HELIA, **21**, Nr. 29, p.p. 1-8, (1998) UDC 631.523;633.854.78

MOLECULAR MAPPING OF THE RECESSIVE BRANCHING GENE *b1* AND THE FERTILITY RESTORATION GENE *Rf1* IN SUNFLOWER

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Received: September 22, 1998
Accepted: October 22, 1998

SUMMARY

PCR-based molecular markers were used to map the recessive branching gene (*b1*) and the fertility restoration gene (*Rf1*) in sunflower. Sixty-one dominant RAPD markers, generated by 31 random primers, as well as 14 STS markers, were analyzed among an F_2 population (C3) of 230 plants derived from cross RPG01 x AEHC1. Fourteen STS markers were analyzed in a second population (C4) of 211 plants from the cross RPG01 x R300V. Ten STS markers were analyzed in both populations. A linkage map of 64.6 cM containing the *b1* locus, 9 RAPD markers and 2 codominant STS markers was identified using JOINMAP based on segregation data. An RAPD marker, UBC105_740, was mapped at 5 cM from the gene *b1*. Another linkage group of 34 cM, containing the male fertility restoration locus *Rf1* as well as one RAPD and one STS marker, was also identified. The STS markers could be easily integrated into the previously established sunflower RFLP maps and should permit to verify the identity of branching and fertility restoration genes of different origins.

Key words: RAPD, STS, branching, fertility restoration, sunflower

INTRODUCTION

Molecular markers linked to useful genes, such as the genes of restoration of cytoplasmic male sterility, have great potential for marker-assisted breeding (Ichikawa *et al.*, 1997; Zhang *et al.*, 1997; Subudhi *et al.*, 1997; Kamps and Chase, 1997). In a previous study (Lu *et al.*, 1998a), PCR-based markers linked to the *Or5* gene (Vranceanu *et al.*, 1980), conferring resistance to broomrape (*Orobanche cumana*) race E, were identified in sunflower. These markers, as well as the resistance gene were integrated into the linkage group 17 of an RFLP map published by Gentzbittel *et al.* (1995) (Lu *et al.*, 1998b). The populations used in that study also segregated for branching and restoration of CMS. In the present paper, we report the PCR-based markers linked to these two segregation traits.

MATERIALS AND METHODS

Two F_2 populations were used in this study: the first one consisted of 230 F_2 plants derived from the cross RPG01 x AEHC1, named C3; the second one consisted of 211 F_2 plants derived from the cross RPG01 x R300V, named C4. AEHC1 and R300V both contain the recessive branching gene (*b1*, Putt, 1964) and the male fertility restoration gene (*Rf1*). Segregations were observed for male fertility restoration and recessive branching in both populations.

The primers, used for RAPD (Random Amplified Polymorphic DNA, Williams *et al.*, 1990) analysis, were purchased from Operon Technologies (Alameda, Calif.) and the University of British Columbia Biotechnology Center (Vancouver, B.C.). The primers for STS (Sequence tagged site, Olson *et al.*, 1989) were developed by Rustica Prograin Génétique (Lu *et al.*, 1998a). Thirty-one RAPD primers were analyzed in C3 population. A total of 18 SCAR primer pairs were analyzed in at least one of the two F_2 populations (14 SCARs for each population). The molecular techniques for analyzing these primers were described by Lu *et al.* (1988a).

Linkage analysis was performed using JOINMAP version 1.4 (Stam, 1993). The Kosambi mapping function (Kosambi, 1944) was used to convert recombination frequency into genetic map distance (in centimorgans). Branching and non-branching, sterile and fertile, were treated as alleles of two extra dominant DNA markers designed separately by *b1* and *Rf1*. Recombination frequencies, pairwise distances between *b1* or *Rf1* and other molecular markers in the linkage groups, and corresponding LOD values were calculated with JOINMAP.

RESULTS

Segregations of branching and fertility in the C3 and the C4 populations fit the expected 3:1 ratio for a single recessive gene for branching and a single dominant gene for male fertility restoration (Table 1). A segregation distortion was observed for fertility restoration in the C3 population (P=0.01) in favor of the male fertile class.

Table 1: Segregation for branching and fertility restoration in C3 and C4 populations

Population	Number of observations	Segregation		Expected ratio	$\chi 2$
C3	212	176 fertile	36 sterile	3:1	7.27*
C4	209	160 fertile	49 sterile	3:1	0.27
C3	221	168 non-branching	53 branching	3:1	0.12
C4	211	170 non-branching	41 branching	3:1	3.49

* significant at P< 0.01

Sixty-one RAPD markers (revealed by 31 random primers) and 14 STS markers (7 dominant and 7 codominant) were scored for segregation in the C3 popula-

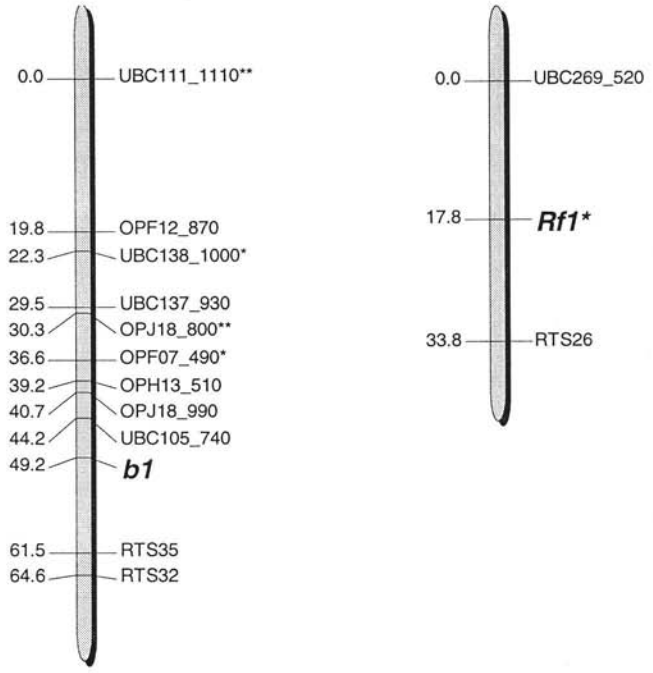

*Figure 1: Molecular maps of sunflower linkage groups containing the recessive branching gene (b1) and the male fertility restoration gene (Rf1). RAPD marker loci were designated by the primer names followed by the size of fragment (bp) "RTS" loci design STS markers derived from RAPD fragments (Table 2). Loci deviating from the expected Mendelian ratios are indicated with * (P<0.05) or ** (P<0.01). The cumulative map distances in centimorgans are shown at the left of each map*

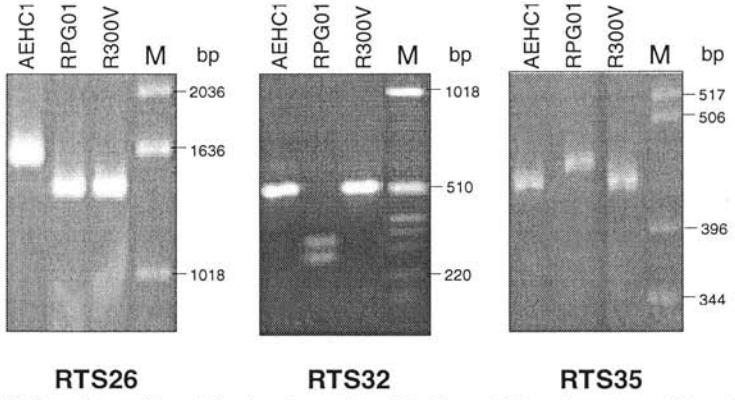

Figure 2: Ethidium bromide stained polyacrylamide (A and C) and agarose (B) gel electrophoretic profiles of STS markers for the three parental lines (indicated on top of each photo). The polymorphism between RPG01 and the other two lines was obtained by using the enzyme TaqI

tion. Fourteen STS markers (8 dominant and 6 codominant) were analyzed in the C4 population. Of the 18 STS markers used, ten (5 dominant and 5 codominant) were analyzed in both the C3 and the C4 population.

Analysis of the segregation data using JoinMap revealed that the 77 loci, scored in the C3 population, were arranged into 13 linkage groups. The number of segregating loci in these groups varied from 2 to 12. Twenty-two other markers segregated independently. The locus *b1* was included into the largest groups together with 9 RAPD and 2 STS markers, while the locus *Rf1* was grouped with 1 RAPD and 1 STS markers. The most probable linkage maps for the 2 groups were presented in Figure 1.

For *b1* locus, all 9 RAPD markers were located on one side and the 2 STS markers on the opposite side. The nearest marker, UBC105_740, was mapped at 5 cM from *b1*. The two STS markers were tightly linked to each other and mapped respectively at 15 and 20 cM from *b1*. Apart from UBC111_1110, the other 10 markers were all significantly linked to *b1* at a LOD score superior to 3 (Table 2).

For *Rf1* locus, an STS marker, RTS26, was mapped at 16 cM from *Rf1* on one side, while an RAPD marker, UBC269_520, was at 18 cM on the opposite side. The two markers were linked to *Rf1* locus at a LOD score superior to 4 (Table 2).

Table 2: Recombination frequency between molecular markers and gene loci

Population	Locus 1	Locus 2	Recombination frequency (%) ± SD	LOD
C3	*b1*	UBC111_1110	35.2 ± 4.9	1.8
C3	*b1*	OPF12_870	24.3 ± 5.5	3.6
C3	*b1*	UBC138_1000	21.6 ± 6.2	3.2
C3	*b1*	UBC137_930	22.4 ± 4.5	6.1
C3	*b1*	OPJ18_800	13.5 ± 5.1	5.7
C3	*b1*	OPF07_490	21.0 ± 6.0	3.6
C3	*b1*	OPH13_510	11.7 ± 2.8	18.4
C3	*b1*	OPJ18_990	13.0 ± 3.5	12.0
C3	*b1*	UBC105_740	10.2 ± 3.0	15.7
C3+C4	*b1*	RTS35	14.4 ± 3.7	10.9
C3+C4	*b1*	RTS32	22.1 ± 2.6	18.2
C3	*Rf*	UBC269_520	22.6 ± 5.2	4.4
C3	*Rf*	RTS26	18.7 ± 4.2	7.8

The primer sequences for STS marker RTS26, RTS32 and RTS35 are given in Table 3.

DISCUSSION AND CONCLUSION

Thirty-one random primers, analyzed in the F_2 population derived from the cross RPG01 x AEHC1, had allowed to identify 61 RAPD dominant markers. Nine of the 61 RAPD markers (15%) were mapped in the chromosomal region containing

Table 3: Three STS markers and their primer sequences. The underlined nucleotides are derived from initial RAPD primers

STS marker	Initial RAPD fragment	Primer	Sequence (5' to 3')
RTS26	OPZ17_1400	F	CCTTCCCACTCCACTATCAATGAT
		R	CCTTCCCACTAGTACACAACAAAA
RTS32	UBC171_500	F	TGACCCCTCCACTTTAAATAAGCT
		R	TGACCCCTCCCTCAATAATTGCAT
RTS35	UBC171_420	F	TGACCCCTCCACTTTAATTAAGCA
		R	TGACCCCTCCCTCAAGAACTACAT

the *b1* locus, while only one RAPD marker (1.6%) was found to be linked to the *Rf1* locus. This indicates an important genetic variation between the 2 sunflower lines for their *b1* chromosomal region. This variation might be the result of introgression of *b1* gene from wild species to sunflower line AEHC1. Contrarily, the *Rf1* gene conferring the restoration of male fertility must have been originated from cultivated sunflower or from wild species after numerous selections.

Recessive branching and male fertility restoration have both been mapped previously on RFLP linkage maps (Gentzbittel *et al.*, 1995; Jan *et al.*, 1998). RFLP markers of 1 cM from *Rf1* and of 14 cM from *b1* had been identified by Gentzbittel *et al.* (1995). Our PCR-based markers for *Rf1* and for *b1* were more distal but more easy to be used for molecular analysis. Analyses of the identified STS markers among the existing RFLP mapping populations would permit to integrate our linkage groups into the existing RFLP maps, and to confirm the identity of branching and fertility restoration genes of different origins. The constant association between the 2 STS markers (RTS32 and RTS35) and recessive branching among both C3 and C4 populations suggested the same *b1* gene in AEHC1 as in R300V.

ACKNOWLEDGEMENTS:

The authors thank Florence Couton, Olivier Naty, Carine Garcia and Sandrine Polain for their technical assistance. Chromosomal maps were drawn with the help of a program written in XVBA developed by Jean-François Rami.

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MAPEO MOLECULAR DEL RAMEADO RECESIVO DEL GEN *b1* Y LA RESTAURACIÓN DE LA FERTILIDAD DEL GEN *Rf1* EN GIRASOL

RESUMEN

Marcadores moleculares basados en PCR fueron utilizados para mapear el gen de ramificación recesiva (b1)y el gen de restauración de la fertilidad (Rf1) en girasol. Sesenta y un marcadores dominantes RAPD generados por 31 primers al azar, así como 14 marcadores STS, fueron analizados entre una población F_2 (C3) de 230 plantas derivadas a partir del cruce RPG01 x AEHC1. Catorce marcadores STS fueron analizados en una segunda población (C4) de 211 plantas a partir del cruce RPG01 x R300V. Diez marcadores STS fueron analizados entre ambas poblaciones. Un mapa de ligamiento de 64.6 cM conteniendo el locus b1, 9 marcadores RAPD y 2 marcadores codominantes STS fueron identificados utilizando el JOINMAP en base a los datos de la segregación. Un marcador RAPD, UBC105-740, fue mapeado a 5 cM del gen b1. Otro grupo de ligamiento de 34 cM conteniendo el locus de restauración de la fertilidad Rf1 así como un marcador RAPD y un marcador STS fueron también identificados. Los marcadores STS pueden ser facilmente integrados en el mapa de girasol RFLP previamente establecido y debería permitir la verificación de la identidad de genes de ramificación y restauración de la fertilidad de distintos orígenes.

CARTOGRAPHIE MOLÉCULAIRE DU GÈNE *b1* DE RAMIFICATION RÉCESSIVE ET DU GÈNE DE LA RESTAURATION DE FERTILITÉ *Rf1*, CHEZ LE TOURNESOL

RÉSUMÉ

Les marqueurs moléculaires basés sur la PCR, ont été utilisés pour cartographier le gène de ramification récessive (*b1*) et le gène de restauration de la fertilité (*Rf1*) chez le tournesol. Soixante et un marqueurs dominants RAPD générés par 31 amorces ainsi que 14 marqueurs STS, ont été analysés dans une population F_2 (C3) de 230 plantes issues du croisement RPG01 x AEHC1. Quatorze marqueurs STS ont été analysés dans une deuxième population (C4) de 211 plantes issues du croisement RPG01 x R300V. Dix marqueurs STS des deux populations ont été analysés. Un groupe de linkage de 64.6 cM incluant le locus *b1*, 9 marqueurs RAPD et deux marqueurs STS codominants a été identifié en utilisant JOINMAP sur la base des données de ségrégation. Un autre groupe de linkage de 34 cM contenant le locus de restauration de *Rf1* ainsi qu'un marqueur RAPD et un marqueur STS a été également identifié. Les marqueurs STS peuvent être aisément intégrés dans les cartes RFLP du tournesol déjà établies, ils devraient permettre de vérifier l'identité de la ramification et des gènes de restauration de la fertilité de différentes origines.