Utilisation of molecular markers for screening of self-incompatible *Brassica napus* genotypes

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ABSTRACT

Brassica napus cultivars, self-compatible (SC) donors of quality and self-incompatible (SI) lines were analysed using identification of *S*-locus. In several examined cultivars one *S*-locus of *SLG* gene was detected as dominant and the second as recessive. In SC individuals the presence of dominant class I *SLG* gene were confirmed. Amplification of class II *SLG* gene screened recessive gene in all analysed SC and SI samples. Expression level of the *SLG* gene did not correlate with the strength of self-incompatibility in the class II *S*-haplotypes. The non-functional *S* allele A10 originated in cultivar Westar was detected using PCR-RFLP approach in all analysed cultivars. The results of PCR and PCR-RFLP analyses indicate that the functional recessive allele replaced the non-functional allele *A*10 in genome C. In A genome of all analysed plants (SC and SI) a non-functional recessive allele is probably localised. The model experimental design - parents - F_1 and F_2 generation was proposed and realised. Theoretically expected F_2 segregation ratio 3:1 (SC: SI) after crossing of SI line and SC quality donor was confirmed by molecular analyses in two model populations.

Key words: *Brassica napus* – self incompatibility – dominant *SLG* gene – recessive *SLG* gene – F_2 segregation

INTRODUCTION

In the *Brassica* family, fertilization can be controlled by a self-incompatibility system that is inherited as a dominant genetic locus called *S*-locus that exhibits a strong self-incompatible phenotype. The second group of alleles demonstrates a weak or leaky self-incompatible phenotypic effect and they are considered to be recessive (Nasrallah et al. 1991). *B. napus*, an allotetraploid, generally occurs as a self-compatible plant (Downey and Rakow 1987). Although some naturally occurring self-incompatible *B. napus* lines have been isolated (Gemmell et al. 1989). These lines had stable recessive self-incompatibility suitable for breeding of hybrid cultivars. Results from recent experiments have shown that *SRK* is the sole determinant of the *S*-haplotype specificity of the stigma. The role of *SLG* is probably to enhance this recognition process (Takasaki et al. 2000).

In the present study we report on the analysis of *SLG* gene *S*-haplotypes of *Brassica napus* cultivars and *Brassica napus* doubled haploid lines.

MATERIALS AND METHODS

Registered cultivars Rasmus, Zorro, Navajo, Lirajet, Mohican, Laser, Capitol, Pilot, Ramiro, Cando, Catonic, Jesper, Sonata, Arabela, Slapská Stela, Solida, Westar (control), doubled haploid SI lines Start, WRG and quality donor 2051 of oilseed rape were analysed. Seed of the cultivars was obtained directly from the breeding stations Opava and Slapy. DH lines were regenerated via a microspore embryogenesis procedure from the SI plants in the Research Institute of Crop Production in Prague.

Genomic DNA of oilseed rape cultivars and DH lines was extracted from young leaves of 2-week-old seedlings by the DNeasy Plant Mini Kit (QIAGEN).

PCR reaction was performed with class-I *SLG*-specific oligonucleotide primers PS5 and PS15 or class-II *SLG*-specific oligonucleotide primers PS3 and PS21 (Nishio et al. 1996). The amplified DNA fragments were digested with *Mbo*I and *Afa*I. Restriction fragments were analysed using agarose gel electrophoresis and stained with ethidium bromid.

For determination of nucleotide sequences, PCR fragments were extracted from gel with QIAquick Gel Extraction kit QIAGEN and ligated with kit TOPO TA Cloning (Invitrogen). The insert of the expected size was analysed using PCR-RFLP and individual clones were sequencing. Sequencing reaction was prepared with Cycle Sequencing Ready Reaction kit (Applied Biosystem). Sequence analyse was performed on the ABI PRISM 310 (Perkin Elmer).

RESULTS

According to the phenotype tested SI DH lines Start and WRG should have recessive type of self-incompatibility. The dominant gene was not detected in SI DH lines. A single DNA fragment (approximately 1.3 kb) was amplified in the 17 *Brassica napus* cultivars and (SC) donor of quality 2051 by PCR with the class-I *SLG*-specific primers, PS5+PS15. Amplification of class-II *SLG* gene screened recessive gene in all analysed SC and SI samples. A single DNA fragment after amplification with combination of PS3 and PS21 primers have been the expected size, approximately 1.1 kb. Using polyacrylamide gel electrophoresis two fragments have been detected, above and under the 1.0 kb. Since one of which was inferred to be functional recessive *S* allele in the genome A and non-functional recessive *S* allele in the genome C. The non-functional dominant *S* allele A 10 originated from the cultivar Westar was detected using PCR-RFLP approach, with restriction endonuclease *Mbo*I, in all analysed cultivars. The results of PCR and PCR-RFLP analyses indicate that the functional recessive allele replaced the non-functional allele A10 in genome C. In A genome of all analysed plants (SC and SI) a non-functional recessive allele is probably localised.

DNA fragments amplified from DH lines Start and WRG and 2051 quality donor with the class-II SLG-specific primers PS3+PS21 were cloned and sequenced. Similarities of the nucleotide sequences of these DNA fragments to those of the recessive genes SLG S15 in *Brassica oleracea* ranged from 93 - 99 %, S2b in *B. oleracea*, 98 %, W2, 99 % and S29, 94 %.

Molecular SI analyses using detection of SLG gene were verified with the model experimental design – parents – F_1 and F_2 generation. Theoretically expected F_2 segregation ratio 3:1 (SC: SI) after crossing of SI line and SC quality donor was confirmed by molecular analyses in two model populations (Fig. 1). This indicates a recessive monogenic disposition of SI in the experimental population. But a part of selected plants in the presumed SI group proved to be semi-SC. Seed test confirmed the strength and stability of SI.

DISCUSSION

Identification of *S*-locus with *SLG* genes has been used to detect self-incompatibility. PCR amplification of *SRK* gene was eliminated during verification of pair of primers PS5+PS15 specificity. PCR amplification of *SRK* gene or *SLR2* gene can be obtained with pair of primers PS3+PS21 using for detection of *SLG* gene class II (Nishio et al. 1996). More than one fragment was detected with polyacrylamide electrophoresis after PCR amplification and these results confirmed the previous data.

Presence of two different *S*-loci in allotetraploid *Brassica napus* makes the analyses of SI more difficult. Robert et al. (1994) characterized dominant and pollen-recessive *SLG* genes in the self-compatible *B. napus* cv. Westar. Non-functional dominant A10 allele in coincidence with detection of this allele in cv. Westar (Goring et al. 1993) was demonstrated in all of 17 cultivars tested.

Although the results from the molecular analyses and seed test are not completely in coincidence, PCR amplification of class I and class II *SLG* genes appears to be suitable approach for screening incompatible individuals in rapeseed hybrid breeding programmes. The level of self-incompatibility response for present recessive *SLG* genes class II has showed to be high. Gaude et al. (1995) described recessive self-incompatibility with a strong tendency to breakdown and variability in the level of self-incompatibility response.

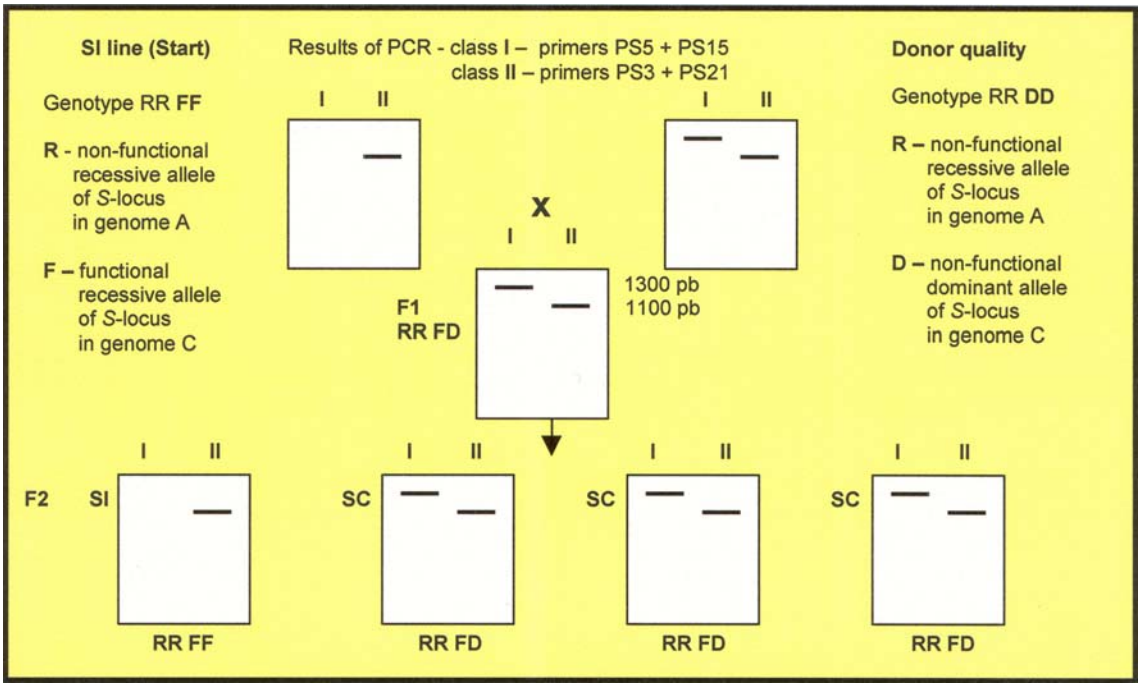

Fig.1. The model of experimental design –parents – F_1 and F_2 generation.

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