Theor Appl Genet (1995) 91: 756–761

N. Foisset · R. Delourme · P. Barret · M. Renard

Molecular tagging of the dwarf *BREIZH* (*Bzh*) gene in *Brassica napus*

Received: 10 March 1995 / Accepted: 17 March 1995

Abstract We mapped the dwarf *Bzh* gene in *B. napus* with RAPD and RFLP markers. Research of the linked markers proceeded in two ways: a random approach through the construction of a detailed genetic map and targeting of the dwarf gene using both near-isogenic lines (NILs) and the bulked segregant analysis (BSA) method. The BSA approach was the most efficient in finding DNA markers linked to *Bzh*, whereas the efficiency of the NILs approach was limited by a too great similarity of the genetic background between the dwarf donor parent and the recurrent lines. Eight RAPD markers were identified as linked to *Bzh*, the closest being at 0.8±0.7 cM. The random genetic mapping approach added markers and extended the linkage group containing *Bzh*. This work represents the first step towards a better understanding of the dwarf mutation, the development of marker-assisted selection, and the cloning of the underlying gene responsible for dwarfing.

Key words *Brassica napus* · Dwarfing gene · Bulked segregant analysis · Near-isogenic lines · Genetic mapping · RAPD markers · RFLP markers

Introduction

Susceptibility to lodging is an important problem in oilseed rape (*Brassica napus* L.) crops which may lead to a significant yield decrease and reduce the final crop dry weight and harvest index (Islam and Evan 1994). During the last few years, the use of plant-growth retardants in oilseed rape fields has been increased due to the planting of some varieties more susceptible to lodging and the availability of new and efficient plant-growth regulator products. Lodging problems might increase in the near future with the orientation of rapeseed breeding towards the development of hybrid varieties; hybrid vigor can lead to a height gain of more than 20 cm in size. Rapeseed breeding for lodging resistance is hampered since this trait is under complex genetic control and its expression is influenced by environmental conditions. This problem could be partially circumvented with the control of plant height by the use of specific dwarfing genes.

Whereas dwarf or semi-dwarf genes have largely been used and studied in species like rice (Liang et al. 1994), barley (Laurie et al. 1993) and wheat (Worland et al. 1994), no dwarfing genetic material has been exploited or proposed in rapeseed breeding other than the spontaneous rapid cycling dwarf mutants used only for physiological studies (Williams and Hill 1986; Zanewich et al. 1991). A few years ago we identified a rapeseed dwarf mutant, obtained from chemical mutagenesis of seeds (MSE 0.50%), in the Primor cultivar, at INRA, Rennes. Genetic analysis of this character revealed that it is under control of a single gene (*Bzh*) with an additive effect, the dwarf plant being *bzh/bzh*. The *bzh* mutated allele was then introduced into many different rapeseed lines by backcrosses and the agronomic performance of the dwarf and semi-dwarf progenies have been compared to that of the recurrent parent. Encouraging results were obtained regarding the level of resistance to lodging as well as yield performance. The final height of the plant, however, was influenced by the genetic background of the recipient line, which complicates the identification of heterozygous plants in segregating progenies. In order to simplify and speed up the introduction of this character in many more breeding lines, and ultimately to gain a better understanding of its function, we initiated molecular tagging of the dwarf *Bzh* gene.

The search for markers linked to the *Bzh* gene was integrated in a *B. napus* mapping project. The mapping population consisted of doubled haploid lines derived from F_1 microspores. This population segregates for different agronomic characters including the dwarf trait. The dwarf gene was targeted using either near-isogenic lines (Young et al. 1988) or the bulked segregant analysis (Michelmore et al. 1991) approach, coupled with a random mapping ap-

Communicated by H. F. Linskens

N. Foisset (✉) · R. Delourme · P. Barret · M. Renard
INRA, Station d'Amelioration des Plantes, BP 29,
35650 Le Rheu, France

proach during the construction of the genetic map (with RFLP and RAPD markers).

In the present paper, we report on the mapping of the linkage group including the *Bzh* gene. The efficiency of the different tagging approaches is also discussed.

Materials and methods

Origin of the *Bzh* gene

The dwarf *Bzh* line, called 'B192', originated from the 'Primor' cultivar through chemical mutagenesis on seeds (MSE 0.50%). 'B192' was first crossed with the 'Jet Neuf' cultivar and the *Bzh* gene was then introduced into different rapeseed lines, such as 'Bienvenu', '2405' and 'Darmor', by backcrossing and selfing.

Mapping population and trait analysis

A doubled haploid (DH) population was derived from isolated microspore cultures (as described by Polsoni et al. 1988) of one F_1 plant from the cross 'Darmor-*bzh*' (B3F3)×'Yudal'. Dwarfism was first scored on primary regenerated H_0 plants by measuring their height (cm) from the ground to the first buds of the main stem at flowering (stage 4.1; Harper and Berkenkamp 1975) in the glasshouse. A correlation between height and the *Bzh* genotype (*Bzh*/*Bzh* or *bzh*/*bzh*) was confirmed during the 93/94 season on 174 H_1 lines produced by self pollination of H_0 plants and arranged in a field experiment with three replicates. The lines were scored at the vegetative stage (stage 3.1; Harper and Berkenkamp 1975) to identify *Bzh*/*Bzh* and *bzh*/*bzh* types. From the DH lines analysed in the field, a total number of 152 were used to construct the genetic map of *B. napus*.

DNA extraction, RAPD and RFLP markers

DNA was extracted by a CTAB procedure (Doyle and Doyle 1990). Procedures for RAPD and RFLP analyses were as described by Quiros et al. (1991) and Landry et al. (1991) respectively. RAPD primers were purchased from Operon and the *Taq* polymerase from Eurobio. RAPD primers are designated by an 'OP' prefix, followed by the kit letter and primer number. RAPD loci are then specified by adding the molecular weight of the corresponding amplified DNA. When RAPD markers are co-dominant, the sufix 'cd1' is added to the name instead of the molecular weight of the two bands. The cDNA probes were kindly provided by B. S. Landry (Agriculture Canada, Quebec). The nomenclature of RFLP loci is similar to that proposed by Landry et al. (1991).

Gene targeting with the near-isogenic lines (NILs)

Three pairs of NILs were used to tag the *Bzh* gene: 'Bienvenu'/'Bienvenu-*bzh*' (B4F4), '2405'/'2405-*bzh*' (B5F5) and 'Darmor'/'Darmor-*bzh*' (B5F4). For each pair of NILs, two pooled DNA samples from eight plants were made (for the dwarf DNA pools, the eight plants were from different F_3 or F_4 mother plant). Segregation analysis was performed on the DH mapping progeny when polymorphism was detected in at least one pair of NILs. However, this was only possible when the marker also segregated between 'Darmor-*bzh*' (B3F) and 'Yudal'.

Gene targeting with the bulked segregant analysis (BSA) approach

DNA bulks were made from the DH lines used to generate the genetic map. DNA samples from eight dwarf and eight normal height DH lines were mixed respectively in two bulks. Each polymorphic locus was first verified on individual DNA samples of the 16 lines of the bulks and complete linkage analyses were then performed on the total mapping progeny when found positive on these 16 DH lines.

Genetic mapping

Linkage analyses were performed using Mapmaker II program (Lander et al. 1987; Lincoln et al. 1992) with linkage criteria of recombination frequency ≤ 0.4 and LOD ≥ 3.0. Goodness of fit to expected Mendelian ratios for each segregating locus was tested by chi-square analysis (α=1%). Arrangements of the linkage groups with a distorted segregation ratio were confirmed by a chi-square test of independance (Mather 1957) and distances were re-estimated with the product formula of Bailey (1949). Centimorgan distances were expressed with the Kosambi function (Kosambi 1944).

Results

Gene targeting with the NILs approach

The three pairs of NILs were simultaneously screened with 128 primers. Fifteen markers were polymorphic between at least one pair of NILs. Eleven of them that segregated on the mapping progeny were found to be false positive and mapped elsewhere in the genome. The three RAPD markers obtained with primers OPP07, OPP08 and OPX12 cannot be confirmed since no segregating progeny from the NILs are available. The remaining marker revealed a DNA band in the *bzh* DNA sample. This polymorphism was detected in only one pair of NILs. Therefore, only one marker (OPM07.730) was confirmed to be linked to *Bzh* (Table 1); OPM07.730 was however tightly linked to *Bzh*.

Gene targeting with the BSA approach

DNA bulks were screened with 306 primers including 100 primers also studied in NILs. They gave 29 products distinguishing the two bulks. Most of them mapped to a region unlinked to *Bzh*. However, eight of them (OPA18.1580, OPB01.990, OPB08.2950, OPC04.1375, OPM07.730, OPW05.2180, OPW08.2120 and OPW09.cd1) were effectively linked to the dwarf locus (Table 1).

Genetic mapping

The genetic map was constructed with 47 RFLP probes and 56 RAPD primers which detected 71 and 112 markers, respectively. Except for the RAPD primers that were selected with the BSA approach, and which identified markers potentially linked to *Bzh*, they were chosen at random from polymorphism studies made on the parents of the progeny ('Darmor-*bzh*'×'Yudal'). The linkage group comprising the *Bzh* gene is 84.5 cM in length and contains five RFLP and 11 RAPD markers (Fig. 1). Segregation distortions ($P \leq 0.01$) were observed at each locus of the group (except 2NH5a), including the *Bzh* locus, and were all skewed towards the alleles of the normal-height parent ('Yudal'). Chi-square values increased progressively from one extremity (2NH5a) with a peak value on 1NG2a (Table 2). These distortions were taken into account and genetic distances re-estimated.

Table 1 Polymorphism of RAPD and RFLP markers belonging to the *Bzh* linkage group and identified through NILs, BSA techniques or genetic mapping. B/B-*bzh*, D/D-*bzh*, 2/2-*bzh* correspond to the three pairs of near isogenic lines ('Bienvenu'/'Bienvenu-*bzh*', '2405'/'2405-*bzh*', 'Darmor'/'Darmor-*bzh*' respectively); Y and D-*bzh* are the parental lines ('Yudal' and 'Darmor-*bzh*') of the genetic map progeny. Markers are listed in the order of their position on the *Bzh* linkage group. /, not studied; + and – symbols correspond to the dominant marker; A and B correspond to alleles of the co-dominant marker

Markers	NILs						BSA		Genetic map	
	B	B-*bzh*	D	D-*bzh*	2	2-*bzh*	Normal bulk	Dwarf bulk	Y	D-*bzh*
OPB07.460	+	+	+	+	+	+	+	+	–	+
1NH6a	/	/	/	/	/	/	/	/	A	B
1NG2a		/	/	/	/	/	/	/	A	B
3NF11a	/	/	/	/	/	/	/	/	A	B
OPP08.cd1	A	A	B	B	A	A	B/A	B/A	A	B
OPW05.750	+	+	–	–	+	+	+	+	+	–
OPA18.1580	+	+	–	–	+	+	+	–	+	–
OPB01.990	–	–	–	–	–	–	+	–	+	–
OPC04.1375	–	–	–	–	–	–	+	–	+	–
OPW05.2180	–	–	–	–	–	–	+	–	+	–
OPB08.2950	–	–	–	–	–	–	+	–	+	–
1NG3b	/	/	/	/	/	/	/	/	A	B
OPM07.730	+	+	+	+	–	+	–	+	–	+
OPW08.2120	+	+	+	+	+	+	–	+	–	+
OPW09.cd1	B	B	B	B	B	B	**A**	**B**	A	B
2NH5a	/	/	/	/	/	/	/	/	A	B

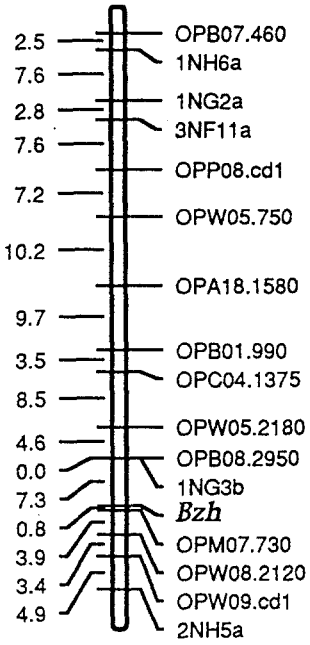

Fig. 1 Linkage group of *Bzh*. Scale in Kosambi cM is shown on the left of the chromosome and loci names are listed on the right

Table 2 Frequency of the over-represented phenotypic class for each distorted locus of the *Bzh* linkage group and the corresponding chi-square value. Loci marked * deviated significantly from a 1:1 ratio at $P<0.01$. Markers are listed in the order of their position on the linkage group

Marker	Frequency of 'Yudal' allele	Chi-square value
OPB07.460	69.4%	19.41*
1NH6a	73.9%	26.35*
1NG2a	81.8%	34.38*
3NF11a	77.4%	33.43*
OPP08.cd1	72.8%	27.36*
OPW05.750	73.4%	30.46*
OPA18.1580	71.7%	26.51*
OPB01.990	69.1%	21.38*
OPC04.1375	69.2%	18.47*
OPW05.2180	64.1%	10.07*
OPB08.2950	64.7%	12.33*
1NG3b	65.5%	10.56*
Bzh	63.3%	10.14*
OPM07.730	63.3%	9.82*
OPW08.2120	63.1%	9.69*
OPW09.cd1	63.2%	8.69*
2NH5a	56.5%	1.18, no distortion

The *Bzh* gene is surrounded by seven co-dominant markers (5 RFLP and 2 RAPD) and nine dominant RAPD markers; two of them revealed a band in coupling with the dwarf allele. Figure 2 shows the amplification of OPW09.cd1, a co-dominant RAPD marker. The nearest flanking marker, called OPM07.730 (Fig. 3), was linked at 0.8 cM to *Bzh*. The furthermost and nearest flanking markers revealed by each method used to tag *Bzh* are indicated in Fig. 4.

Discussion

The NILs approach, proposed by Young et al. (1988) to target specific genes, has been widely and successfully used particularly for genes controlling disease resistance as reviewed by Lefebvre and Chevre (1995). Another approach, called BSA, has been proposed more recently by Michelmore et al. (1991) and is becoming widely applied (Barua et al. 1993; Kesseli et al. 1993; Chaparro et al. 1994; Delourme et al. 1994). Both methods were used here with RAPD markers in order to rapidly find DNA markers tightly linked to a dwarfing gene mutation named *Bzh*.

The NILs approach was, however, slightly modified in its concept since the DNA of eight nearly isogenic plants was pooled. This was done in order to reduce the probability of false positive detection taking advantage of DNA pooling to obtain a randomized genetic background of unlinked loci. This also might reduce the length of the targeted segment to a region common to the eight dwarf pooled

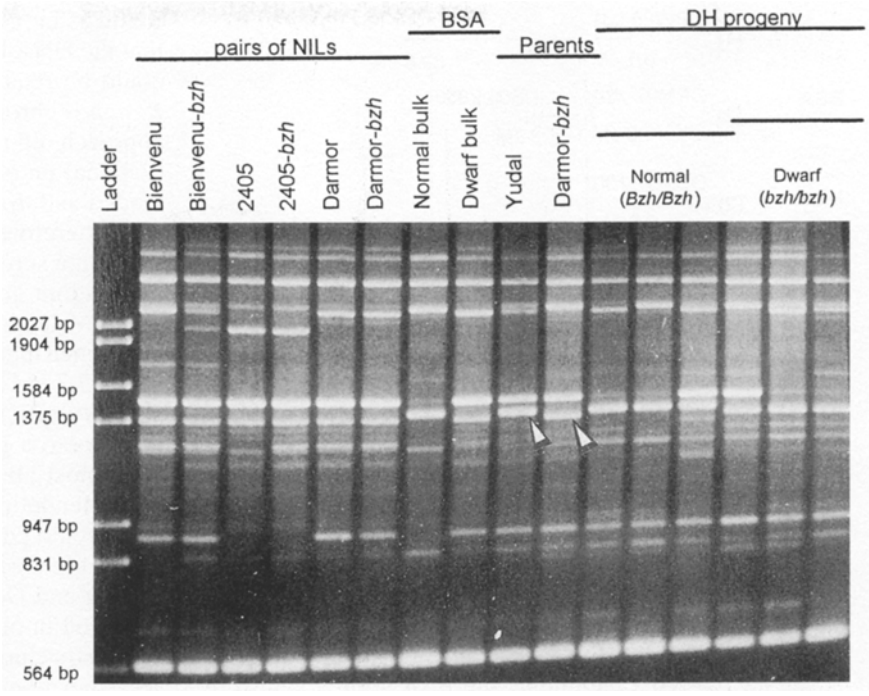

Fig. 2 RAPD patterns for the primer OPW09 on NILs, BSA and DH progeny from the cross 'Darmor-*bzh*' 'Yudal'. The *arrows* indicate the position of the alleles of the co-dominant marker OPW09.cd1 which is linked to the *Bzh* gene at 8.1 cM

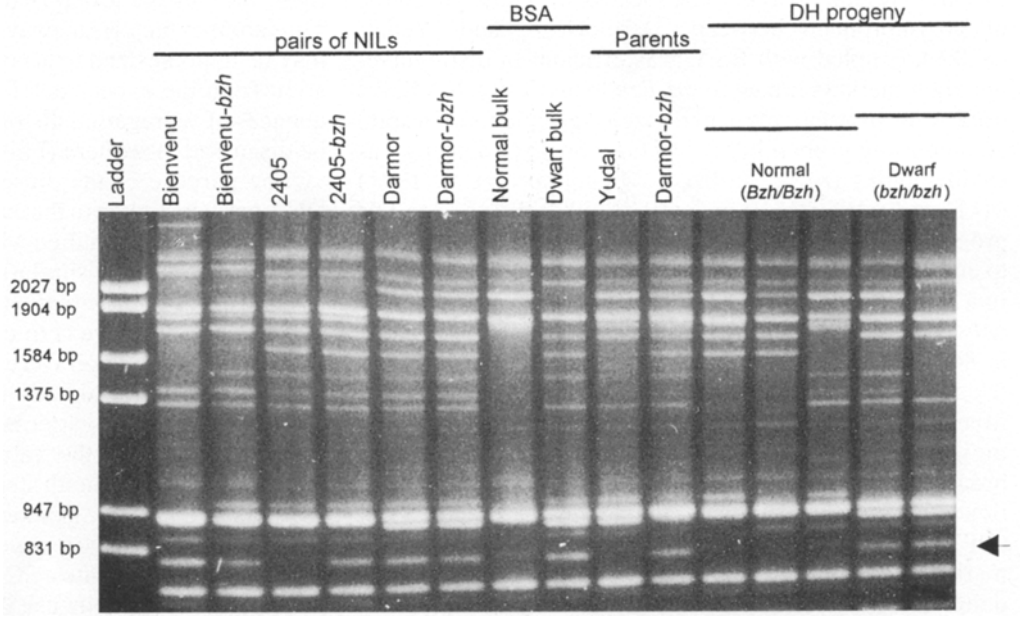

Fig. 3 RAPD patterns for the primer OPM07 on NILs, BSA and DH progeny from the cross 'Darmor-*bzh*' 'Yudal'. The arrow indicates the position of the marker OPM07.730 which is linked to *bzh* at 0.8 cM

plants. However, by this method the goal is only reached when the dominant allele comes from the recurrent parent.

Except for one marker, OPM07.730, which was also the nearest flanking marker to the *Bzh* locus, all the positive markers identified with the BSA approach failed to be confirmed with the NILs approach. The low frequency of success with NILs is in fact due to a too great similarity of the genetic background between the donor parent and the recurrent lines (the donor parent and the recurrent lines originated from a common genetic base). This has already been indicated as an important limiting factor in the use of near-isogenic lines for gene tagging (Muehlbauer et al. 1988; Young et al. 1988). It has been theoretically demonstrated that the target gene would be flanked by an introgressed segment extending about 5 cM in both directions even after 20 backcross generations (Stam and Zeven 1981). The fact that OPM07.730 was detected on only one pair of NILs ('2405'/'2405-*bzh*'), although its dominant allele is linked in coupling to the dwarf allele and at a very short distance from *Bzh* (0.8 cM), is a clear demonstration of the low level of divergence between the donor and recurrent genomes. This is confirmed by the results of the BSA method.

Three other primers gave one polymorphic band in one pair of NILs. They still remain potential markers but were

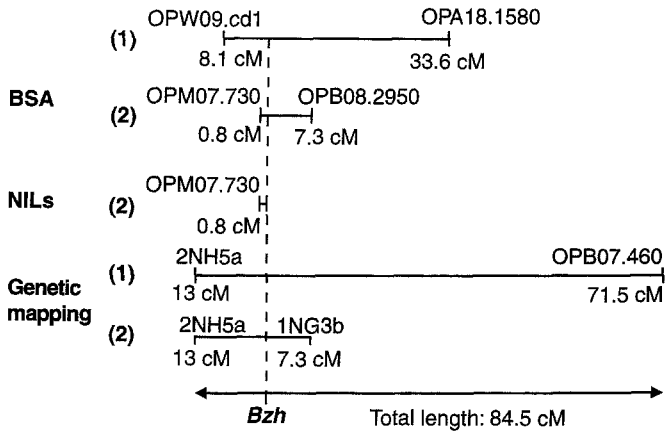

Fig. 4 Furthermost (*1*) and nearest (*2*) flanking markers revealed by the different methods used to tag the *Bzh* gene

not verified since they did not segregate in the DH progeny. Unfortunately no segregating progeny from the NILs was available.

As for the NILs technique, the BSA approach also requires a good genetic divergence between both parents in the target region in order to be successful. The high degree of polymorphism between 'Darmor-*bzh*' and 'Yudal' (>70%), coupled with BSA, was efficient in distinguishing eight markers linked to the *Bzh* locus. The DNA bulks used were of relatively small size (eight plants). Despite an increasing probability (p) of false positive detection as smaller bulks (n) are utilized (Michelmore et al. 1991) (n=15 p=6×10^{-5}; n=10 p=2×10^{-3}; n=8 p=8×10^{-3} on DH progeny), bulks of eight plants were constructed in order to ensure a maximum chance of detection (p') of a marker in a large chromosomal area (r=recombination frequency between the marker and the targeted locus; r=0.1 n=8/p'=0.43 n=15/p'=0.21; r=0.2 n=8/p'=0.17 n=15/p'=0.04 on DH progeny). A number of 21 false positives were effectively obtained with the BSA method; those bands mapped to other linkage groups. The BSA approach was efficient in identifying eight markers positioned around the *Bzh* locus, the nearest being at 0.8 cM (OPM07.730) and the furthermost at 33.6 cM. One RAPD marker was co-dominant (OPW09.cd1). The others were dominant with two of them (OPM07.730 and OPW08.2120) having a DNA band associated with the dwarf allele. Michelmore et al. (1991) showed that markers can be reliably identified in a 25-cM window on either side of the target locus, as a function of bulk size and with a decreasing sensitivity as genetic distances increase. In the present study, one marker was detected as far as 33.6 cM from the dwarf gene (OPA18.1580). Michelmore et al. (1991), however, indicated that the sensitivity of BSA may vary with the sequence amplified and the segregating population used. They also assumed that if bulks are made with enough individuals, the genetic window detected will be symmetrical around the target. In the present case, no marker was identified at more than 8.1 cM (OPW09.cd1) on one side whereas, as mentioned above, markers were regularly found as far as 33.7 cM on the other side. Despite the fact that the DNA bulks were of small size, this disequilibrium could be related to the position of the *Bzh* locus on the *B. napus* chromosome since the random genetic mapping approach did not identify any markers further than 13 cM (2NH5a) on one side, whereas the linkage group was extended as far as 71.5 cM on the other side of the *Bzh* locus. Therefore, we hypothezise that the *Bzh* locus is located in a sub-telomeric region.

Random genetic mapping on the DH progeny gave a 84.5-cM-length linkage group containing the *Bzh* gene and positioned markers at regular intervals. The closest marker was at 7.3 cM (1NG3b) which confirmed that BSA or NILs are very efficient methods for identifying tightly linked markers to a gene of interest. Distortions were observed for almost all markers in favor of the alleles of 'Yudal'. Non-Mendelian segregations are commonly observed on androgenic DH progeny, whatever the species, for a relatively high percentage of markers studied (reviewed in Foisset and Delourme 1995). The segregation distortions observed in our study were not related to the *Bzh* mutant allele since normal segregation of this gene has been observed in other crosses and also in view of the fact that distortions increase from the *Bzh* region to the other extremity of the linkage group (with a peak value near 1NG2a). Thus another factor, far away from *Bzh* and close to 1NG2a, may be hypothesized to account for the origin of the deviation from the expected 1:1 Mendelian ratio. The consequences of segregation distortions in genetic mapping will be discussed elsewhere (Foisset et al., in preparation).

The purpose of the present study was to rapidly find DNA markers linked to the dwarf *Bzh* gene. This succeeded well with the BSA method whereas the NILs approach was limited by a too great similarity of the genetic backgrounds of the dwarf donor parent and the recurrent lines.

Our next objective is to continue saturation of the *Bzh* locus with more markers on each side of the gene. The nearest RAPD markers already identified have been assayed on a set of rapeseed varieties (data not shown) in order to obtain estimates of the value of these markers in other crosses since one difficult step is the transfer of useful polymorphisms from the genetic map to breeding programs. With this perspective in mind, RAPD marker OPM07.730 will be transformed into a SCAR marker (Paran et al. 1991) in order to facilitate its use in other lines.

This study is also the first step towards the development of marker-assisted selection and provides a starting point to clone the *Bzh* gene of *B. napus*.

Acknowledgements The authors thank A. M. Chèvre and B. S. Landry for their critical review of the manuscript. The work was supported by G. I. E. PROCOLZA and SERASEM. N. Foisset was supported by a grant from INRA-PROMOSOL.

References

Bailey NTJ (1949) The estimation of linkage with differential viability. II and III. Heredity 3:220–228

Barua UM, Chalmers KJ, Hackett CA, Thomas WTB, Powell W, Waugh R (1993) Identification of RAPD markers linked to a

Rhynchosporium secalis resistance locus in barley using near-isogenic lines and bulked segregant analysis. Heredity 71:177–184

Chaparro JX, Werner DJ, O'Malley D, Sederoff RR (1994) Targeted mapping and linkage analysis of morphological isozyme, and RAPD markers in peach. Theor Appl Genet 87:805–815

Delourme R, Bouchereau A, Hubert N, Renard M, Landry BS (1994) Identification of RAPD markers linked to a fertility restorer gene for the *Ogura* radish cytoplasmic male sterility of rapeseed (*Brassica napus* L.). Theor Appl Genet 88:741–748

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissues. Focus 12:13–15

Foisset N, Delourme R (1995) Segregation distortion in androgenic plants. In: Mohan Jain S, Sopory SK, Veilleux RE (eds) In vitro haploid production in higher plants. Chapter 9, vol 2. Kluwer Academic, Dordrecht, Netherlands (in press)

Harper FR, Berkenkamp B (1975) Revised growth-stage key for *Brassica campestris* and *B. napus*. Can J Plant Sci 57:657–659

Islam N, Evan EJ (1994) Influence of lodging and nitrogen rate on the yield and yield attibutes of oilseed rape (*Brassica napus* L.). Theor Appl Genet 88:530–534

Kesseli R, Witsenboer H, Stanghellini M, Vandermark G, Michelmore R (1993) Recessive resistance to *Plasmopara lactucae-radicis* maps by bulked segregant analysis to a cluster of dominant disease resistance genes in lettuce. Mol Plant-Microbe Interact 6:722–728

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eug 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg, I (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Landry BS, Hubert N, Etoh T, HaradaJJ, Lincoln SE (1991). A genetic map for *Brassica napus* based on restriction fragment length polymorphisms detected with expressed DNA sequences. Genome 34:543–552

Laurie DA, Pratchett N, Romero C, Simpson E, Snape JW (1993) Assigment of the *denso* dwarfing gene to the long arm of chromosome 3(3H) of Barley by the use of RFLP markers. Plant Breed 111:198–203

Lefebvre V, Chèvre AM (1995) Tools for marking plant disease and pest resistance genes: a review. Agronomie 15:3–19

Liang CZ, GU MH, Pan XB, Liang GH, Zhu LH (1994) RFLP tagging of a new semidwarfing gene in rice. Theor Appl Genet 88:898–900

Lincoln S, M Daly, E Lander (1992) Constructing genetic maps with MAPMAKER/EXP 3.0. Whitehead Institute Techn Rep 3rd edn.

Mather K (1957) The measurement of linkage in heredity. Methuen, London

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Muehlbauer GJ, Specht JE, Thomas-Compton MA, Staswick PE, Bernard RL (1988) Near-isogenic lines – a potential resource in the integration of conventional and molecular marker linkage maps. Crop Sci 28:729–735

Paran I, Kesseli R, Michelmore R (1991) Identification of restriction-fragment-length-polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce, using near-isogenic lines. Genome 34:1021–1027

Polsoni L, Kott LS, Beversdorf WD (1988) Large scale microspore culture technique for mutation-selection studies in *Brassica napus*. Can J Bot 66:1681–1685

Quiros CF, Hu J, This P, Chevre AM, Delseny M (1991) Development and chromosomal localization of genome-specific markers by polymerase chain reaction in *Brassica*. Theor Appl Genet 82:627–632

Stam P, Zeven AC (1981) The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing. Euphytica 30:227–238

Williams PH, Hill CH (1986) Rapid cycling populations of *Brassica*. Science 232:1385–1389

Worland AJ, Sayers EJ, Börner A (1994) The genetics and breeding potential of *Rht12*, a dominant dwarfing gene in wheat. Plant Breed 113:187–196

Young, ND, Zamir D, Ganal MW Tanksley SD (1988) Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the *TM-2a* gene in tomato. Genetics 120:579–585

Zanewich KP, Rood SB, Southworth CE, Williams PH (1991) Dwarf mutants of *Brassica*: responses to applied gibberellins and gibberellin content. J Plant Growth Regul 10:121–127