# SEARCH FOR RAPD MARKERS ASSOCIATED WITH RECESSIVE BRANCHING GENE *b* IN THREE SUNFLOWER NEAR-ISOGENIC LINES

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## ABSTRACT

**Three pairs of sunflower near isogenic lines (NIL) differing by recessive branching gene *b*, were used to search for RAPD markers linked to the *b* locus, associated with branching. Two strongly amplified markers, E 11-1000bp and A 17-1200bp, were found to differentiate branched and unbranched genotypes. If confirmed by additional study, these markers will make possible to conduct genotypic selection and thus, facilitate the production of branched paternal inbred lines.**


**Key words**: recessive branching, RAPD markers, sunflower

## INTRODUCTION

The inheritance of branching in sunflower is complex, but several 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; Vrânceanu et al., 1985). This recessive branching gene, designated *b*, is often used in sunflower breeding programmes to produce branched paternal inbred lines (Vrânceanu et al., 1985). The branching trait serves to extend flowering time, and thus facilitates pollination in hybridization plots (Vrânceanu et al., 1985).

We have recently converted several single-headed inbred lines into branched plants (Vrânceanu et al., 1985), by phenotypic recurrent selection. Although this approach has been successful, it requires evaluation of nearly mature plants prior to selection and thus is labor, space and time intensive. Genotypic recurrent selection represents a more efficient alternative strategy because a progeny genotype test forms the basis of selection. Implementation of this strategy requires the availability of genetic markers that are closely linked to the *b* gene. Unfortunately, appropriate markers are not yet available for sunflower.

This paper presents the results of a search for molecular markers associated with the *b* gene in sunflower. We chose to work with random amplified polymorphic DNA (RAPD) markers since this method provides a large number of molecular markers with minimal marker development time. RAPDs are efficiently assayed, inexpensive, and highly reproducible if reaction conditions are held constant (Williams et al., 1990). This approach is now well accepted as a valuable tool for genetic mapping (Mouzeyar et al., 1995; Qiullet et al., 1995; Rieseberg et al., 1993), the rapid identification of markers linked to traits of interest (Braham and Friedt, 1995; Rafalski et al., 1996), genetic fingerprinting (Dehmer and Friedt, 1992; Mosges and Friedt, 1994; Weising et al., 1995), and diagnostic application (Weising et al., 1995).

## MATERIALS AND METHODS

The conversion of single-headed inbred lines into branched plants (Vrânceanu et al., 1985) resulted in the releasing of several pairs of near isogenic lines (NILs) with and without the *b* gene. Three of these lines, representing the end products of seven up to eight generations of backcrossing and selection, were chosen for study: VF-1721, VF-1721b BC6, A-1566, A-1566b BC8, SP-4559, SP-4559b BC6. Achenes from each pair of lines were planted in the greenhouse, and fresh leaves were used for DNA extraction.

### DNA isolation

One gram of fresh leaf tissue was ground to fine powder in liquid nitrogen, using a mortar and pestle. The powdered tissue was mixed with extraction buffer, following the protocol described by Bervillé (Faivre-Rampant et al., 1989), modified by increasing the proportion of CTAB to 3% and extracting with chloroform: isoamyl alcohol 24:1. The DNAs were purified using a EluQuic Kit (Shleicher & Schulle) and redissolved in TE (100 mMTris,

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0.1mM EDTA, pH 7.5).

### PCR primers, reaction conditions and gel electrophoresis

A hundred and twenty 10 mers oligodeoxynucleotide primers were used to amplify DNA from two replicates of each pair of NILs. The primers were obtained from Operon Technology (A,B,C,D,K,F,M,U,X) and British Columbia Biotechnology Laboratory (sets: 200 and 500).

RAPD amplifications were performed in 25 μL volumes using 10 ng DNA template, 2mM MgCl2, 20mM Tris HCl ph 8.4, 100 mM each dNTP, 15ng 10 mers primer, and 0.5 units *Taq* DNA polymerase. Reactions were overlaid by mineral oil (one drop) 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, followed by a seven minute extension at 72°C. Amplification products were separated by electrophoresis on 1.5% TBE agarose gels, and detected by staining with ethidium bromide 10 μg/mL.

## RESULTS AND DISCUSSIONS

DNA from three pairs of NILs differing for *b* gene were used as template for amplification with each of 120 RAPDs primers. On an average, seven amplified products per primer were detected. Polymorphic PCR products were produced by 32 (27%) of these primers: Operon Technologies OP-A 8, A 17, A20, B1, C1, C20, D3, D7, D11, D13, E11, E12, E16, E18, E20, F8, F9, M9, U7, U8, U10, U14, U17, U19, X1, X4, X9, X13; University of British Columbia Biotechnology Lab UBC106, UBC156, UBC409, UBC440. Five of these were informative and separated the pairs of NILs according to branching genotype: E 11, A 17, E 12, F 9 and X 1.

The primer E11 generated a 1000bp fragment, which was present in branched genotypes (Figure 1 columns 2 and 4), but absent in unbranched genotypes (Figure 1 columns 1 and 3) for NIL VF 1721. However, this fragment was absent in NIL A-1566 and monomorphic in NIL SP-4559. Likewise, the product A 17-1200bp differentiated branched and unbranched genotypes for NIL VF 1721 (Figure 2 columns 4 and 6). However, amplification pattern for NIL SP 4559 were inconsistent (Figure 2 columns 1, 2 ,7 and 8).

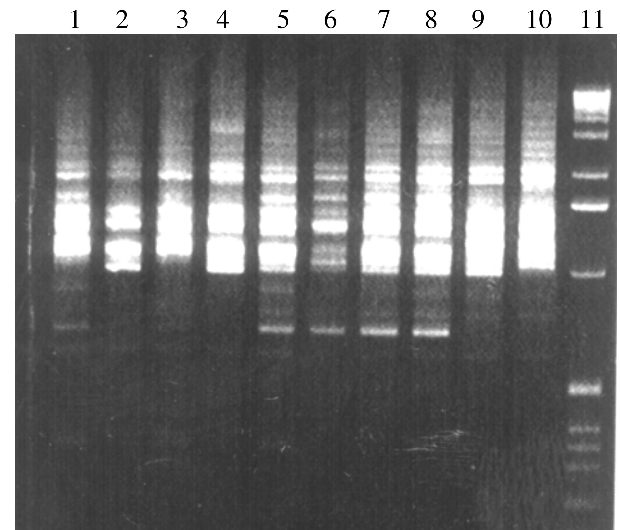


*Figure 1.* Sunflower RAPD markers: OP E11-1000bp (2,4); E11-980bp (9); VF-1721 (1,3); VF-1721b (2,4); A 1566 (6,8); A 1566b (5,7); SP 4559 (9); SP 4559b (10); 1Kb (11) (Indiana University Bloomington)

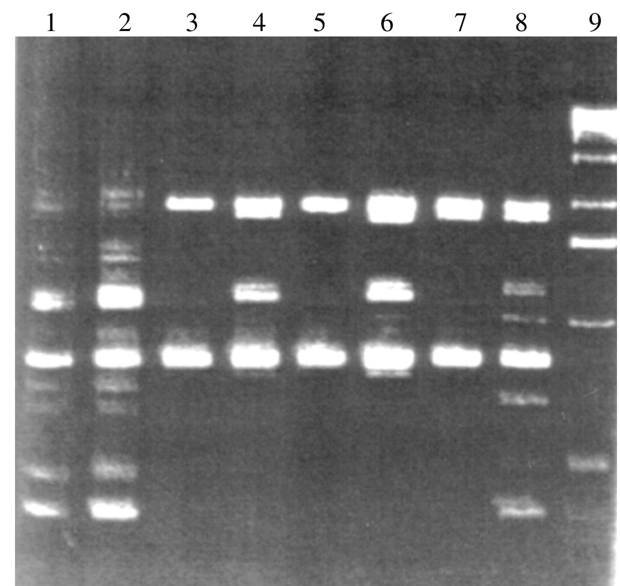


*Figure 2.* Sunflower RAPD markers: OP A17: 800bp, 1200bp, 1800bp (4,6); 376bp and 710bp (8); VF 1721 (3,5); VF 1721b (4,6); SP 4559 (1,7); SP 4559b (2,8); 1Kb (9) (Indiana University, Bloomington)

Similar patterns were observed for RAPD primers E 12 and F 9. RAPD marker E 12-1250bp (Figure 3 columns 2 and 4) and F 9-600bp (Figure 3 columns 3 and 13), are posi-

tively associated with branching genotype in NIL VF 1721. However, NIL SP 4559 and NIL A 1566 are not consistently differentiated by these primers, although some faint bands do appear to separate the branched and unbranched genotypes for SP 4559 (Figure 3 columns 5 and 8).

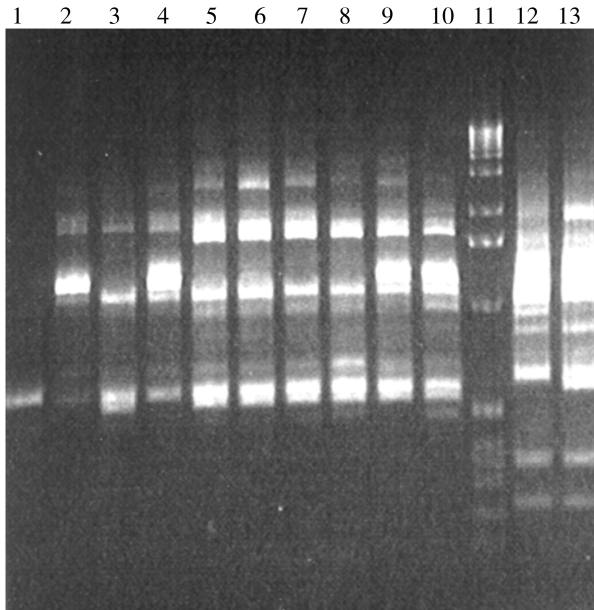


*Figure 3.* Sunflower RAPD, markers: OP E12-1250bp and 2000bp (2,4), 1018bp (10); OP F9-600bp (13); VF 1721 (1,3,12); VF 1721b (2,4,13); A 1566 (6,8); A 1566b (5,7) SP 4559 (9); SP 4599b (10); 1Kb (11), (Indiana University, Bloomington)

Finally, marker X1-984bp is positively associated in NIL SP 4559. No polymorphism were observed for NILs A 1566 (Figure 4 columns 6 and 7).

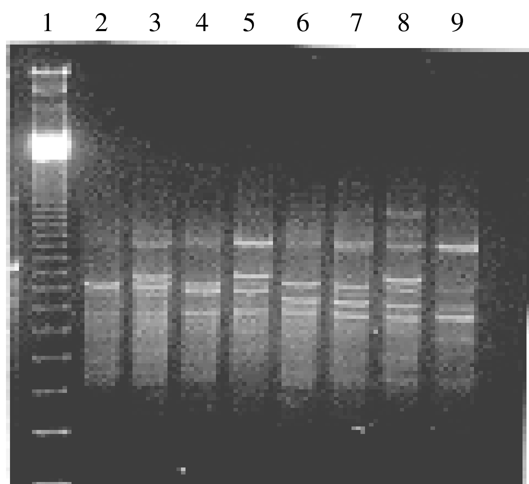


*Figure 4.* Sunflower RAPD markers: OP X1-984bp (3,5), 990bp (8); VF 1721 (2,4); VF 1721b (3,5); A 1566 (6); A 1566b (7); SP 4559 (8), SP 4559b (9), 123bp (1), (Fargo North Dakota)

Unfortunately, none of the informative RAPD markers consistently discriminated between the branched and unbranched inbred genotypes in all three NILs. Nonetheless, some of the RAPD markers found here, such as E11-1000bp and A17-1200bp, may be partially useful for discriminating between branched and unbranched genotypes. In general, only positively associated markers will be useful for selection, since negatively associated markers such as E 11-980 are likely to arise due to drift rather than physical linkage. Likewise, markers with intermediate or variable intensity are unlikely to be use due to high error rates in scoring.

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