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Mapping of RFLP and qualitative trait loci in *Brassica rapa* and comparison to the linkage maps of *B. napus*, *B. oleracea*, and *Arabidopsis thaliana*

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Abstract A linkage map of restriction fragment length polymorphisms (RFLPs) was constructed for oilseed, *Brassica rapa,* using anonymous genomic DNA and cDNA clones from *Brassica* and cloned genes from the crucifer *Arabidopsis thaliana*. We also mapped genes controlling the simply inherited traits, yellow seeds, low seed erucic acid, and pubescence. The map included 139 RFLP loci organized into ten linkage groups (LGs) and one small group covering 1785 cM. Each of the three traits mapped to a single locus on three different LGs. Many of the RFLP loci were detected with the same set of probes used to construct maps in the diploid *B. oleracea* and the amphidiploid *B. napus*. Comparisons of the linkage arrangements between the diploid species *B. rapa* and *B. oleracea* revealed six LGs with at least two loci in common. Nine of the *B. rapa* LGs had conserved linkage arrangements with *B. napus* LGs. The majority of loci in common were in the same order among the three species, although the distances between loci were largest on the *B. rapa* map. We also compared the genome organization between *B. rapa* and *A. thaliana* using RFLP loci detected with 12 cloned genes in the two species and found some evidence for a conservation of the linkage arrangements. This *B. rapa* map will be used to test for associations between segregation of RFLPs, detected by cloned genes of known function, and traits of interest.

Key words *Arabidopsis* · *Brassica* · Comparative maps Erucic acid · Restriction fragment length polymorphism (RFLP)

Introduction

The genus *Brassica* includes both diploid and amphidiploid species with a variety of different morphotypes. *B. rapa* (n=10, AA genome) is one of the diploid species and includes the vegetables turnip, Chinese cabbage, pak choi, and the oilseeds turnip rape and sarson. *B. rapa* is closely related to the diploid *B. oleracea* (n=9, CC) (Song et al. 1988; McGrath and Quiro 1991; Warwick and Black 1991) and these two species are the hypothesized progenitors (U 1935) of the amphidiploid *B. napus* (n=19, AACC). However, organellar restriction fragment length polymorphism (RFLP) data from a recent study suggested that *B. montana* (n=9), or a close relative, may be the maternal parent of most oilseed *B. napus* types as well as the progenitor of *B. rapa* and *B. oleracea* (Song and Osborn 1992).

Additional information on the relationships of *Brassica* genomes could be obtained by comparing linkage maps of RFLPs. This approach has been used to compare the genomes of tomato and potato (Bonierbale et al. 1988), maize and sorghum (Hulbert et al. 1990), and as well as rice, maize and wheat genomes (Ahn and Tanksley 1993; Ahn et al. 1993). Genetic linkage maps of RFLPs have been provided for *B. oleracea* (Slocum et al. 1990; Kianian and Quiros 1992; Landry et al. 1992; Camargo 1994), *B. rapa* (Song et al. 1991; Chyi et al. 1992), and *B. napus* (Landry et al. 1991; Ferreira et al. 1994). Detailed RFLP linkage maps also have been generated for the related cruciter *Arabidopsis thaliana* (Chang et al. 1988; Hauge et al. 1993). Since *A. thaliana* has been chosen as a model system for investigating questions in plant biology a great deal of information is available for this species. It should be possible, therefore, to incorporate cloned genes from *A. thaliana* into *Brassica* maps and then look for associations between the segregation of RFLPs detected by genes and traits of interest using QTL (quantitative trait locus) analysis. This will provide information on the potential function(s) of specific genes in traits of interest and make it possible to use advances in *A. thaliana* for *Brassica* crop improvement.

This paper reports on an RFLP linkage map for an oilseed, *B. rapa,* population and the mapping of genes controlling simply inherited traits. It also provides the first reported comparisons among *B. rapa*, *B. napus,* and *B. oleracea* RFLP linkage maps constructed with a set of the

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same DNA probes. Cloned genes previously mapped in *A. thaliana* were used to detect homologous RFLPs for the *B. rapa* map, also allowing preliminary comparisons between these genomes.

Materials and methods

Plant materials

Single plants of the *B. rapa* biennial cultivar Per and annual cultivar R500 were selected as the female and male parent, respectively. These parents were self-compatible and differed for morphological and seed quality traits. F_1 hybrids were made by bud emasculation and a single F_1 plant was selected and self-fertilized to produce an F_2 population of approximately 100 plants. Each F_2 plant was self-pollinated to produce seed for F_3 families. For seed production, plants of Per, F_1, and F_2 were first vernalized for 6 weeks in a cold room at 5°C with a 16-h photoperiod from fluorescent bulbs and then transferred to the greenhouse. All plants flowered under these conditions. For DNA isolation, plants from self-pollinated seed of the parents, the F_1 hybrid, and from F_3 families, were grown in 12-packs (Com-Pack) for 2–3 weeks. Young leaves were harvested and bulked from 24 individuals in each of 91 F_3 families. The leaves were kept on ice during the harvest and then lyophilized.

DNA was extracted from the lyophilized tissue following the CTAB procedure as described by Kidwell and Osborn (1992). Five or ten micrograms of DNA digested with either *Hin*dIII, or *Eco*RI or *Eco*RV were electrophoresed on 1% agarose gels. Southern blots of these gels were prepared by neutral transfer onto Magna NT nylon membranes (MSI, Westboro, Mass.). DNA was fixed to the membrane through UV crosslinking (Stratagene UV Stratalinker 2400) followed by 2 h in a 90°C vacuum oven. DNA probes were radiolabeled by random priming in the presence of ^{32}P-dCTP (Feinberg and Vogelstein 1983) and then hybridized to the Southern blots. The details of the method of RFLP analysis are described by Osborn et al. (1987), except that the blots were hybridized in rotating glass bottles at 60°C. The stringency of the final wash varied from 0.1 to 0.4×SSC (all at 60°C), depending on the homology of the probe with the *B. rapa* DNA (0.2×–0.3 × for *A. thaliana* probes, 0.2×–0.4 × for *S. oleracea* probes). After the probed blots were exposed to X-ray film, the blots were treated with 0.2 M NaOH to remove the probe and then neutralized in 0.5 M Tris/HCl, 0.1 × SSC, 0.1% SDS (pH 7.5). These blots then were re-probed up to 20 times.

The majority of the probes were from three libraries: (1) a *Pst*I genomic DNA library (WG clones) and (2) a cDNA library (EC clones) from the *B. napus* cultivar Westar (Thormann et al. 1994), and (3) an *Eco*RI genomic DNA library from the *B. rapa* cultivar Tobin (TG clones) that was constructed and screened essentially as described for the Westar genomic DNA library. Probes from these libraries were screened by hybridization to blots containing DNA from the parental cultivars digested with *Hin*dIII and *Eco*RI. Cloned genes and heterologous probes were also screened for their detection of polymorphisms between the parental cultivars using *Hin*dIII, *Eco*RI, and *Eco*RV.

Map construction

For each F_3 family, co-dominant RFLP loci were scored as homozygous for Per alleles, homozygous for R500 alleles, or heterozygous. Dominant loci were scored for the presence or absence of one of the parental alleles. The RFLP loci were named after the probe used for hybridization. Probes which hybridized to multiple RFLP loci were named identically, but were given the suffix 'a' or 'b'. Only probes which provided genotype data for at least 70% (64) of the 91 F_2 plants were included in the mapping data set. Segregation ratios for each locus were compared using Chi-square analysis, to Mendelian ratios expected for an F_2 population.

Map construction was performed using the MAPMAKER program (Lander et al. 1987) for the Macintosh v2.0 (supplied by Scott Tingey, DuPont, Co.). The map was constructed with a recombination fraction maximum of 0.3 and a LOD threshold minimum of 3.0. Two-point estimates were used to designate preliminary linkage groups, and multipoint analyses were used for determination of the most-likely locus orders. These initial locus orders were used to identify individuals scored as having double-crossover events and then the autoradiographs were re-checked for genotype scoring errors for these same individuals. After correcting scoring errors, the loci were re-ordered and checked again using the 'ripple' command (LOD difference threshold 4.0) of the MAPMAKER/EXP 3.0b program (Lincoln et al. 1992) which re-orders overlapping groups of five loci consecutively down the linkage group. The final locus order for each group was determined by comparing the likelihood of the different map orders suggested by the 'ripple' command. Map distances in centimorgans (cM) were calculated from recombination frequencies using the Kosambi mapping function.

Trait measurements

The parents, F_1, and F_3 families were measured for three simply inherited traits. Seed color of the F_3 seed was determined from visual inspection of lots of 24 seeds from each family. Only seeds which were pure yellow were scored as 'yellow', while all dark seeds were scored as 'brown'.

Erucic acid content in the F_3 seed was determined for lots of 24 seeds per family. Briefly, lipids were extracted with heptane, transmethylated with 0.5 N sodium methoxide in methanol, and then compositions were determined using a Hewlett-Packard 3890 gas chromatograph with an FID detector (modified from Christie 1982).

Lots of 12 plants from each F_3 family were scored for the presence of hairs on the leaf surface (pubescence) 3 weeks after planting. The families were scored as either all individuals having leaf hairs, all individuals lacking hairs, or with the family segregating for presence of leaf hairs.

Results

Probe selection

Thirty percent of 573 WG clones, 43.4% of 122 TG clones, and 31.5% of 308 EC clones, detected polymorphisms between the parental DNAs cut with *Eco*RI; 29.5% of WG, 50.8% of TG, and 37.3% of EC clones detected polymorphisms for DNAs cut with *Hin*dIII; and 38.9% of WG, 59.8% of TG, and 44.5% of EC gave polymorphisms for either *Eco*RI or *Hin*dIII. A subset of clones (48 WG, 12 TG, 25 EC) were selected as mapping-probes because they detected fairly simple polymorphic patterns between the *B. rapa* parents and between the parents of other *Brassica* mapping populations. Thirty-one cloned genes hybridized to RFLPs between the two parents and were used as mapping probes (Table 1).

Map characteristics

A total of 144 segregating RFLP loci were scored from hybridizations with 116 probes. Of these, 139 marker loci

Table 1 Summary of cloned genes from the three species used to map RFLP loci in the *B. rapa* 'Per' × 'R500' F_2 population

Probe name	Type[a]	Gene encoded	Reference
Arabidopsis thaliana			
Arab. PR-2	c	β-1,3-glucanase	Uknes et al. 1992
Athsp21	c	Heat shock protein	Chen and Vierling 1991
AtUBC13	c	Ubiquitin-conjugating enzyme	Van Nocker and Vierstra 1991
AtUBC3	c	Ubiquitin-conjugating enzyme	M. Sullivan (personal communication)
COR6.6	c	Cold-regulated	Hajela et al. 1990
COR15	c	Cold-regulated	Hajela et al. 1990
COR47	c	Cold-regulated	Hajela et al. 1990
COR78	c	Cold-regulated	Hajela et al. 1990
pCT4.2	c	5s rDNA	Campell et al. 1992
DHS2	c	DAHP synthases	Keith et al. 1991
EZ3	g	Subclone of cosmid g8261	H. Goodman (personal communication)
GAP-A, GAP-B, GAP-C	c	Glyceraldehyde-3--phosphate dehydrogenase	Shih et al. 1991
GS-KB6	c	Glutamine synthase	Peterman and Goodman 1991
pJ5-3	c	Lipid transfer protein	Thoma et al. 1993
m456	g	Random clone	Chang et al. 1988
PG11	c	Random clone	Hauge et al. 1993
PG26	c	Random clone	H. Goodman (personal communication)
pCSODRH	c	Superoxide dismutase	Hindges and Slusarenko 1992
TMK-1	c	Transmembrane kinase	Chang et al. 1992
tt4/pCHS3.8	c	Chalcone isomerase	Feinbaum and Ausubel 1988
U2R9	c	U2.9 snRNA	Vankan and Filipowicz 1988
UBQ4	c	Polyubiquitin	Burke et al. 1988
Brassica napus			
BN9	c	Stearoyl ACP desaturase	Slocombe et al. 1992
BN59	c	Cold-induced ATPase	Orr et al. 1993
BNC24A	c	Cold-induced	Saez-Vasquez et al. 1994
pC1	c	Cruciferin	Simon et al. 1985
PEP/4	c	Phosphoenol-pyruvate--carboxykinase	M. Delseny (personal communication)
Spinacia oleracea			
pWB2	c	Lipid transfer protein	Bernhard et al. 1991
pVA2	c	Glycolate oxidase	Volokita and Somerville 1987

[a] The type of probe was either cDNA (c) or genomic DNA clone (g)

were assembled into ten linkage groups with eight or more loci and one additional group (A) containing just three loci (Fig. 1). The total map distance was 1785 cM with an average distance between loci of 13.5 cM. Five loci were unlinked. The mapped loci included 121 scored as having codominant alleles and 23 scored as having dominant alleles (16%) with the Per fragment absent for 13 loci and the R500 fragment absent for ten loci. Although a single segregating locus was scored for most probes, two loci were scored for each of 28 of the 116 probes (24%). Some probes hybridized to additional bands, but were not scored for greater than two loci due either to ambiguous banding patterns or to the faintness of the additional bands. Many of the loci (23%) had segregation ratios which deviated significantly ($P < 0.05$) from the Mendelian ratios expected for an F_2 population (Fig. 1). Of the 33 loci with distorted segregation ratios, 30% had an abundance of R500 alleles, 40% were skewed towards the Per allele, while 30% had an excess of heterozygotes.

The 24 *A. thaliana* probes (Table 1) detected 29 segregating loci in *B. rapa* (Fig. 1). These loci were distributed throughout the *B. rapa* genome, although some loci mapped close to each other. For example, COR15 and COR78 detect RFLPs only 14 cM apart on LG 3, while PG11 and TMK-1 mapped 13.1 cM apart on LG 7. The two cDNAs from *Spinacia oleracea* and four cDNAs from *B. napus* (Table 1) each detected one segregating locus, and each of the loci mapped to a different LG (Fig. 1).

Loci controlling three simply inherited traits were included in the map (Fig. 1). Leaf hairs (pubescence) were present in Per, absent in R500, and present in the F_1. This trait segregated in a 1(presence):2(segregating): 1 (absence) ratio among the F_3 families. The locus controlling presence or absence of leaf hairs (*Pub*1) mapped to LG 4. Erucic acid was absent in Per seeds, R500 seeds had greater than 50% erucic acid, while the F_1 was intermediate between the two levels. The F_3 families were grouped into categories of undetectable, intermediate, and high levels

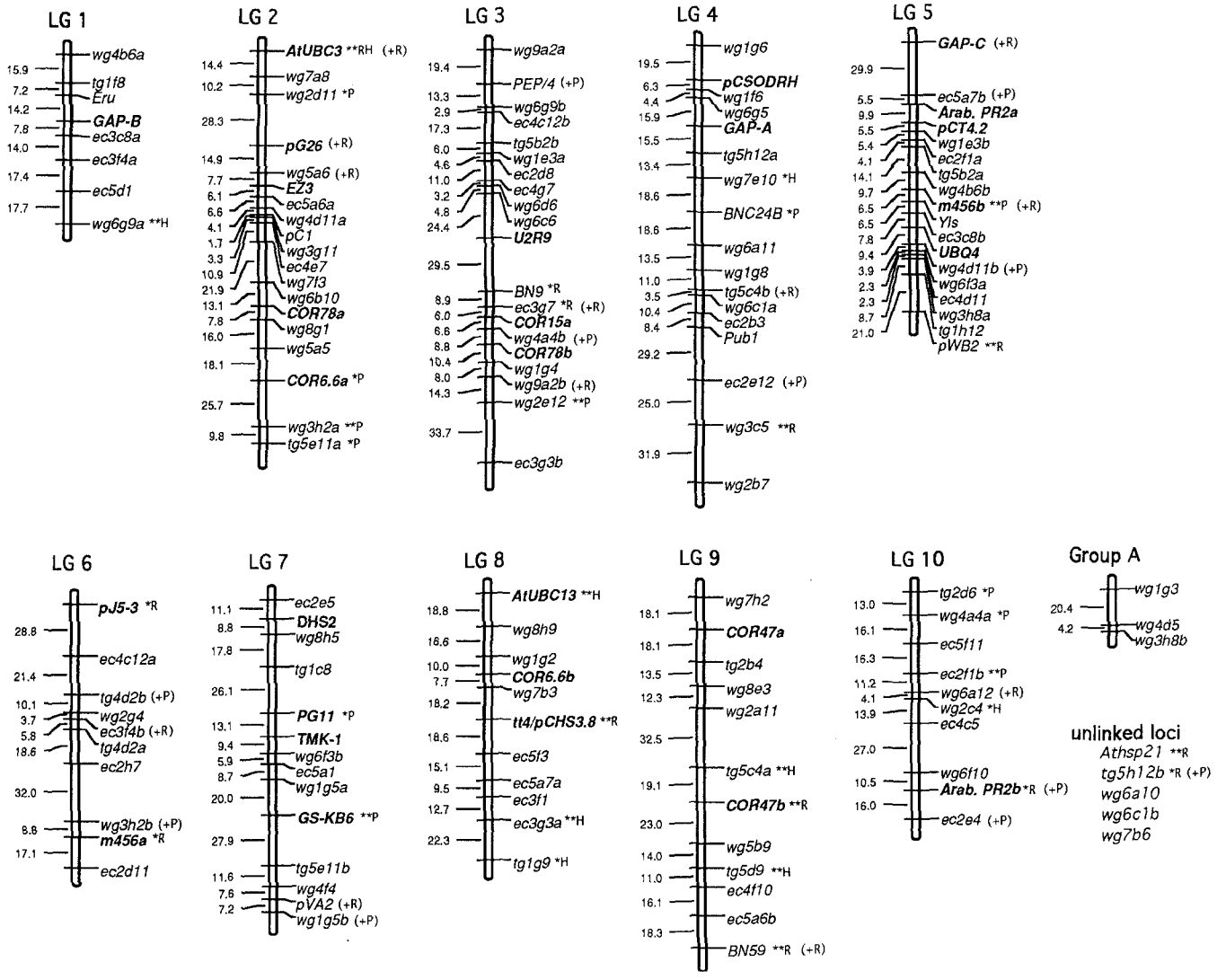

Fig. 1 RFLP linkage map for the *B. rapa* Per × R500 F_2 population. Map distances in cM are indicated on the left side of linkage groups and locus names on the right. RFLP loci detected with DNA probes from *A. thaliana* are indicated with *bold type*. Loci with segregation ratios significantly distorted from Mendelian ratios are indicated with asterisks (* for $p < 0.05$, ** for $p < 0.01$). The allele in excess is indicated after the asterisks (*P*=Per, *R*=R500, *H*=heterozygote). (+*P*) and (+*R*) indicate loci scored as having dominant alleles, with the dominant allele coming from Per or R500, respectively

of erucic acid and segregated in a 1:2:1 ratio. The locus (*Eru*) controlling the presence or absence of erucic acid in the seed mapped to LG 1. The parents, F_1, and F_3 families were also scored for seed color. R500 seeds were yellow, while seeds of Per and the F_1 were brown. The color of F_3 seeds segregated as a dominant trait with a 3:1 ratio of brown- to yellow-seeded families. The locus (*Yls*) controlling yellow vs brown seed color mapped to LG 5.

Comparison to linkage maps of related species

The *B. rapa* map was compared to a *B. oleracea* map (Camargo 1994) using RFLP loci (46 loci in *B. rapa*, 46 loci in *B. oleracea*) detected by 38 probes in both populations (Fig. 2). Six *B. rapa* (BR) LGs shared at least two loci with individual *B. oleracea* (BO) linkage groups. BR LG 7 and BO LG 3 had the most loci (five) in common. All the shared loci were in the same order in the two maps, except for loci in BR LG 6 and BO LG 2. In general, the distances between loci were very different between the two species in all eight pairs, with 71% (10 of 14) of the intervals having greater distances between loci in the BR map.

The *B. rapa* map was compared to a *B. napus* (BN) map (Ferreira et al. 1994) using RFLP loci (63 loci in *B. rapa*, 68 loci in *B. napus*) detected by 50 probes in both populations (Fig. 3). Nine BR LGs had at least two loci in common with individual BN LGs. BR LG 2 had six marker loci in common with both BN LG 1 and BN LG 9, and the locus orders were preserved in all three LGs. The only case where common loci were in different orders was between BR LG 5 and BN LG 20, where the positions of *wg4d11* and *wg3h8* were reversed in the two maps. The map dis-

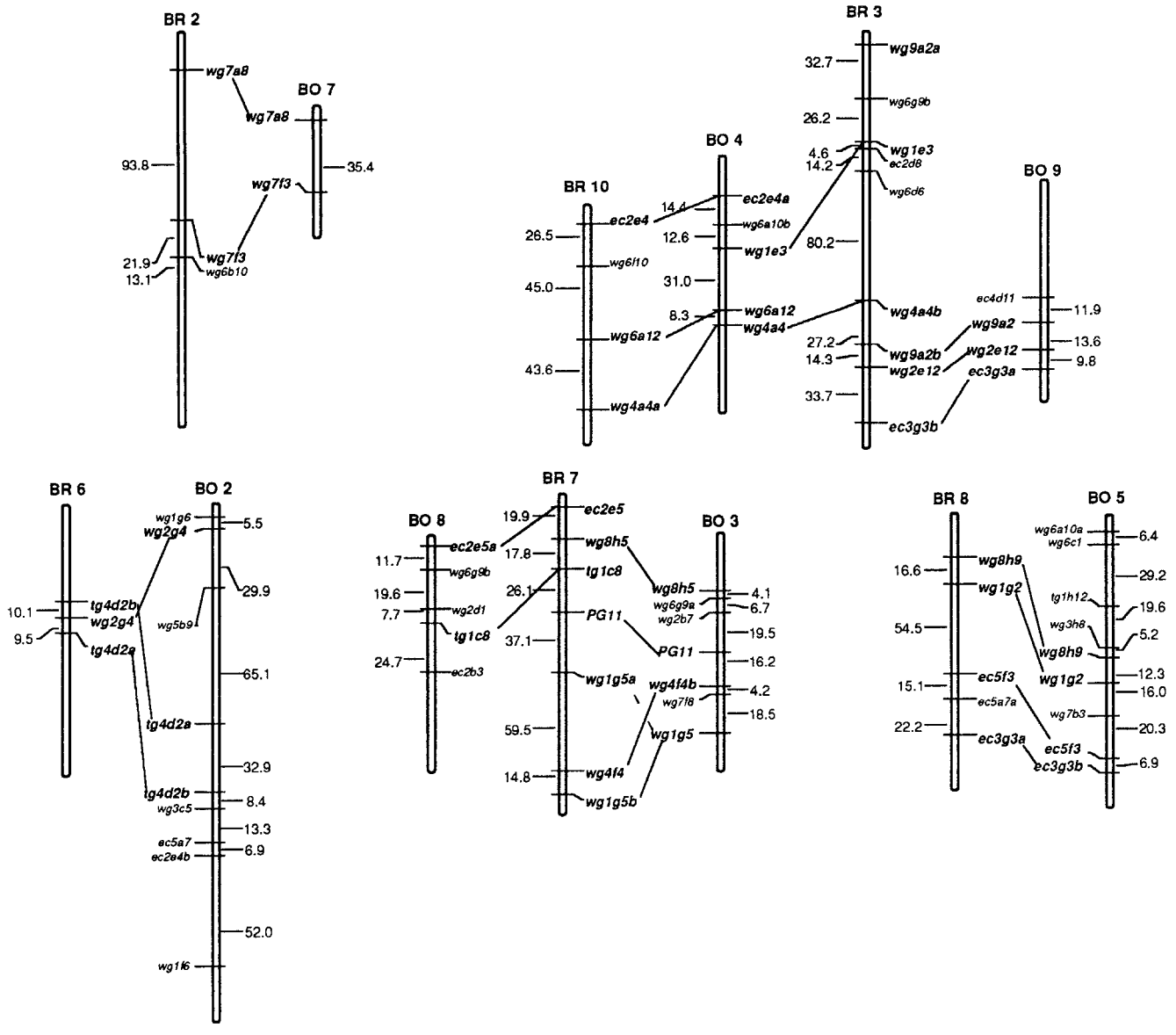

Fig. 2 Comparison between linkage maps of *B. rapa* (*BR*) and *B. oleracea* (*BO*). Only RFLP loci detected by probes in both species, and linkage groups which shared two or more loci in common, are included on the comparative maps. Map distances in cM are indicated on the left side of linkage groups and locus names on the right. *Lines* connect loci detected by the same probe in the two species

tances between adjacent loci were very different for the two species and were larger in the BR map for 91% (21 of 23) of the intervals between linked loci.

The *B. rapa* map positions of 14 RFLP loci detected by 12 of the 24 *A. thaliana* probes were compared to the map positions of 12 loci detected by these probes using an integrated map of *A. thaliana* (Hauge et al. 1993). Only two pairs of probes (DHS2 – PG11 and COR6.6 – tt4/pCHS3.8) detected linked RFLP loci in both species (Table 2).

Discussion

Map characteristics

The 144 RFLP loci segregating in this *B. rapa* grouped into ten large LGs and one triplet of linked markers (Group A). Since the haploid chromosome number of *B. rapa* is 10 and ten LGs were reported previously for other *B. rapa* maps (Song et al. 1991; Chyi et al. 1992), the addition of more marker loci would probably result in linkage of Group A and unlinked markers to the ten large LGs. The map covered a distance of 1785 cM, which is similar to the size of previously reported *B. rapa* maps (1850 cM with 280 loci, Song et al. 1991; 1876 mu with 360 loci, Chyi et al. 1992).

The percentage of loci with significant segregation distortion (23%) is similar to the level that was found in a *B. rapa* RFLP map constructed with one of the same parental lines, R500 (Chyi et al. 1992). There was no consistent pattern of distortion towards one parental allele. This is in

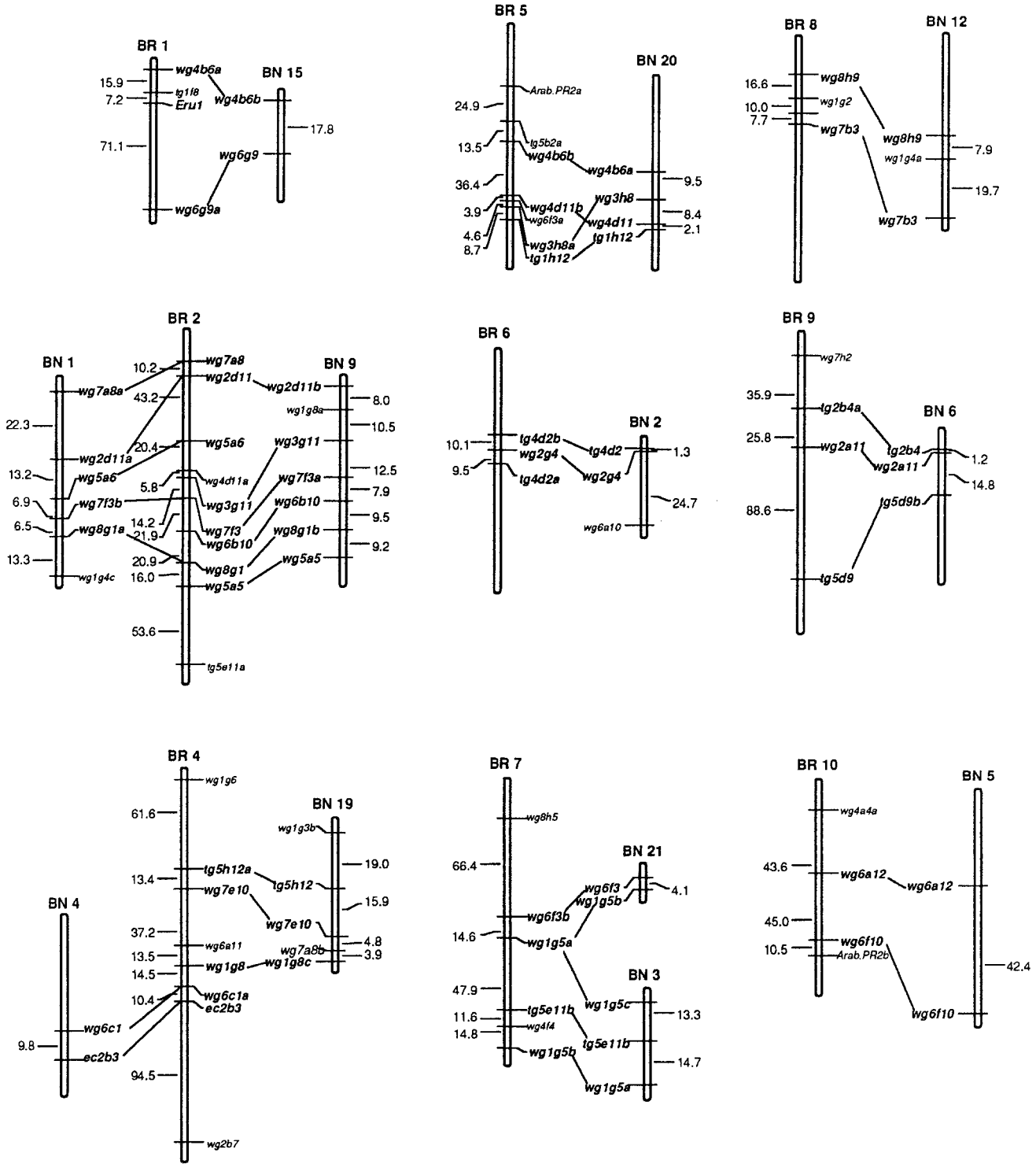

Fig. 3 Comparison between linkage maps of *B. rapa* (*BR*) and *B. napus* (*BN*). Only RFLP loci detected by probes in both species, and linkage groups which shared two or more loci in common, are included on the comparative maps. Map distances in cM are indicated on the left side of linkage groups and locus names on the right. *Lines* connect loci detected by the same probe in the two species

contrast to the results of Chyi et al. (1992) who found overall skewedness against R500 alleles in distorted regions. These authors also reported that the majority of the skewed loci were clustered to regions of six LGs. We found that 39% (13 of 33 loci) of skewed loci were adjacent to another skewed locus, but only one region (at the end of LG 2) contained a cluster of three or more loci with distorted segregation ratios. All three of these loci had a lack of the homozygous R500 genotype, suggesting post-fertilization selection against this genotype. Distortion in other regions

Table 2 Comparison of the chromosome (*A. thaliana*) and linkage group (*B. rapa*) locations of RFLP loci detected by *A. thaliana* cloned genes in both species. Probes are grouped according to the map position of RFLPs detected in *A. thaliana*

Probe name[a]	*A. thaliana*[b]	*B. rapa*
GAP-B	1	1
EZ3	1	2
TMK-1	1	7
GAP-A	3	4
GAP-C	3	5
GS-KB6	3	7
U2R9	4	3
m456	4	5, 6[c]
DHS2	4	7
PG11	4	7
tt4/pCHS3.8	5	8
COR6.6	5	2, 8[c]

[a] See Table 1 for the source and cloned gene of each probe
[b] *Arabidopsis* map data from Hauge et al. (1993), except COR6.6 (L. Medrano and E. Myerowitz, personal communication)
[c] Two segregating loci mapped in *B. rapa*

may be due to lethality, the lower fitness of gametic or zygotic genotypes, or to chance.

The level of locus duplication detected in this *B. rapa* population (24% of probes) was similar to that reported previously by Chyi et al. (1992) for *B. rapa* (20.1%), but was lower than others have reported for *Brassica* species: 35–44% in *B. oleracea* (McGrath et al. 1990; Slocum et al. 1990; Kianian and Quiros 1992); 37% in *B. rapa* (Song et al. 1991). Differences in estimates are expected since the level of duplication detected is dependent on the population, the specific hybridization conditions, and the scoring regime used. In our map, some probes hybridized to multiple segregating loci, but only one locus was scored since the banding patterns of the additional loci were not clear enough to score accurately.

The duplicated loci identified in this *B. rapa* map were scattered throughout the genome with little evidence for conserved linkage arrangements, suggesting that either small chromosomal regions were duplicated or else that larger chromosomal regions were rearranged after duplication. Dispersed arrangements of duplicated loci have been reported for other *B. rapa* RFLP maps; however, evidence for large duplicated chromosome fragments containing 3–10 loci was also observed (Song et al. 1991; Chyi et al. 1992). The lack of such long blocks of duplicates in this *B. rapa* map may be because we did not score as many segregating loci per probe.

A single gene controlling the presence or absence of hairs on the leaf surface (*Pub*1) was mapped to LG 4. A gene with the same effect (*Pub*) was mapped previously by Song et al. (1994) in a cross between Chinese cabbage and Spring broccoli, but it is not known whether this is the same gene as *Pub*1.

Yellow seed color segregated as a maternally dependent, recessive trait with a ratio of 3 brown to 1 yellow seeds in F_3 families. Seed color was controlled by three genes in *B. napus* but only one gene in *B. carinata*, yet there was incomplete dominance of brown over yellow leading to yellow-brown heterozygotes in both amphidiploids (Shirzadegan 1986; Getinet et al. 1987). In this *B. rapa* population, all dark seeds were scored as brown, so we could not distinguish the heterozygotes from the brown homozygotes. Development of pure yellow-seeded oilseed *Brassica* cultivars is an important breeding objective, because yellow seeds have increased oil and protein content due to the thinner seed coat (Abraham and Bhatia 1986; Downey and Röbbelen 1989). Since this is a maternally dependent, recessive trait it might be helpful for breeding purposes to have molecular markers linked to the locus responsible for this trait. The locus controlling yellow seed color (*Yls*) in our population mapped to LG 5 less than 10 cM from flanking marker loci. These markers, or more closely linked markers identified by additional screening, could be used to select for yellow-seededness.

Rapeseed oil which lacks detectable levels of the fatty acid erucic acid is more desirable for food uses and can be designated as 'canola' quality if the oil contains less than 2% erucic acid (and low glucosinolates in the meal) (Shahidi 1990). High erucic acid oils are more desirable for industrial uses. Dorrell and Downey (1964) found a single locus that controlled the presence or absence of erucic acid in *B. rapa*. Our F_2 population segregated in a 1:2:1 ratio of non-detectable:intermediate:high levels of erucic acid and the locus controlling this variation (*Eru*) mapped to LG 1. The two alleles of this locus had additive effects resulting in the intermediate erucic acid level of the heterozygous class. This is similar to previous results with *B. rapa* where erucic acid content was controlled by the additive effects of multiple alleles at a single locus (Jönsson 1977).

Comparison to linkage maps of related species

Comparison between the two diploid species *B. rapa* (BR) and *B. oleracea* (BO) revealed many regions with conserved linkage arrangements. Four regions had at least three loci in common between the two species in which all loci were in the same order (Fig. 2). These results support a close evolutionary relationship between *B. oleracea* and *B. rapa,* as suggested from other studies (Song et al. 1990; McGrath and Quiros 1991; Warwick and Black 1991; Song and Osborn 1992). The map distances between loci were larger for BR in 71% of the intervals. This could be due mainly to an overall higher level of recombination in BR, which is consistent with the larger linkage maps reported for BR (see above) compared to BO (820 mu with 258 markers, Slocum et al. 1990; 747 cM with 108 loci, Kianian and Quiros 1992; 1112 cM with 198 loci, Landry et al. 1992; 980 cM with 116 loci, Camargo 1994). In some regions, deletions or insertions could have occurred after divergence of these two species to create the differences in interval lengths. In addition, chromo-

some repatterning which occurred during or after speciation could be responsible for the fact that many of the loci had altered syntenic associations between the two species, as suggested by other comparative maps (McGrath and Quiros 1991).

Comparison between the *B. napus* (BN) and *B. rapa* (BR) maps revealed nine BR LGs with at least two loci having conserved linkage arrangements in the BN map (Fig. 3). The locus orders were maintained in all comparisons except between BR 5 and BN 20. The map distances between loci with conserved linkages were again larger in the BR map. Hoenecke and Chyi (1991) also compared RFLP maps of these two species based on a common set of genomic clones and found 11 conserved linkage segments with at least four loci in common between the two species. In contrast to our results, most of the conserved blocks had similar distances between markers and more than half of the conserved segments (6 of 11) had the order of loci rearranged, although their comparison included more loci in common. Overall, both comparative maps found a high degree of conserved linkage arrangements between the two species even though the maps were constructed with different cultivars. These results support the hypothesis that *B. rapa,* or a closely related ancestor, was one of the progenitor species of the amphidiploid *B. napus* (U 1935; Song and Osborn 1992).

If *B. rapa* and *B. oleracea*, or close relatives, were the progenitors of *B. napus*, each BN LG should have more linkage-order conservation with either a BR LG or a BO LG. Our results, and those of Camargo (1994), do indicate a higher degree of conserved linkage arrangement between some BN LGs and either BR or BO LGs. However, since our comparisons are based on a small number of loci, and not all loci detected by a probe were mapped, we cannot determine with any certainty if one diploid species contributed each *B. napus* LG. Using a synthetic *B. napus*, Lydiate et al. (1993) reported that the ancestral genome source of each LG could be determined and that there was little rearrangement of the LGs within the amphidiploid. Comparative mapping of additional molecular-marker and trait loci in the three species may help clarify the origin of BN LGs and provide more information on genome relationships.

Cloned gene sequences from *A. thaliana* hybridized to *B. rapa* at fairly high stringency conditions (0.2 × SSC), suggesting that there is a high degree of sequence homology between these species. However, only 4 of the loci mapped with 12 probes in both species showed conserved linkage arrangements. These markers were over twice as far apart in *B. rapa* than in *A. thaliana*, but this would be expected since the *A. thaliana* map is three-fold smaller than the *B. rapa* map. Since we have compared the locations of only 14 loci, and have no information on undetected duplicated loci, we cannot make strong conclusions about chromosomal conservation. However, our preliminary results suggest that there is some synteny between the two species.

The presence of RFLPs at homologous loci of cloned genes in this *B. rapa* map will make it possible to determine if alleles of these candidate genes are associated with variation for important traits. Most of the mapped genes encoded proteins, such as -1,3-glucanase, superoxide dismutase (SOD), whose expression is induced by plant stresses and by cold temperatures. We plan to use this map and QTL analysis to investigate the possible role of these candidate genes in the stress responses of *B. rapa*, such as freezing tolerance.

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