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A MARKER GENES COLLECTION AND RAPD MARKERS FOR RECESSIVE BRANCHING IN SUNFLOWER

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SUMMARY

The paper presents the marker gene collection and the possibilities to use them in sunflower breeding programs. There are two classes of markers: morphological and physiological. The marker genes are in connection with colors of ligulate flowers, anther and pollen grains, shape of ligulate flowers, branching of the stem, pigmentation, seed characteristics. Five different sunflower inbred lines with marker genes were crossed between them, in order to identify new linkages. Between F_2 progenies, double and triple recessive genotypes were identified. A hundred of 10 mer primers were used to amplified DNA from three pairs of NILs differing in the recessive branching gene "b". Few RAPD markers were identified by polymorphisms analysis and three RAPD markers D7-1800 bp, C1-750 bp and C1-1450 bp, are presented, but none of them discriminated consistently between branched and unbranched inbred lines in all three NILs, as we expected. For practical breeding work it is desirable to identify only positive markers associated with branched genotype.

Key words : Sunflower, gene character, mutants, RAPD, linkage

INTRODUCTION

The gene diversity estimated by the available variability for cultivated sunflower, is one of the lowest described in crop plants. It is difficult to anticipate the utilization of different genetic resources, thus, germplasm collections should be large enough. For broadening the genetic variability of cultivated sunflower increased breeding efforts are required. It is relevant for sunflower breeding how new methods as DNA fingerprinting, PCR and gene transfer can contribute to overcome problems of a narrow genetic base (Rafalski *et al.*, 1994). Varieties, populations, intra and interspecific hybrids, inbred lines, marker genes, nuclear and cytoplasmic male sterile genotypes and wild species are the main germplasm collections at the Research Institute for Cereals and Industrial Crops Fundulea in Romania.

Table 1: Sunflower marker genes

Nr	Character	Symbol	Action	Reference
1	Multiple alleles for ligulate flowers	Yellow L lemon white 11 orange 12	dominant recessive recessive	Vranceanu-Stoenescu, 1974; Fick, 1976; Leclercq, 1968
2	Atrophied ligulate flowers (tubular)	fl	recessive	Placek, 1930; Zimmerman, 1958; Lazar-Stoianova,1964; Vranceanu-Stoenescu, 1974
3	White anthers	ag	recessive	Heiser, 1954
4	White pollen	pa	recessive	Placek, 1930
5	Xantha leaves -at the top - total	y	recessive	Hockett-Knowles, 1970
6	Chlorina apicalis(carotenoid deficiency)	nd 1	recessive	Barotti, Fambrini, 1995
7	Chlorophyll deficiency mutants		recessive	Mihaljcevic, 1995
8	Anthocyanic pigmentation	T	dominant	Satiperov, 1914
9	No nthocyanic seeds of anthocyanic plants	tf	recessive	Leclercq, 1968
10	Striped achenes	S	recessive	Satiperov, 1914
11	Phytomelanin pericarp	P	recessive	Satiperov, 1914; Ananieva, 1936
12	Fasciation	F	recessive	Petrov, 1974; Stoenescu,1974; Škaloud, Kovacik, 1981
13	Sessily (short petiol)	SS	dominant	Vranceanu-Iuoras, 1983
14	Branched stem(wild type)	Br	dominant	Putt, 1940; Heiser, 1954; Hockett, Knowles, 1970
15	Multiple alleles for branching	b (1-4)	recessive	Putt Hockett Knowles; Sandu, 1997
16	Nuclear male sterility (1-5)	ms	recessive	Stoenescu, Vranceanu, 1977
17	Dwarfness (1-3)	na	recessive	Vranceanu, Stoenescu, 1974

Marker genes collection comprises different monogenic characters and some polygenic features (Table 1). Few linkage groups are known in case of sunflower. Based on the already known linkage groups, the relationship between marker genes were studied in a diallel cross and new linkages have been revealed (Table 2).

Table 2: Sunflower (*Helianthus*) linkage group

N	Symbol	Genetic linked character	% of cross- overs	Author, year
1	T ms1	Plant anthocyanin colour and genetic male sterility	1.3± 0.2	Leclercq 1966, Stoenescu, Vranceanu 1977
2	Y b	Xantha leaves at theop, recessive branching	11.6±1.0 38.5±5.1	Hockett, Knowles, 1970 Iouras, Stoenescu, 1982
3	L T	Yelow ligulate flowers and anthocyanic plant	10	Kovacik, Škaloud, 1982
4	l1 ag	Lemon white ligulate flower and white anther	20.7±6.9	Iuoras, Stoenescu,1982
5	Y pg	Xantha leaves at top and white pollen	38.2±0.3	Iuoras, Stoenescu,1982
6	b pg	Recessive branching and white pollen	27.9±0.5	Iuoras, Stoenescu, 1982

Double and triple recessive genotypes were isolated from this linkage study (Table 3). These genotypes are useful for localization of an unknown gene, based on

association score with recessive genes. Unfortunately, few marker genes have a practical value.

Table 3: Double and triple recessive branching genotypes in sunflower

Character	Symbol
Recessive branching, white pollen	bb papa
Recessive branching, lemon white liqulate flower	bb 1111
Recessive branching, tubulate flower, lemon white ligulate flower	bb flfl 11 1
Recessive branching, white anther	bb agag
Lemon white and tubular ligulate flower	fl fl 11 11
Orange and tubulat ligulate flower	fl fl 1212

The nuclear male sterility gene "ms 1" linked with anthocyanic pigmentation on the hypocotile of the plantlets, gene "T", were used to produce first commercial sunflower hybrids (Stoenescu and Vranceanu, 1977). Kovačik and Škaloud (1982) proposed a pattern for evaluation of gene linkage and its use in determination of heredity of the trait. The inheritance of branching in sunflower is complex, but few genes with major effects have been identified. In some lines, a single dominant gene, designated Br, results in branching over the entire stem (Putt, 1940), whereas, in other lines, branching is recessive (Putt, 1964). This recessive branching gene, designated "b', is often used in sunflower breeding programs to produce paternal inbred lines, which serves to extend flowering time, and thus, facilitates pollination inside of large CMS populations. Vranceanu *et al.* (1985) converted several single headed inbred lines into branched plants, by phenotypic recurrent selection. Genotyping recurrent selection is a more efficient alternative strategy because the progeny genotype test forms the bases of selection. This strategy requires the availability of genetic markers closely linked to the "b" gene, but, appropriate molecular markers are not available for sunflowers.

To broaden the basis for the selection of appropriate breeding material's, both, sunflower lines as well as wild *Helianthus* species and subspecies, have to be described in more details. Such a thorough characterization will have to focus on molecular markers in addition to morpho-physiological and biochemical traits, aiming at the construction of detailed genetic maps of cultivated sunflower materials. The construction of genetic maps of sunflower can be successfully performed by RAPD as has been demonstrated by Rieseberg *et al.* (1993) for *H. anomalus*, or by RFLPs. The first linkage map on 180 RFLP markers identifying 18 linkage groups was published by Gentzbittel *et al.* (1995). Berry *et al.*, in 1995, published a construction of an RFLP linkage map for cultivated sunflower. Recently, Jan *et al.* (1998) communicated large linkage maps for sunflower.

The identification of different *Helianthus* genotypes has been reported by Mosges and Friedt (1994) using biochemical and molecular markers on two sets of NILs, in connection with downy mildew resistance gene. Dehmer and Friedt (1996) identified the band F15-690 (6.8 cM) associated with locus Ol1=high oleic in

homozygous genotype. This marker should facilitate the selection of homozygous genotype Ol1 in early segregating generations for high oleic oil. For linkage maps, the authors consider that it is necessary to saturate the region Ol1 with other markers RAPD and AFLP, as well. Rieseberg *et al.* (1991) published a study of an infarred from chloroplast DNA and isozymes variation in the frame of *Helianthus* section.

This paper presents few results of a search for molecular markers associated with the "b" gene, in sunflower.

MATERIALS AND METHODS

Three pairs of near isogenic lines (NlLs) with and without "b" gene, products of 7-8 generations of backcross and selection, were studied: VF 1721, A 1566, SP 4559 (Vranceanu *et al.*, 1985).

DNA isolation followed a protocol described by Faivre-Rampant *et al.* (1989), with modifications. PCR reaction were performed in 25 µl, using 10 ng as DNA template, 2 mM $MgCl_2$, 20 mM Tris-HCl, pH 8.4, 100 mM each dNTPs, 15 ng 10 mer primer and 0.5 U Taq DNA polymerase. Reaction were overlaid by mineral oil and placed in a MJ Research Thermal Cycler, programmed for 1 min 94°C, 1 min 36°C, 2 min 72°C, during 40 cycles, and 7 min end extension at 72°C.

Amplification products were separated by electrophoresis on 1.5% TBE 0.5x agarose gel, and detected by 10 mg/mL BET staining.

RESULTS AND DISCUSSION

Fifty-three 10 mer primers were used to amplify DNA extracted from leaves of the three sunflower NlLs. Polymorphic PCR products were produced by 27% of these primers. Three of them were informative and separated the pairs of NlLs, according to branching genotype: D7-1800, C1-750 and C1-1450.

Primer D7 generated a 1800 base pairs fragment, which was present in branching genotype (Figure 1, lines 3,5), but absent in unbranching genotype (Figure 1, lines 2,4). This fragment identified for NIL VF 1721, was absent in NIL SP 4559 (Figure 1, lines 10,11), and inconsistent for NIL A-1566 (Figure 1, lines 6,9).

Similar patterns were observed for primer C1. RAPD markers C1-750 (Figure 2, lanes 2, 4) and C1-1450 (Figure 2, lanes 2,4) are associated with unbranched genotype, in case of NIL VF-1721. The pairs of the other two inbred A-1566 and SP 4559, were not differentiated by this primer.

The other primers produced polymorphisms, but did not differentiate the branched and unbranched genotypes. Unfortunately, none of the informative RAPD markers, discriminated consistently between the branched and unbranched inbred lines in all three NlLs, as we expected, assuming that it is only one recessive allele "b". For practical breeding work, it is desirable to identify only positive markers, associated with branched genotype.

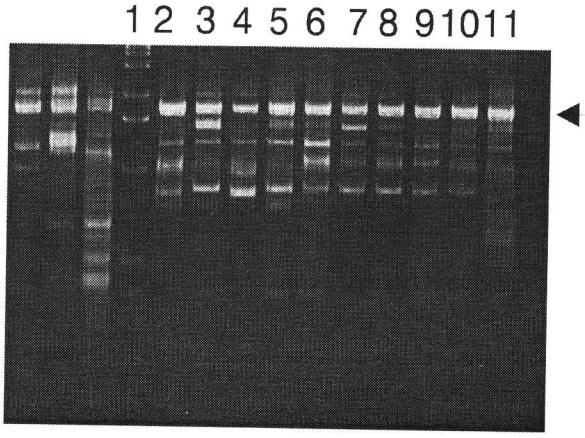

Figure 1 : Polymorphism of RAPD markers: D7-1800 (3,5) 1-1 Kb, 2, 4 - VF 1721 unbranched, 3, 5 - VF 1721 branched, 6-9-A 1566, 10-11-SP-4559.

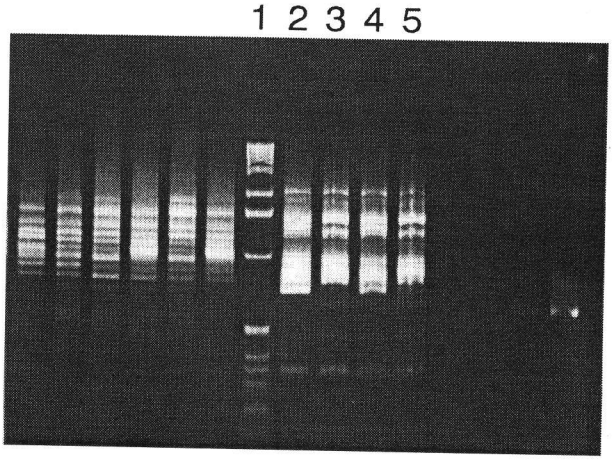

Figure 2: Sunflower RAPD markers: OP-C1- 750, OP-C1-1450 (2, 4) VF 1721 unbranched (2, 4), VF 1721 branched (3, 5), 1 Kb (1)

CONCLUSIONS

Sunflower germplasm collections should be large enough and at hand, for breeding programs.

Particular marker genes are useful for unknown gene identification. Identification of other molecular markers as RAPD D7-1800 is expected for differentiation in case of branched and unbranched genotypes.

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COLECCION DE GENES-MARCADORES Y RAPD MARCADORES DE LA RAMIFICACION RECESIVA DE GIRASOL

RESUMEN

El estudio presenta la descripcion de la coleccion de genes-marcadores y trata de las posibilidades de su utilizacion en la seleccion de girasol. Hay dos clases de marcadores: morfologicos y fisiologicos. Los genes-marcadores son liados con el color de flores liguladas, estambre y polen, la forma de flores liguladas, ramificacion del tronco, pigmentacion, las caracteristicas de semillas. Cinco lineas consanguineas de girasol fueron cruzados mutualmente con diversos genes-marcadores para comprobar nuevas conexiones. En la descendencia de F_2 fueron identificados los genotipos recesivos doblemente y triplemente. Una centena de 10 mer primers ha sido aplicada al ADN aumentado de tres pares de NILs que se difenciaban segun el gen para la ramificacion recesiva. Solo pocos RAPD marcadores fueron identificados por el analisis de polimorfismo. Solo tres, D7-1800 bp, C1-750 bp y C1-1450 bp fueron presentados, pero aun ellos, en contra de nuestras expectaciones, no podian distinguir consecuentemente las lineas consanguineas ramificadas y no ramificadas en todos tres NILs. Para la seleccion practica, es deseable de identificar solo los marcadores positivos liados con el genotipo ramificado.

COLLECTION DE GÈNES MARQUEURS ET MARQUEURS RAPD DE RAMIFICATION RÉCESSIVE DANS LE TOURNESOL

RÉSUMÉ

Cet article présente la collection de gènes marqueurs et la possibilité de les utiliser pour la culture du tournesol. Il existe deux classes de marqueurs: morphologiques et physiologiques. Les gènes marqueurs sont liés à la couleur des fleurs ligulées, à l'anthère et au pollen, à la forme des fleurs ligulées, à la ramification de la tige, à la pigmentation, aux caractéristiques de la semence. Cinq lignes inbred de tournesol de différents gènes marqueurs ont été croisées entre elles dans le but de confirmer de nouveaux liens. Des génotypes récessifs doubles et triples ont été identifiés dans les progénies F_2. De trois paires de NIL différant par ramification récessive de gène "b", une centaine de 10 mer primers ont été appliqués à l'ADN amplifié. Quelques marqueurs RAPD seulement ont été identifiés par l'analyse des polymorphismes. Trois marqueurs RAPD, D7-1800 bp, C1-750 bp et C1-1450 bp sont présentés mais, contrairement à notre attente, aucun d'entre eux n'a pu différencier substantiellement les lignes inbred ramifiées et non ramifiées dans les trois NIL. Dans le travail de sélection pratique, il est souhaitable de pouvoir identifier seulement les marqueurs positifs associés au génotype ramifié.