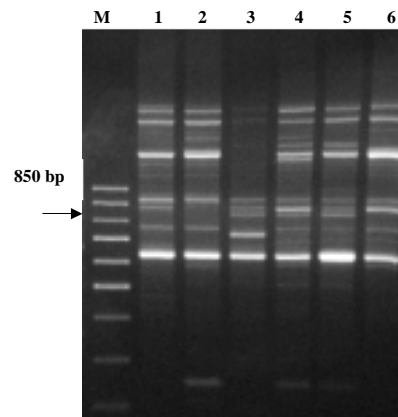


Fig. 8. RAPD banding patterns of different lines and cultivars *Helianthus annuus* (1 – 6) showing specific amplification product (indicated by arrow) in three of the resistant lines (3, 4, 5), generated by primer OPB-13. M – 100 bp DNA size marker (Amersham Biosciences, USA); 1 – cv.Peredovik; 2 – cv.VNIIMK 8931; 3 – MX 655; 4 – 6116A; 5 – cv.Vega; 6 – 7009R.

TABLE 1

List of primers, used in RAPD analyses and presence or absence of specific bands in susceptible and resistant lines and cultivars *Helianthus annuus*.

Primer	5'to 3?sequence	Bands, specific for susceptible cultivars		Bands, specific for resistant to race E lines and cultivars <i>Helianthus annuus</i>			
		cv.Peredovik	cv.VN IIMK 8931	MX 655	6116A	cv.Vega	7009R
OPA-02	TGCCGAGCTG	-	-	+	+	+	-
OPA-11	CAATCGCCGT	-	-	+	+	+	+
OPB-01	GTTTCGCTCC	-	-	+	+	+	+
OPB-10	CTGCTGGGAC	-	-	+	+	-	+
OPB-13	TTCCCCGCT	-	-	+	+	+	-
OPB-18	CCACAGCAGT	-	-	+	+	+	+
RAPD analysis primer 1	GGTGCGGGAA	+	+	-	-	-	-
OPK-17	CCCAGCTGTG	-	-	+	+	+	+

dominant genes are involved in the control of resistance to *O. cumana* in sunflower, the inheritance of resistance may be more complex that previously expected. Other than

single dominant genes may also be involved in some very useful resistances.

In our case the model of inheritance to broomrape will be studied in F1 to F3 gen-

erations of the crosses VNIIMK 8931 x MX655 and VNIIMK 8931 x 6116A.

The applied RAPD analysis is the first attempt to characterize the mutation derived resistant lines by molecular markers. The obtained RAPD polymorphisms that discriminate between susceptible and resistant lines and cultivars could be converted into SCAR (sequence characterized amplified regions) markers by isolation of RAPD bands, sequencing and design of specific primers. These primers could be used for direct and rapid screening and selection of resistant to *O. cumana* sunflower lines for the purposes of breeding.

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