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RFLP mapping of loci controlling self-incompatibility in *Brassica campestris* and their comparative mapping with *B. napus* and *B. oleracea*

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Abstract

RFLP analysis of a cDNA probe *SLG6*, governing self incompatibility (SI) in *Brassica oleracea*, using a recombinant inbred population of *Brassica campestris* followed by genetic linkage analysis led to the detection of two marker loci, *SLG6a* and *SLG6b* controlling SI. *SLG6a* was mapped in linkage group (LG) 9 and was flanked by the RFLP markers *ec4f10* (6.4 cM) and *wg5b9* (4.2 cM). *SLG6b* positioned in LG 2 and was flanked by the RFLP markers *wg2d11* (9.9 cM) and *ec4e7* (26.9 cM). These results indicated the scope of marker-aided introgression of these genes into self-compatible genotypes for production of SI lines suitable for hybridization in *B. campestris*. Comparative mapping of LG 9 containing *SLG6b* with corresponding linkage groups of *B. oleracea* (BO 2) and *B. napus* (BN 16) led to the detection of small homologous regions with *SLG6* locus linked with another RFLP locus. This evidenced for homology of the *SLG* genes across *Brassica* species and possibility of using any single cloned *SLG* gene for development of SI lines in any *Brassica* species.

Additional key words: linkage analysis, molecular map, RI population, *SLG* gene.

Introduction

Brassica campestris L. syn. *B. rapa* is an important oilseed *Brassica* species having somatic chromosome complement of 2n = 2x = 20 and it comprises the 'AA' genome. Development of F_1 hybrids to attain higher yields through heterosis is a major breeding objective in this crop. Production of hybrid seeds on a commercial scale depends on simple and efficient methods to force out-crossing in this primarily self-pollinating crop. One of these methods includes the use of self-incompatibility (SI) through S-alleles from *B. oleracea* and *B. campestris*, which are responsible for pollen rejection at stigma surface whenever the pollen and stigma surface bear identical S-alleles (Nasrallah *et al.* 1991). SI system is widely used for heterosis breeding in vegetables of *Brassicaceae* family because F_1 hybrid seeds can be harvested from both the parents using this strategy.

The SI system in *Brassica*, especially that of *B. oleracea*, has been well studied (Nasrallah *et al*. 1985, 1988, Trick and Flavell 1989, Scutt *et al*. 1990, Boyes *et al*. 1991, Stein *et al*. 1991, Scutt and Croy 1992) and has been described as being under sporophytic control, which occurs when the phenotype of the pollen is determined by the diploid genotype of the pollen producer, the sporophyte (Bateman 1955). SI system in *Brassica* is controlled by the single multiallelic S-locus. Recently, in this locus several closely linked genes including S-locus receptor kinase (SRK), S-locus glycoprotein (SLG), small cystine-rich protein (SCR) and unlinked S-locus related (SLR) genes have been identified. The S-locus in *B. oleracea* corresponds to about 200 kb region containing several transcriptional units co-segregating with SI phenotypes.

Genetic mapping using molecular markers has been used as potential for mapping, monitoring and cloning of oligogenes of agronomic importance (Kole and Gupta 2001). This technique has also facilitated detection

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Abbreviations: LG - linkage group; MAB - marker assisted breeding; SCR - S-locus cystein rich protein; SI - self incompatibility; SLG - S-locus glycoprotein; SRK - S-locus receptor kinase.
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of quantitative trait loci (Landjeva *et al.* 2008) and their Mendelization leading to precise genetic dissection and manipulation as tacitly as oligogenes (Kole *et al.* 2001). The SI genes have already been mapped in *B. oleracea* (Kianian and Quiros 1992a,b, Camargo *et al.* 1997, Cheung *et al*. 1997) and *B. napus* (Cheung *et al*. 1997, Lombard and Delourme 2001). Loci controlling SI (SLG) has been mapped in *B. campestris* using F_2 mapping populations by Tanhuanpaa *et al*. (1995) and Nozaki *et al.* (1997). Mapping of SI controlling locus/loci in permanent mapping populations such as recombinant inbred (RI) population in *B. campestris* could facilitate precise genetic monitoring of the gene(s) through marker-assisted breeding (MAB).

Comparative mapping has emerged as a useful strategy in molecular genetics to decipher homologous genes and gene clusters of economic interests across taxa (Kole and Gupta 2001). A homologous gene can be cloned and used for genetic tailoring of a number of species through genetic transformation. Comparative mapping of *Brassica* S-locus region and its homologue in *Arabidopsis* has already been done using physical maps (Conner *et al*. 1998, Kusaba and Nishio 1999). Such comparative mapping based on genetic maps has been done between *B. oleracea* and *B. napus* (Cheung *et al*. 1997). Available literature does not, however, evidences for any report on such comparative mapping using genetic maps for SI of *Brassica campestris* to another species or genus.

We report here on genetic linkage mapping of the loci controlling self-incompatibility in *Brassica campestris* using a heterologous probe and its comparative mapping to *B. oleracea* and *B. napus*.

Materials and methods

A recombinant inbred (RI) population consisting of 87 F_7 families developed by single-plant-descent of individual F_2 plants derived from a cross between an winter turnip rape (*Brassica campestris* L.) cv. PER and a spring sarson (*Brassica napus* L.) cv. R500 was used as the mapping population (Kole *et al.* 1997) in this study. These two parents differ contrastingly with regard to their response to several characters including vernalization requirement, flowering time, winter survival, freezing tolerance besides self-incompatibility.

DNA was extracted from leaf tissues following the CTAB procedure as described by Kidwell and Osborn (1992). DNA (5 µg) from each RIL digested with either *Hind*III or *Eco*RI was electrophoresed in 0.8 % agarose gels. RFLP loci were detected with the heterologous cDNA probe of the cloned gene *SLG6* from *B. oleracea* encoding *S*-locus specific glycoprotein. RFLPs were analyzed as described by Teutonico and Osborn (1994).

Linkage analysis of 151 RFLP loci including the genotypes data for *SLG6* was done using the *MAPMAKER/EXP 3.0* program (Lincoln *et al*. 1992). A minimum logarithm of odd (LOD) threshold of 3.0 and a maximum recombination fraction (RF) of 0.4 were used for grouping of the marker loci into potential linkage groups. The order and relative distance of the marker loci for each linkage group were determined using three-point and multipoint analyses. The final linkage orders were verified with the 'ripple' command. Map distances in cM were expressed in Haldane mapping function (Haldane 1919). The map position of *SLG-6* locus in the present map of *B. campestris* was compared with the map of *B. oleracea* (Camargo *et al.* 1997) and of *B. napus* (Kole 1997).

Results

Hybridization of *Hind*III digested DNA samples of 87 recombinant inbred (RI) lines (Kole *et al.* 1997) with heterologous probe *SLG6* detected two co-dominant segregating polymorphic fragment (Fig. 1), which were later on treated as marker locus *SLG6a* and *SLG6b*. Segregation of PER and R-500 allele for both the marker locus were tested for 1:1 ratio based upon the homozygous individuals of RI population. The RI lines exhibited monogenic segregation for *SLG6b* ($\chi^2 = 0.209$, $P = 0.660$) and distorted segregation for *SLG6a* ($\chi^2 = 6.259$, $P = 0.013$) respectively. *SLG6a* exhibited skewness towards the R500 parent. In fact all the eight marker loci including *SLG6a* in a cluster in this genomic region were skewed towards R500.

Linkage analysis of 151 RFLP marker loci that included the two new loci detected by the *SLG6* in the present study and four trait loci (seed colour, leaf pubescence and resistance to white rust caused by *Albugo candida* race 2 and 7) employing the computer program *MAPMAKER/EXP 3.0* led to the development of 10 major linkage groups (LG) spanning over 44.4 to 130.4 cM containing 9 to 22 loci and two short LGs with two or three marker loci (Table 1). Two linkage groups were found to contain the RFLP loci detected by the probe *SLG6*, one in LG 9 (*SLG6a*) and another in LG 2 (*SLG6b*). The locus in LG 2 (*SLG6b*) was positioned 9.9 cM apart from *wg2d11* (Fig. 2). In LG 9, *SLG6a* was flanked by *ec4f10* and *wg5b9* at 6.4 and 4.2 cM apart, respectively (Fig. 2). The *SLG6a* locus exhibited distorted segregation skewed towards the R500 parent. In fact all the eight marker loci including *SLG6a* in a cluster in this genomic region were skewed towards R500.

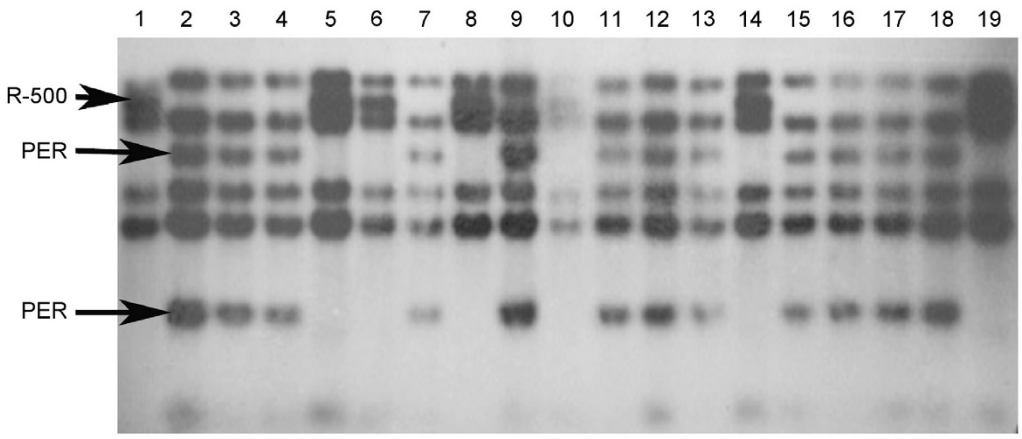

Fig. 1. An autoradiogram developed from hybridization of *Hind-III* digested DNA of some RI lines and their parents with *SLG6*, a cDNA probe from *B. oleracea*. *Lanes* 1 and 2 represent the parents R-500 and PER respectively and *lanes* 3 - 19 for RIL-01 to RIL17. The *arrow* indicates the polymorphic fragment segregating in co-dominant manner.

Table 1. Information on the 10 major and two short linkage groups produced from linkage analysis of 151 RFLP loci including *SLG6a* and *SLG6b* and four trait loci in the RI population of *B. campestris* (* - linkage group contains marker locus detected by *SLG6* probe)

Linkage group	Number of marker loci	Map length [cM]	Marker interval [cM]	Log-likelihood value
1	11	99.1	9.009	-114.01
2*	18	114.7	6.372	-173.87
3	22	130.4	5.927	-205.74
4	21	92.5	4.404	-182.76
5	13	55.4	4.261	-130.5
6	12	126.7	10.558	-167.46
7	15	86.0	5.733	-173.01
8	14	123.1	8.792	-136.62
9*	15	112.0	7.467	-160.75
10	9	44.4	4.933	-97.56
A	3	8.8	2.933	-41.83
B	2	0.0	0.0	0.0

Intragenomic comparative mapping between LG 2 and LG 9 revealed that in addition to the loci detected by *SLG6*, another pair of loci detected by *ec5a6* was also common between the groups LG 2 and 9 (Fig. 3). The linkage group containing the *SLG6a* locus, LG 9 was compared to the corresponding linkage group developed by Tanhuanpaa *et al.* (1995) using some probes common to the present study. This led to the detection of homology of three loci detected by *wg2a11*, *SLG6* and *wg5b9* in the same order (Fig. 4). This finding confirmed the map position of *SLG6a* in the present investigation.

Comparative mapping was done for LG 9 and LG 2 of *B. campestris* with the LG 2 of *B. oleracea* (Camargo *et al.* 1997) and LG (BN) 16 of *B. napus* (Kole 1997) containing the loci detected by *SLG6*. LG 9 shared two loci (*SLG6a* and *wg5b9*) with *B. oleracea* and two loci (*SLG6a* and *tg5d9*) with *B. napus* (Fig. 5). The *B. oleracea* linkage map also shared two common loci *SLG6* and *wg5a1* with that of *B. napus*.

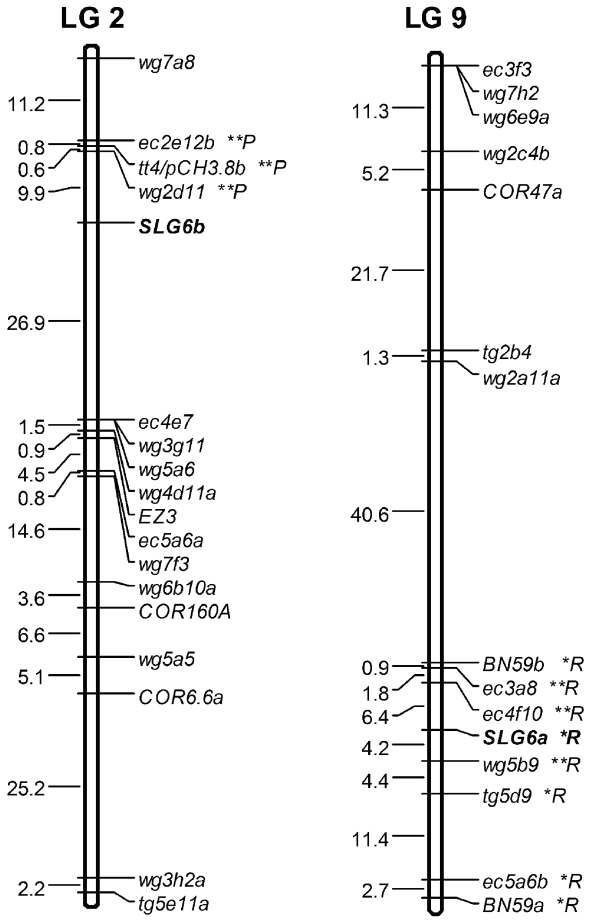

Fig. 2. RFLP linkage maps for two linkage groups containing *SLG6a* (LG9) and *SLG6b* (LG2) marker loci (marker loci *on the right*, distances in cM *on the left*; * and ** deviated significantly from 1:1 segregation ratio at $P < 0.5$ and $P < 0.01$ respectively; *R* indicate that R-500 genotypes predominated.

Discussion

Self-incompatibility (SI) in the family *Brassicaceae* is controlled by the single multiallelic S-locus. The specificity of the *S*-locus is determined by the production of *S*-locus specific glycoprotein (*SLSG*) through the expression of the *SLG* gene (Nasrallah *et al.* 1987). Recently some homologous proteins involved in self incompatibility have also been isolated from buckwheat (Miljuš-Dukić *et al.* 2004). *SLG* from plants with different alleles have been cloned and sequenced, and extensive sequence divergence has been observed (Nasrallah *et al.* 1988, Ebert *et al.* 1989, Yamakawa *et al.* 1994). Introduction of *S*-alleles in self-pollinating crops could be a potential means to prevent self-pollination and to facilitate the production of F_1 hybrid seeds for higher yield through heterosis. *S*-alleles from *B. campestris* and *B. oleracea* have already been introduced into breeding lines of *B. napus* for this purpose (Dwyer *et al.* 1991, Nasrallah *et al.* 1991, Toriyama *et al.* 1991). *S*-alleles from the few self-incompatible cultivars of *B. campestris* or related *Brassica* species could be introduced into the self-compatible cultivars for the above purpose. This can be achieved by mapping the gene followed by marker-assisted backcross breeding. Otherwise a homologous gene from an allied species can be used by genetic transformation. Genes controlling self-incompatibility were mapped firstly in *B. oleracea* by Kianian and Quiros (1992a,b). Later on, Camargo *et al.* (1997) mapped the *SLG6* locus in this species. Cheung *et al.* (1997) mapped *S*-loci in *B. oleracea* and *B. napus* and studied their homology. In *B. campestris*, Tanhuanpaa *et al.* (1995) mapped a locus *SLG* in the same linkage group as in the present study. *SLG6* mapped to the linkage group N16 (LG 21) in a *B. napus* map (Kole 1997). The loci controlling S-glycoprotein and NS-glycoprotein were also mapped to LG 1 and LG 2 of *B. campestris* (ssp. *oleifera*) by Nozaki *et al.* (1997). Lombard and Delourme (2001) also detected a locus for *SLG* (LG 10) in their integrated *B. napus* map.

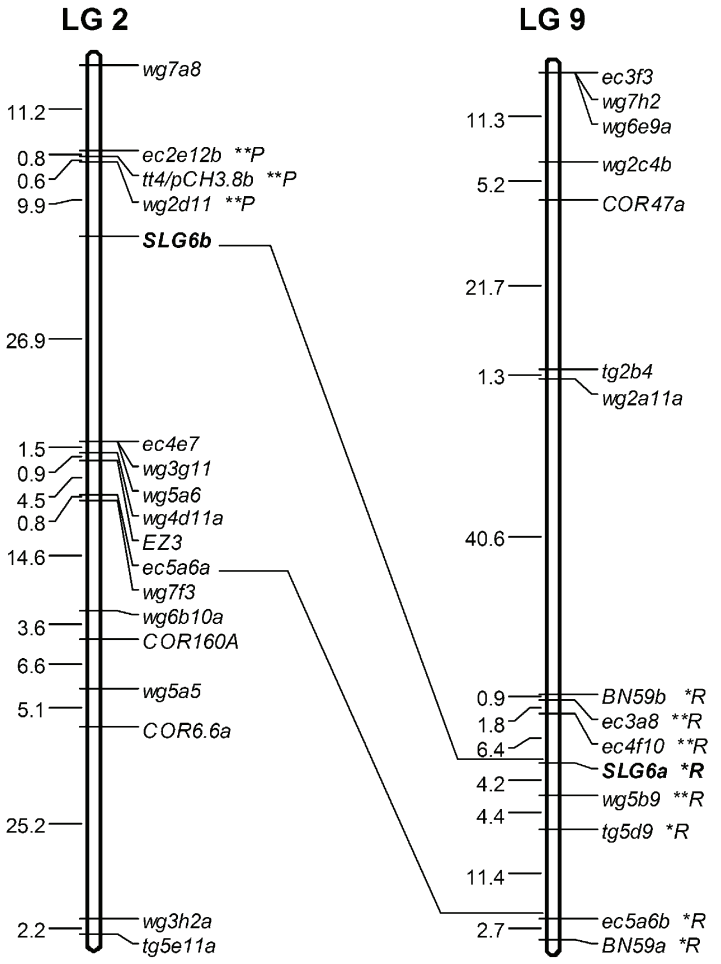

Fig. 3. Intra-genomic comparative mapping of LG 2 and LG 9 of *B. campestris* evidencing duplicated regions. *Lines* connect the duplicated marker loci including *SLG6.*

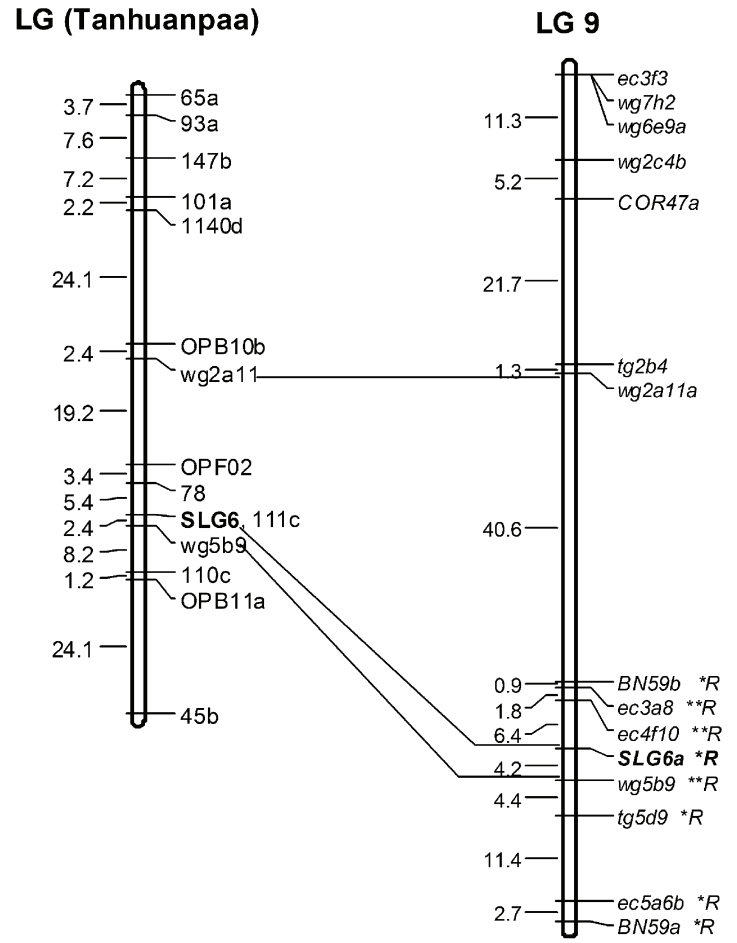

Fig. 4. Intra-specific comparative mapping for *SLG6* locus between LG 9 and corresponding LG of Tanhuanpaa *et al.* (1995a). Lines connect the homologous marker loci mapped in each case.

In the present study, duplicated *SLG6* loci have been detected in LG 2 and LG 9. The *SLG6a* locus (LG 9) was tightly linked to an RFLP marker locus *wg5b9* (4.2 cM) and closely linked to another RFLP locus *ec4f10* (6.4 cM). The *SLG6b* locus (LG 2) was 9.9 cM apart from *wg2d11*. These markers could be used for introgression of the SI gene into the self-compatible genotypes of *B. campestris* to prevent self-pollination and to produce F_1 hybrid seeds. The RI lines exhibited distorted segregation for the *SLG6a* locus, skewness being towards the R500 parent. All the eight marker loci including *SLG6a* present at the lower half of this linkage group were skewed towards the R500 parent evidencing for presence of deleterious PER alleles at this genomic region.

Comparative mapping using marker loci and/or trait loci has been effectively employed in the identification of homologous chromosomal regions and also genes, and facilitated the way for their direct use rather than cloning another set of copies. In the present study, LG 9 containing *SLG6a* locus was compared to the corresponding linkage group developed by Tanhuanpaa *et al.* (1995). Three marker loci *wg2a11*, *SLG* (*SLG8*) and *wg5b9* in their map were homologous to *wg2a11a*, *SLG6a* and *wg5b9* in the present map, the order being the same. Again, two tightly linked (4.2 cM) marker loci in LG 9 of *B. campestris*, *SLG6a* and w*g5b9* were detected in LG 2 of *B. oleracea* map of Camargo *et al.* (1997) at a distance of 3.5 cM. Similalarly, LG 9 of *B. campestris* has been found to be homologous also to BN16 of *B. napus* for homology of two loci, *SLG6a* and *tg5d9*. These findings were evidenced for high sequence homology of *SLG* genes across *Brassica* species and conservation of homologous genes across species in the *Brassica* genus. Besides, the results indicate the possibility of using the same cloned heterologous genes (*SLG6/ SLG8*), for raising of self-incompatible lines through genetic transformation besides marker-assisted breeding aiming at production F_1 hybrid seeds.

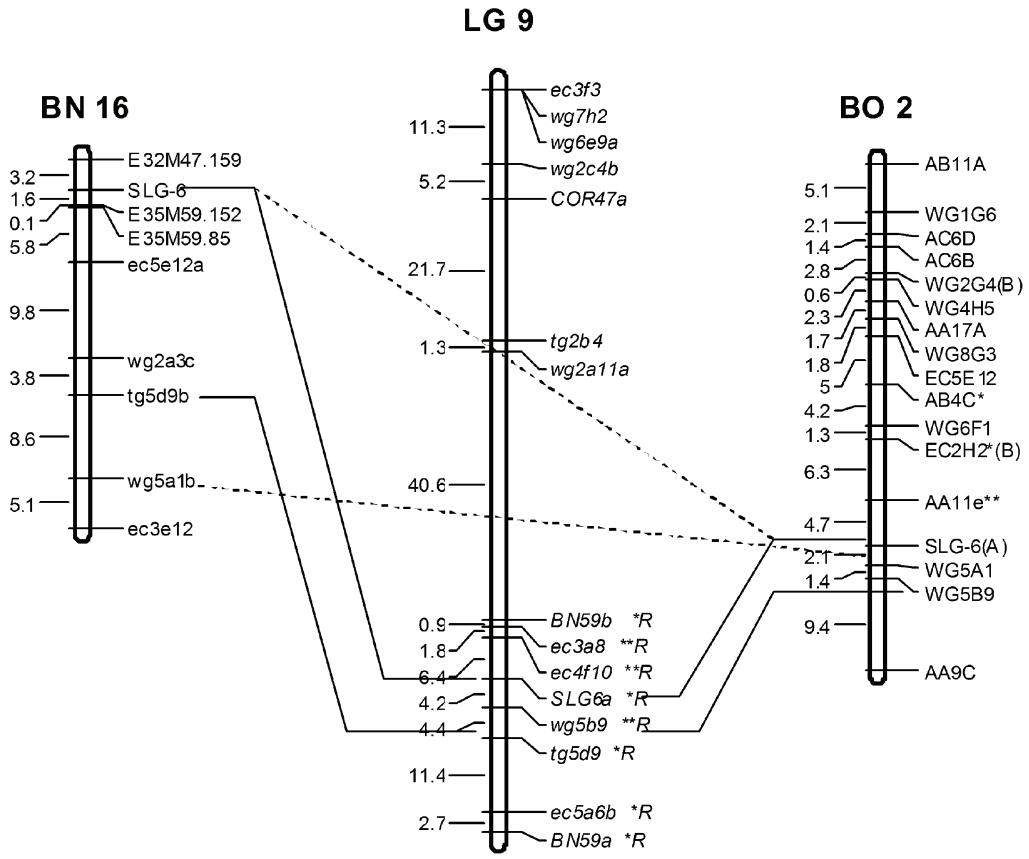

Fig. 5. Inter-specific comparative mapping for *SLG6* locus between LG 9 of *B. campestris*, BN 16 of *B. napus* and BO 2 of *B. oleracea*. Lines connect the homolgous marker loci mapped in each l
inkage group.

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