ANALYSIS OF SLG GENE – THE MOLECULAR MARKER IN HYBRID BREEDING OF OIL SEED RAPE

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ABSTRACT

Oil seed rape (*Brassica napus* L.) cultivars, donors of quality (SC) and self-incompatible (SI) lines have been analysed using identification of *S*-locus. In several *Brassica napus* cultivars one *S*-locus SLG gene was detected as dominant and the second *S*-locus as recessive. Amplification class II SLG gene screened recessive gene in all analysed samples (SC and SI). The DNA fragment of recessive gene corresponded to SLG gene W found in cv. Westar. *S*-haplotypes were analysed by PCR-RFLP. Different *Brassica napus* cultivars had an identical electrophoretic profile conforming with nonfunctional A10 allele in *B. campestris*. In *B. napus* A10 allele is localised in genome A. The functional recessive SLG gene is probably localised in genome C. Model of their segregation was suggested. SC and SI plants segregated in F_2 generation at the ratio of approximately 3:1. This indicates a recessive monogenic disposition of SI in the experimental population.

KEY WORDS: *Brassica napus* **L., Self-Incompatibility, SLG Gene, PCR-RFLP, Functional Recessive Allele**

ABSTRAKT

Autoinkompatibilita u odrůd, donorů kvality a autoinkompatibilních linií *Brassica napus* byla analyzována použitím identifikace S lokusu. U několika odrůd *B. napus* jeden *S* lokus genu SLG byl detekován jako dominantní a druhý S lokus jako recesivní. Amplifikací SLG genu třídy II byl odhalen recesivní gen ve všech analyzovaných vzorcích (autokompatibilních i autoinkomatibilních). DNA fragment recesivního genu sekvenčně souhlasil s SLG genem W, který byl objeven u odrůdy Westar. S haplotypy byly analyzovány metodou PCR-RFLP. Různé odrůdy *B. napus* měly identická elektroforetická spektra, která odpovídala nefunkční A10 alele v *B. campestris*. V *B. napus* A10 alela je lokalizována v genomu A, který pochází z *B. campestris*. Funkční recesivní alela SLG genu je pravděpodobně lokalizována v genomu C. Byl navržen model segregace. Autokompatibilní a autoinkompatibilní rostliny segregovaly v F_2 generaci v poměru přibližně 3:1. To potvrzuje recesivní monogenní založení autoinkomaptibility v experimentálních populacích.

KLÍČOVÁ SLOVA: *Brassica napus* **L., autoinkompatibilita, SLG gen, PCR-RFLP, funkční recesivní alela**

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INTRODUCTION

Self-incompatibility (SI) prevents the self-fertilization and promotes out-crossing in hermafrodite seed plants [5]. In the family *Brassicaceae*, the sporophytic form of self-incompatibility is controlled by a single multi-allelic locus termed the *S*-locus.

Molecular analysis of the *S*-locus region shows that this locus is a complex locus spaning many kilobases and containing several physically linked transcriptional units that cosegregate perfectly with SI phenotype [1, 3]. A subset of genes within the *S*-locus complex („*S* haplotype“) is highly polymorphic as expected for genes involved in recognition, and specific combinations of allelic forms of lack of these genes are thought to define different SI specificities.

Recognition of *S*-haplotype specificity has recently been shown to involve at least two *S*-locus genes, the *S*-receptor kinase (SRK) and *S*-locus protein 11 or *S*-locus Cys-rich (SP11/SCR). SRK encodes a polymorphic membrane-spanning protein kinase, which is the sole female determinant of the *S*-haplotype specificity [15]. SP11/SCR encodes a highly polymorphic Cys-rich small basic protein specifically expressed in the anther tapetum and in pollen [12, 14, 17]. While it seems that the function of SRK and SP11/SCR is relatively clear, the function of the third *S*-locus gene *S*-locus glycoprotein (SLG) is not yet known. SLG encodes an abundant, secreted glycoprotein located in the cell wall of the papillar cell of the stigma [16] and shares extensive sequence similarity with SRK extracellular *S*-domain [13]. Although it is not required for *S*-haplotype specificity of the stigma, SLG enhances the self-incompatibility response; however, how this is accomplished remains controversial [7, 12, 15].

SLG was the first identified specific *S*-haplotype protein in the *Brassica* stigma. With respect to sequence similarity, SLG genes have been classified into two groups: class I and class II [4]. Interestingly, all of the class II *S*-haplotypes identified thus far are pollen recessive, whereas all of the class I *S*-haplotypes are dominant [10]. The simple method for identifying SLG alleles - *S*-haplotypes was developed, which is called PCR-RFLP. This consists of specific amplification of SLG alleles, using the polymerase chain reaction (PCR) with a pair of SLG-specific primers, and electrophoretic analysis of the PCR products after cleavege with one or more restriction endonucleases [2, 9].

In the present study we recognized the self-incompatibility plants of oil seed rape in the F_2 generation when plants are founded in the first developmental stage (the first young leaves) so they were not damaged and could be grown to full age. The determination of self-incompatibility on the molecular levelcan speed up the process of hybrid breeding of oil seed rape.

MATERIAL AND METHODS

Registered *B. napus* cultivars ´Rasmus´, ´Zoro´, ´Navajo´, ´Lirajet´, ´Mohican´, ´Laser´, ´Capitol´, ´Pilot´, ´Ramiro´, ´Cando´, ´Catonic´, ´Jesper´, ´Sonata´, ´Arabela´, ´Slapská Stela´, ´Solida´, ´Westar´ (control), double haploid SI lines ´Start´, ´WRG´ and ´Tandem´ and quality donor ´2051´ were analysed. Seed of the cultivars was obtained directly from the breeding stations Opava and Slapy, Czech Republic. DH lines were regenerated via a microspore embryogenesis procedure from the SI plants with objective to fix SI phenotype and low content of glucosinolates in the Research Institute of Crop Production in Prague.

In 2002 plants of two populations (designated 3 and 4) were sown. These populations originated from hybridization of two plants – SI Start lines and SC Slapska Stela cultivar. SI Start line is characterized by the high stability of self-incompatibility of recessive form.

Genomic DNA of *B. napus* 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 [9]. Plant genomic DNA approximately 50 ng was mixed with a pair primers, 10 pmoles, 10x buffer (10 mM Tris HCl pH 8.3, 50 mM KCl, 3 mM $MgCl_2$, 1% Triton X-10), 100 μM dNTP, 1U DyNAzyme II Taq polymerase (Finzyme) in a final volume of 25 μl. The PCR conditions were 30 cycles of 1 min at 94°C, 2 min at 57°C and 3 min at 72°C. The amplified DNA fragments were digested with MboI and AfaI. For digestion 22 μl of PCR product was mixed with 2.5 μl of restriction endonuclease 10x buffer, respective and 0.5 μl restriction endonuclease (10 U/μl, TaKaRa). The mixture was incubated at 37°C for 2 h. Restriction fragments were analysed using agarose and polyacrylamide gel electrophoresis and stained with ethidium bromide.

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

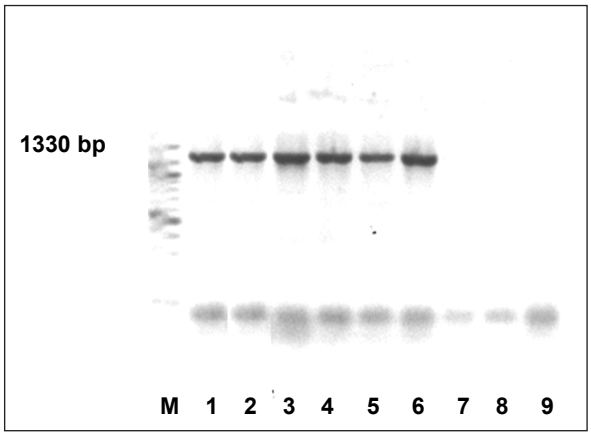

Figure 1: PCR fragments after amplification with primers PS5+PS15 for SLG gene class I cultivars – (1) Lirajet, (2) Sonata, (3) Arabela, (4) Slapská Stela, (5) Westar, (6) QD 2051, SI DH lines (7) Start, (8) WRG, (9) Tandem, M - 100bp DNA ladder

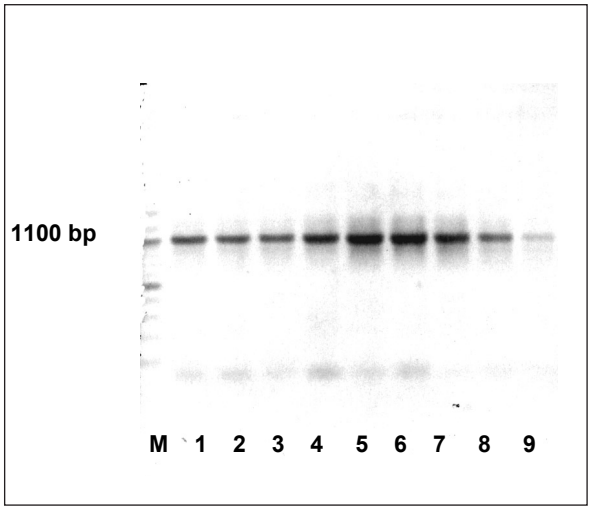

Figure 2: PCR fragments after amplification with primers PS3+PS21 for SLG gene class II cultivars – (1) Lirajet, (2) Sonata, (3) Arabela, (4) Slapská Stela, (5) Westar, (6) DQ 2051, SI DH lines (7) Start, (8) WRG, (9) Tandem, M - 100bp DNA ladder

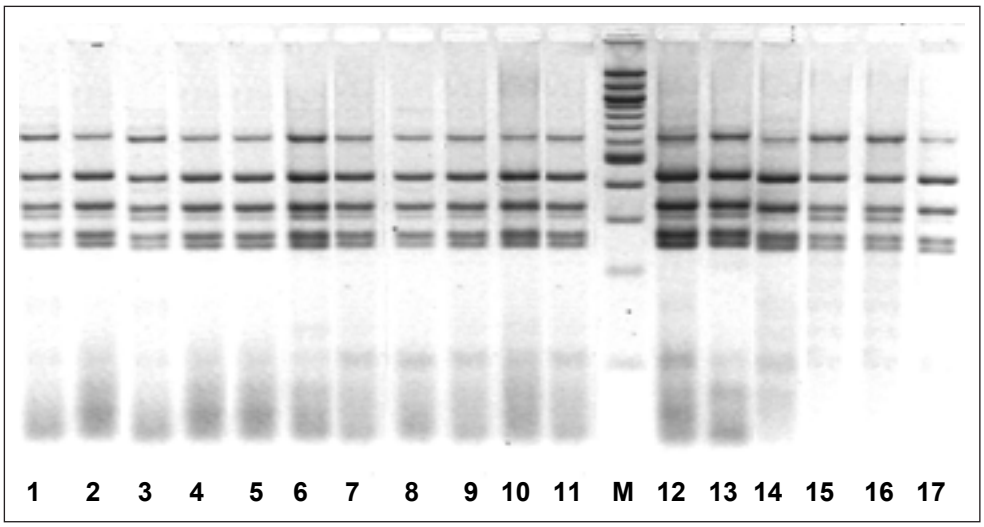

Figure 3: PCR amplification of class I SLG gene and restriction digest with MboI in different *Brassica napus* cultivars ´Rasmus´ (1), ´Zoro´ (2), ´Navajo´ (3), ´Lirajet´ (4), ´Mohican´ (5), ´Laser´, ´Capitol´ (6), ´Ramiro´ (7), ´Pilot´ (8), ´Ramiro´ (9), ´Cando´ (10), ´Catonic´ (11), ´Jesper´ (12), ´Sonata´ (13), ´Arabela´ (14), ´Slapska Stela´ (15), ´Solida´ (16), ´Westar´ (17), M - 100 bp DNA ladder

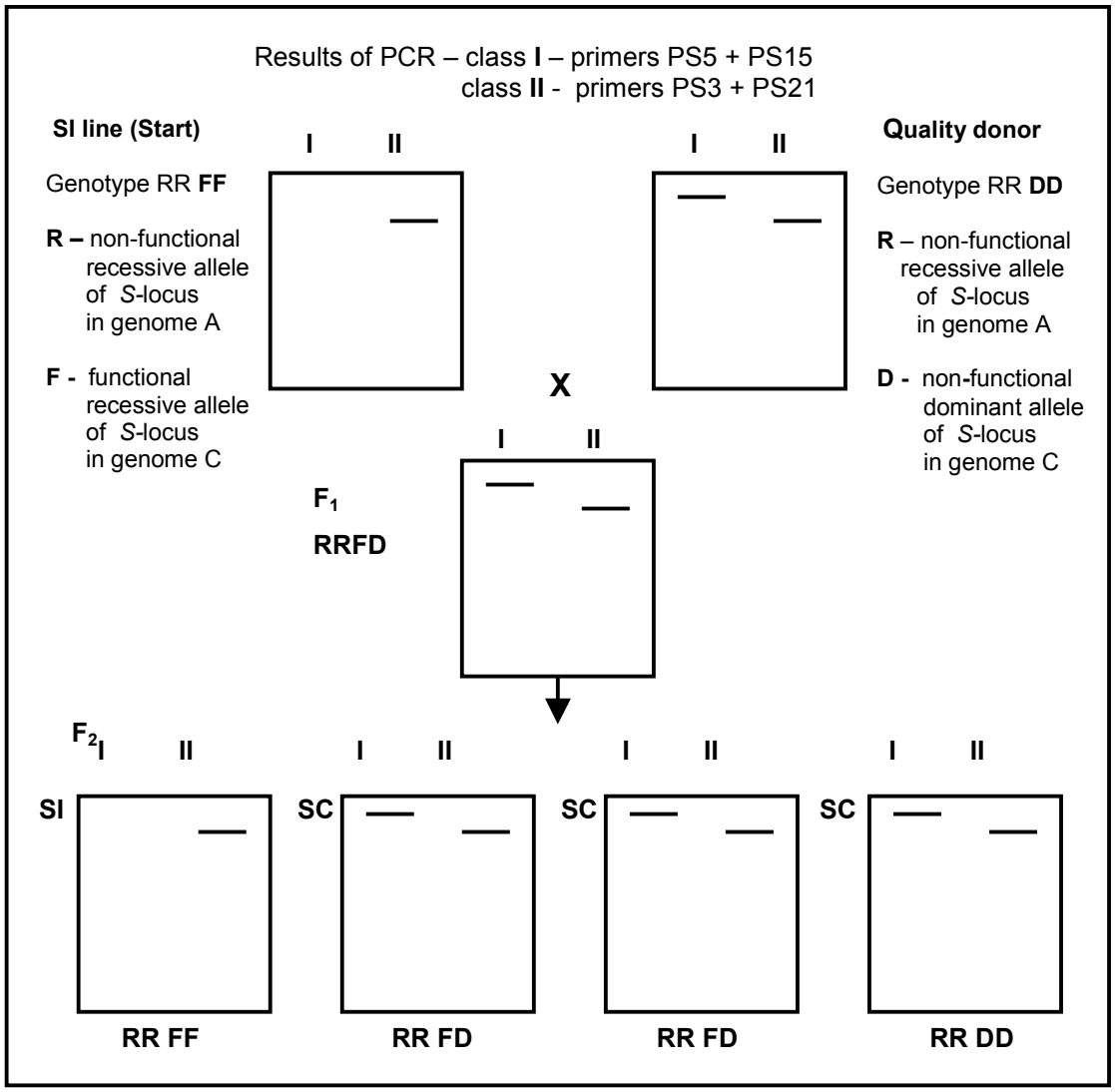

Figure 4: Segregation of *S*-locus in F_2 for case when dominant *S*-locus of quality donor and *S*-locus AI line are in different genomes (Sobotka 2001)

RESULTS AND DISCUSSION

According to the phenotype tested SI DH lines ´Start´, ´WRG´ and ´Tandem´ 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 (Figure 1). 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 [8].

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 of the expected size, approximately 1.1 kb (Figure 2). Using polyacrylamide gel electrophoresis two fragments have been detected, above and under the 1.0 kb. Since one of which was inferred to be a functional recessive *S* allele in the genome A and a non-functional recessive S allele in the genome C. Presence of two different *S*-loci in allotetraploid *Brassica napus* makes the analyses of SI more difficult. Robert et al. [11] characterized dominant and pollen-recessive *SLG* genes in the self-compatible *B. napus* cv. ´Westar´. The non-functional dominant S allele A 10 originated from cultivar ´Westar´ was detected using PCR-RFLP approach, with restriction endonuclease MboI, in all analysed cultivars (Figure 3). 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 that of the recessive genes *SLG* S_{15} in *Brassica oleracea* ranged from 93% to 99%, S_{2b} in *B. oleracea*, 98%, W2, 99% and $S_{29,}$ 94% .

Molecular analyses of SI using the detection of *SLG* gene were verified with the model experimental design – parents – F_1 and F_2 generation. Theoretically expected F_2 segregation ration 3:1 (SC:SI) after crossing of SI line and SC quality donor was confirmed by molecular analyses in two model populations (Figure 4). This indicates a recessive monogenic disposition of SI in the experimental population. Seedling test confirmed the strength and stability of SI.

Although results from the molecular analyses and seedling test are not 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 programs. The level of self-incompatibility response for present recessive *SLG* genes class II have showed to be high. Gaude et al. [6] described recessive self-incompatibility with a strong tendency to breakdown and variability in the level of self-incompatibility response.

CONCLUSIONS

Identification of SI in *Brassica napus* has been performed using analysis of *SLG* gene. Amplification class II *SLG* gene screened recessive gene in SI lines, although the dominant gene was not identified in these lines. The functional recessive *SLG* gene is probably localised in genome C. 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. 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.

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