PRELIMINARY STUDIES RELATED TO THE USE OF MARKER ASSISTED SELECTION FOR RESISTANCE TO *OROBANCHE CUMANA* Wallr. IN SUNFLOWER

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ABSTRACT

Sunflower resistant cultivars are the best solution to control the parasitic weed *Orobanche cumana* Wallr. Most Romanian hybrids that are highly tolerant to broomrape, have the gene of resistance, at the locus *Or5* present in the inbred LC 1093, utilised as the source of resistance in all hybrid combinations. The development of sunflower inbreds resistant to *Orobanche* should be accelerated by Marker Assisted Selection (MAS). The molecular markers could help early selection, by screening the presence of specific DNA fragments in recombinant inbreds. Five RAPD markers (UBC 73-460, UBC 318-350, UBC 685-700, UBC 264-680 si OP A17-690) discriminated parental inbred lines LC 1093Or5 and LC 1004or5, but not very clearly between BC1 segregating population, for all markers. The three SSR markers (ORS 1114, ORS 1036 and ORS 1222) tested on BC1 plants scored for resistance in the field of Baraganu - Romania with a highly natural infestation by broomrape, segregated in different proportion (56%, 81% and 65%) for resistant gene *Or5*. None of SSR markers identified so far, are strongly linked or near *Or5* locus so, MAS is limited to the centromeric side of the locus Or5. The validation of the markers ORS 1114, ORS 1036 and ORS 1222 in a different location as Baraganu - Romania with high natural infestation by broomrape plants, should be very important for sunflower breeding using marker asisted selection.

Key words: broomrape, molecular marker, sunflower

INTRODUCTION

Broomrape (*Orobancche cumana*) a parasitic Angiosperme infects the roots of sunflower crop causing severe losses. Breeding for resistant sunflower cultivars is the most effective method to control the parasitic weed. Vrânceanu et al (1980) identified five physiological races (A-E) of *O. cumana* in field highly infected with broomrape plants (Braila - Romania) using five different genotypes, differentiating the five genes associated with the five races (Table 1). These results have been utilised in many other experimental studies for molecular markers identification associated with the genes for resistance.

Cultivation of resistant or highly tolerant sunflower hybrids challenge the evolution of parasite physiological races toward the defeat of known resistant genes so, new more aggressive races have been reported (Tang et al., 2003, Perez-Vich et al 2004).

Sunflower Romanian hybrids resistant or highly tolerant to the attack of *Orobanche cumana* contain resistant alleles at the locus *Or5* in LC 1093 inbred line, which has been used as resistant sources in all hybrid combinations. Backcross and selection cycles could be accelerated using molecular markers, as RAPD (Random Amplified Polymorphic DNA), AFLP (Arbitrary Fragments of Length Polymorphism) RFLP (Restriction Fragments of Length Polymorphism) and SSR (Simple Sequence Repeat) (Tang et al., 2003; Burke et al., 2002; Tang et al., 2002; Lu et al., 2000; Gedil et al., 2001; Jan et al., 1998; Gentzbittel et al., 1995, 1999; Berry et al., 1995, 1996, 1997, 1999; Perez-Vich et al., 2004; Yu et al., 2003).

Tang et al. (2003) tried to identify SSR markers tightly linked to *Or5* and to place the *Or5* locus on the public molecular genetic linkage map of sunflower. They consider that this region is responsible for sunflower genetic resistance to *O. cumana* race E. The authors, retorically asked why several hundreds DNA markers had to be screened to identify loci linked to *Or5*, why no DNA markers other than an unconfirmed RAPD-SCAR marker for *Or5*, and the closest SSR marker is 6.2 cM downstream of *Or5*. The scarcity of DNA markers near *Or5* could be due to the low DNA polymorphisms. But, *Orobanche* resistance genes have been introgressed from wild sunflowers, which should be very polymorphic in comparison with DNA sequences found in elite inbred lines. The *Or5* is telomeric or near telomeric, located in a region of apparently high recom-

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Table 1. The physiological races of Romanian broomrape populations (Vrânceanu et al., 1980; Pacureanu et al., 1998)

Differentiating genotypes	Broomrape races						Resistance	Genes for resistance
	A	B	C	D	E	F		
AD 66	S	S	S	S	S	S	RO	-
Kruglik A-41	R	S	S	S	S	S	R1	*Or1*
Jdanov 8281	R	R	S	S	S	S	R2	*Or2*
Record H-8280	R	R	R	S	S	S	R3	*Or3*
S-1358, O-7586	R	R	R	R	S	S	R4	*Or4*
P-1380-2	R	R	R	R	R	S	R5	*Or5*
LC1093	R	R	R	R	R	R	R6	*Or6*
Population ratio %	59.4	24.4	6.9	5.9	3.4	<1		

R= resistant, S= susceptible

binations. On the other hand, the are phenotypical errors, (susceptible escapes misclassified as resistant) and introducing spurious recombinants and inflating map distance. Among 1600 RFLP and SSR markers maped so far, only four are into the interval of 6.2 - 11.2 cM to the locus *Or5*. The markers situated in the Linkage Group 3 (LC3) in the interval 6.2 - 11.2 cM are the following: ORS 1040 (11.2 cM) ORS 1036 (6.2 cM) CRT 392 (6.2 cM) and CRT 314 (7.8 cM). The others markers were identified in the LG3 as: ORS 1114 (74.3 cM) and ORS 1222 (29.5 cM) .

The goal of our research was to test the identified molecular markers for selection in order to obtain broomrape resistant sunflower inbreds.

MATERIAL AND METHODS

One hundred BC1 descendants of the cross between LC 1093 *Or5* and LC 1004 *or5* sunflower inbred lines were screened together with the parental inbred lines for resistance/ susceptibility, using RAPD and SSR markers. A field experiment was conducted in the field of Baraganu, Braila County, Romania, with a high natural infestation by *Orobanche cumana* seeds. The resistant and susceptible sunflower plants were recorded in the field, at blooming stage.

DNA extraction was performed using SDS based method (Delaporta et al., 1983). The amount of 0.5 g of fresh leaves was grounded in liquid nitrogen with the extraction buffer (Tris 0.1M pH 8.0, EDTA 0.05M, NaCl 0.5M). The PCR amplification followed the clasical protocol with 55°C annealing temperature for RAPD and 63/53°C for SSR in a M.J. Research termocycler, for 35 cycles in RAPD and 10 cycles and additional 30 cycles for SSR . The UBC decamer primers purchased from British Columbia University, Canada for RAPD markers and specific primers for SSR markers (ORS 1114, ORS 1222 and ORS 1036), sinthetised for us by Mycrosynth, Switzerland according to the structure of the Oregon State University data base (professor S. Knapp team) were used in PCR amplification with a DNA matrix of 30 ng/reaction, and 2U of Stoffel Fragment as Taq DNA polymerase (Perkin Elmer). The PCR products were evaluated by electrophoresis on 1.5% agarose gels, staining with ethidium bromide 10mg/ml and vizualization at a Bioprint equipment.

RESULTS AND DISCUSSION

The evaluation of plant reaction to the parasite attack was done by counting the number of broomrape plants around sunflower plants, at blooming stage, assuming that all broomrape plants were present around susceptible plants. The recorded results are presented in the table 2. In the case of recombinant BC1 only four plants out of the 100, have had 1-3 broomrape plants around. In the same field at Baraganu, susceptible sunflower hybrid plants had 3-35 broomrape plants around.

Table 2. Number of *O. cumana*/sunflower plants

Genotype	No. of sunflower plants	No. of broomrape/sunflower plants
LC- 1093 *Or5*	20	0
LC 1004 *or5*	18	5-25
BC1	100	0/96 1-3/4

Out of 60 random decamer primers used for DNA-PCR amplification, only five revealed the polymorphism between parental inbred lines, by the band present in resistant genotypes and absent in susceptible ones. The analysis of recombinant BC1 plants revealed the segregation of genotypes related to the characteristic band of each primer, as: UBC 73-460 present in 26 out of the 30 genotypes and UBC 418-350 present in 58 out of the 60 genotypes. All other markers UBC 685-700, UBC 264-680 and OP A 17-690 identified the bands associated with resistant gene. The marker UBC 120-660 reported easier (Lu et al., 200) as associated with the resistant genotype, was not confirmed in our experimets (Table 3).

Among SSR markers tested in order to identify the association with broomrape resistance, on breeding material from Fundulea tested in the field at Baraganu Romania with a high infestation by broomrape plants, only the marker ORS 1036 is located into the interval of 6.2- 11.2 cM distance from *Or5* locus (Tang et al., 2003). The band of 245 bp was identified in the resistant genotype LC 1093 *Or5*. In the susceptible inbred LC 1004 *or5* the specific band was not present. The band 245 bp segregated among the BC1 genotyps as: 73 resistant: 17 susceptible (Table 4).

The SSR marker ORS 1222 situated at 29.5 cM distance from *Or5* locus, identified the specific band of 100 bp in 52 out of 80 analysed genotypes, in spite of the field test recorded as 96 resistant out of 100 plants. The marker ORS 1114 situated at 74.3 cM far away from the locus *Or5*, identified the band of 280 bp in 35 out of 62 genotypes, characterized as resistant in the field, with no broomrape plants around.

The SSR markers applied to backcross genotypes segregated unexpectedly, according to phenotypical characterization performed in the field with a high natural infestation of broomrape. The marker ORS 1222 identified the band of 100 bp in 65% of genotypes characterized as resistant, the marker ORS 1114 identified the band of 280 bp at 56% of analysed genotypes characterized as resistant and the marker ORS 1036 identified the band 245-246 at 81% of analised genotypes and characterized as resistant. A possible explanation should be phenotypical errors which introduced spurious recombinants, assuming that there were broomrape plants underground, which did not appeare out.

Because none of the DNA markers described so far, are tightly linked to or flank *Or5*, Marker Assisted Selection is limited to the centromeric side of the *Or5* locus, and *Or5* genotypes cannot be precisely identified from RAPD-SCAR or SSR markers.The efficiency of MAS depends strongly on a precise QTL mapping and the effects of each individual QTLs.

It should be of interest to validate at a different location the test of *Orobanche* resistance for sunflower and to test additional markers for saturating the region of possible QTLs associated with broomrape resistance should also be useful.

We cannot say when the MAS will be realy available for sunflower selection and breeding for

Table3. RAPD markers for sunflower resistance to broomrape

Primers		% plants - parental inbreds		BC1				
	Band bp	LC 1093 *Or5*	LC 1004 *or5*	No. of plants	Resistant plants		Susceptible plants	
					No.	%	No.	%
UBC 73	460	100	0	30	26	86.7	4	13.3
UBC 318	350	100	0	60	58	96.7	2	3.3
UBC 685	700	100	0	32	32	100	0	-
UBC 264	680	100	0	30	30	100	0	-
OP A 17	690	100	0	18	18	100	0	-
UBC 120	660	/	/	/	/	/	/	/

Table 4. SSR markers for sunflower resistance to *O. cumana*

Marker	cM to *Or5*	Band pb	LC 1093 *Or5*	LC1004 *or5*	BC1				
					No. plant	+		-	
						No.	%	No	%
ORS 1222	29.5	100	+	-	80	52	65	28	35
ORS 1114	74.3	280	+	-	62	35	56	27	44
ORS 1036	7,5	245-246	+	-	90	73	81	17	19

broomrape resistance. This objective is desired by all breeders and geneticists, because the marker assisted selection could accelerate broomrape resistant inbreds selection by identification and removing the heterozygots, the phenotypical errors, and seedling selection of resistant genotypes, as well. Sunflower breeding needs MAS procedures with reduced costs, reduced technical analysis required by SSRs - PCR markers for gene mapping and selection. SNPs (Single Nucleotide Polymorphism) no PCR, no gel based assay, should be a powerful tool for diagnostics of genes and QTLs in the future.

CONCLUSION

The identified RAPD markers for sunflower resistance to *Orobanche cumana* clearly discriminated the two parental inbreds, but only three of them: UBC 685-700, UBC 264-680 and OP A17-690 identified the bands in all BC1 segregating genotypes , characterized as resistant. The markers UBC 73-460 and UBC 318-350 identified the bands in 86.7% respective 96.7% of BC1 genotypes.

The SSR markers applied to backcross genotypes segregated differently from the phenotypical characterization performed in a field with a high natural infestation by broomrape. The marker ORS 1222 identified the band of 100 bp in 65% of genotypes characterized as resistant, the marker ORS 1114 identified the band of 280 bp in 56% of analysed genotypes characterized as resistant and the marker ORS 1036 identified the band 245-246 in 81% of analysed genotypes, characterised as resistant. A possible explanation may be phenotypical errors which introduced spurious recombinants, assuming that there were broomrape plants underground, which did not appeare out.

Because none of the DNA markers described so far, are tightly linked to or flank *Or5*, Marker Assisted Selection is limited to the centromeric side of the *Or5* locus, and *Or5* genotypes cannot be precisely identified from RAPD-SCAR or SSR markers. A saturation of the region with possible QTLs is needed, and then, an evaluation of MAS comparative with traditional breeding methods for broomrape resistance.

It should be of interest to validate at a different location the test related to *Orobanche* resistance for sunflower. The SSR markers ORS 1222, ORS 1114 and ORS 1036 tested on BC1 plants, that were phenotipically scored in a field with a high natural infestation broomrape, at Baraganu – Romania, open new ways for markers evaluation and is important for sunflower breeders interested in practical marker assisted selection.

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