Marker-assisted selection of self-incompatible oilseed rape plants.

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Abstract

Self-incompatibility (SI) could be used as a pollination control system for *Brassica napus*, if a sufficient number of S-alleles is available in this species. Unlike self-incompatible (SI) *Brassica rapa* and *Brassica oleracea*, two ancestor species, *Brassica napus* is naturally self-compatible (SC). However, occasionally SI also occurs in rapeseed cultivars. SI oilseed rape plants selection was performed with doublehaploids derived from crosses between lines with recessive self-incompatibility and self-compatible donors of quality. SI in Brassicaceae plants is sporophytically controlled by a single multi-allelic locus (*S*-locus), which contains at least three highly polymorphic genes expressed in the stigma (*SLG* and *SRK*) and in the pollen (*SCR* or *SP11*). We have used two *S*–genes, *SLG* gene class I and *SCR* gene class II, as molecular markers in order to screen segregating doublehaploid population for SI plants. A molecular marker on the basis of *SCR* class II gene was designed to determine specific *SCR* II allele connected with self-incompatibility. This marker specifically determines SI lines derived from line 'Tandem' only. In contrast to *SCR* II marker gene, universal *SLG* I marker gene enables to detect SC plants in all SI lines. Theoretically expected segregation ratio 1:1 (SI:SC) of doublehaploids derived from the SI line and the SC quality donor was confimed by molecular analysis in two model populations. Both marker genes determined the same SI/SC phenotype.

Introduction

Self-incompatibility (SI) is a natural mechanismus of plants that prevents inbreeding and promotes outcrossing. This system naturally occurs in *Brassica oleracea* and *Brassica campestris*, which are diploid ancestors of amphidiloid *Brassica napus,* but despite of this fact is *Brassica napus* self-compatible (Olsson 1960).

In the *Brassica* SI system is controlled by a single polymorphic locus termed *S*-locus (Bateman 1955). There are three highly polymorphic genes at the *S* locus: *SRK* (*S*-locus receptor kinase) (Stein et al. 1991), *SP11/SCR* (*S*-locus protein 11/*S*-locus cysteine rich protein) (Schopfer et al. 1999, Suzuki et al. 1999), and *SLG* (*S*-locus glycoprotein) (Nasrallah et al. 1987).

S haplotypes have been classified into two classes based on the nucleotide sequence similarity of *SLG* alleles. Class-I *S*-haplotypes are known to be generally dominant to class-II *S*-haplotypes in the pollen (Nasrallah et al. 1991, Nasrallah 1993). SP11s have two classes which common to general feature of *S*-locus. Class-II SP11s originating from *B. olerecea* and *B. rapa* form a distinct group separated from class-I SP11s (Shiba et al. 2002). In contrast to SLG protein, SCR is supposed as a male determinant with essential function in pollen–stigma recognition (Suzuki et al. 1999, Schopfer et al. 1999). However, SLG seems not to play essential role in pollen-stigma recognition (Okazaki et al. 1999, Suzuki et al. 2000, Suzuki et al. 2000).

Material and Methods

A segregating doubled haploid (DH) populations of oilseed rape (*Brassica napus*) was derived from four crosses between self-compatible (SC) cultivar 'Lisek' and self-incompatible (SI) line 'AIK 6', SC cultivar 'Rasmus' and SI line 'AIK 6', SC cultivar 'Rasmus' and SI line 'AIK 3′, and finaly SC line 'OP BN-03′ and SI line 'AIK 3′. 'AIK 3′ and 'AIK 6' SI lines were derived from SI line 'Tandem' with recessive type of self-incompatibility. This population was consisted of 118 plants. Seeds of the cultivars and DH lines was obtained directly from the breeding stations Opava and Slapy, Czech Republic. DH populations were regenerated via a microspore embryogenesis procedure from F_1 generation after crossing with an objective to fix SI phenotype and low content of glucosinolates in the Research Institute of Crop Production in Prague.

Genomic DNA was extracted from young leaves of 2-week-old seedlings by the DNeasy Plant Mini kit (QIAGEN). The PCR reaction was performed with class-I *SLG*-specific primers PS5 and PS15 (Nishio et al.1996). *SCR* gene was amplified with class-II *SCR*-specific oligonucleotide primers designed for functional allele originating from SI line 'Tandem' termed allele 2 (5′-TTGGACTTTGACATATGTTC-3′ and 5′-CTCTGAAGTGGGTTTTACAG-3′).

Results

Two marker genes were used for SI plants selection. PCR with class-I *SLG*-specific primers has resulted in approximately 1300 bp fragment together with cca 1000 bp long, probably nonspecific fragment (fig. 1.). This fragment was specifically present in plants considered to be self-compatible. This marker gene have been detected in spectrum of naturally self-compatible oilseed rape cultivars whereas in self-incompatible lines not.

The second marker system specifically targets allele of class-II *SCR* gene. This allele was found in self-incompatible lines derived from line 'Tandem'. Amplified fragment of class-II *SCR* gene allele was 280 bp long and specifically occured in plants considered to be self-incompatible (fig. 2.).

The two marker systems segregated in ratio 1:1 as was expected and they exactly correlated each other. On the basis of

molecular marker selection, young doubled haploid plantlets of oilseed rape were selected and further subjected to the phenotypical examination.

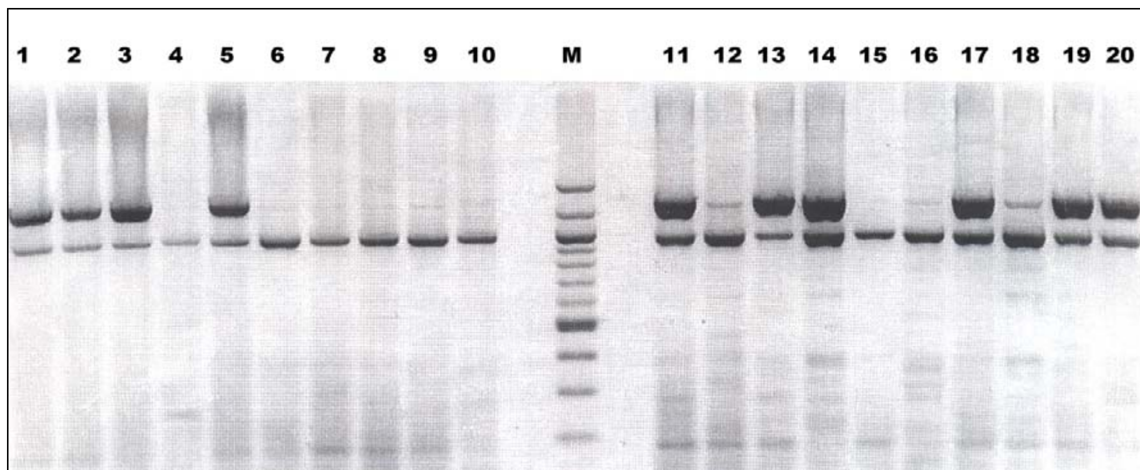

Fig. 1. Amplification of class-I *SLG* gene. The upper band of 1300 bp was amplified in putative self-compatible oilseed rape plants of segregating doubled haploid population.

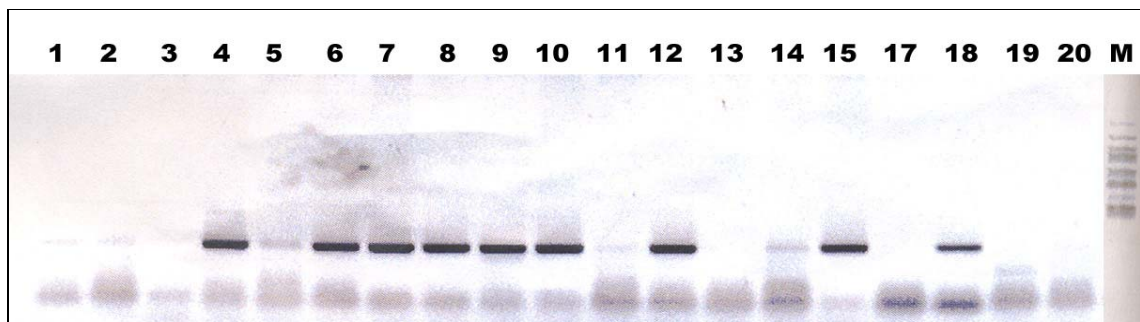

Fig. 2. Amplification of class-II *SCR* gene. An 280 bp band was amplified in putative self-incompatible oilseed rape plants of segregating doubled haploid population.

Discussion

It was proved that *SLG* gene is not essential in pollen-stigma recognition reaction (Okazaki et al. 1999, Suzuki et al. 2000, Suzuki et al. 2000). As the self-incompatibility of used oilseed rape lines was of recessive type, dominant class-I *SLG* gene was not amplified in putative SI plants because of likely dysfunction of this gene which could cause self-compatibility of common oilseed rape plants. Considering that SLG protein is not essential in pollen-stigma recognition reaction, absence of class-I *SLG* gene could suggest absence of the whole class-I *S*-locus, which could be in favour of recessive class-II *S*-locus.

The second approach was to use a functional allele of essential gene in SI reaction as a marker gene. This approach was supposed to be more accurate than application of *SLG* marker gene. After comparison of the first marker system (*SLG* marker gene) with the second (*SCR* marker gene) the results were the same. But we could not exclude the possibility that there are another oilseed rape cultivars with functional class-II *SCR* gene and dysfunctional class-I *SLG* gene simultaneously. In that event, class-I *S*-locus containing *SLG* gene, considered as a dominant, should suppress functional class-II *S*-locus with *SCR* gene resulting in common self-compatible oilseed rape plant.

As a more useful appears to be universal class-I *SLG* marker gene than specially developed *SCR* marker originating from particular SI line, which is more expensive. Development of *S*-haplotype specific marker could be useful as a prevention of contamination definite SI line with SI line carrying different *S*-allele.

Conclusion

The two marker genes were used to select self-incompatible plants from segregating doubled haploid populations of oilseed rape. The *S*-locus specific marker, allele of class-II *SCR* gene, and the universal marker, class-I *SLG* gene, exactly correlated with segregation ratio of self-incompatibility in doubled haploid population. Both marker systems would be used for marker-assisted selection in hybrid oilseed rape breeding.

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