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MARKER ASSISTED SELECTION AS A TOOL FOR DETECTION OF *BRASSICA NAPUS* PLANTS CARRYING SELF-INCOMPATIBILITY ALLELES, IN HYBRID BREEDING PROGRAMS

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ABSTRACT

Self-incompatibility (SI) is one of the most important pollination systems for hybrid seed production in *Brassica napus*. In this study we performed molecular analysis of the *S* locus related genes to understand function of the male and female *S* determinants of SI plants. Specific molecular markers were used for detection of SC/SI plants in current breeding program. We studied three genes for their structure, function, role in SI reaction and dominance relationships, having effect on self-incompatibility expression in plant phenotype with the aim to find effective molecular markers for selection of SI plants to accelerate hybrid breeding process. Molecular markers based on analysis of *SLG* I, II, *SCR* I and *SRK* I can be successfully used for selection of SI plants and undesirable SC plants are eliminated at early ontogenetic stage from breeding population. Additionally comparative analysis of all of these molecular markers for individual genes can achieve successful selection of functional carriers of recessive *S* alleles during breeding programmes.

Key words: *Brassica napus*, self-incompatibility, *S* locus, *SLG*, *SRK*, *SCR*.

INTRODUCTION

Many modern plant breeding programmes are focused on production of elite F_1 hybrid cultivars. In self-pollinating plants such programmes encounter limitations to overcome and prevent pollination by own pollen and hybrid breeding is using different approaches to protect maternal plants from self-pollination. Self-incompatibility (SI) system is successfully used for the seed production of F_1 hybrid cultivars of *Brassica napus*. In comparison with cytoplasmic male sterility (CMS), SI hybrids have higher F_1 seed production without obvious negative cytoplasm effect and an abundance of restorers (Fu et al., 1975). SI prevents self-fertilization by rejecting pollen from plants with the same genotype. The specificity of pollen-stigma interaction in self-incompatibility system is controlled sporophytically by multiple alleles of a single Mendelian polymorphic locus, designated as the *S* locus (Bateman, 1955). Two female determinants, SLG and SRK, located at the *S* locus are involved in the SI plant response. A *SLG* gene (*S* locus glycoprotein) encoding SLGs by respective *S* alleles accumulated in the mature papilla cell wall, where the inhibition of self-pollen tube development occurs (Kishi-Nishizawa et al., 1990). The SLGs consisted of a cleavable signal peptide, several *N*-glycosylation sites, three hypervariable regions, and twelve conserved cysteine residues towards the C-terminus (Watanabe et al., 2003). Gain-of-function experiments have demonstrated that SLG enhances the recognition reaction of SI. The coding region of second *S*-linked gene *S* receptor kinase gene, *SRK* (Stein et al., 1991) is 2.6 kb in length and is divided by six introns (Sato et al., 2002). The expression of *SRK* was specifically detected in stigma tissues (Watanabe et al., 1994). Loss of the function of SRK was found to result in breakdown of SI (Goring et al., 1993). Extracellular S domain sequences of *SRK* alleles are similar to *SLG* sequences of the

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same class (Cabrillac et al., 1999) and contains 12 cysteine residues found in all members of the S-gene family in the *Brassicaceae*. This domain is connected via a single pass transmembrane domain to a protein kinase catalytic center. Analysis of *SRK* gene suggests that it encodes an integral membrane protein (~98 kD). The S domain plus 9 residues encoded by exon 2 would be located extracellulary, and the putative kinase domain and flanking regions would be oriented toward the cytoplasm. Of 10 potential *N* glycosylation sites, 7 would occur extracellularly (Stein et al., 1991). This structure supposes existence of receptors with possible serin/threonin kinase specificity (Nasrallah, 1997). The interaction between the S domain of *SRK* and *SCR/SP11* gene is expected to trigger a phosphorylation cascade in the papilla cell that leads to the rejection of self-pollen.

An anther-specific gene *SCR/SP11* encodes a small cysteine-rich basic protein. The sequence of SCR/SP11 contains six completely conserved cystein residues which could be important for the tertiary structure of SCR/SP11. Pollination bioassay and gain-of-function experiments have indicated that SCR/SP11 is the male S determinant. Recent biochemical analysis has suggested that SCR/SP11 operates as a sole ligand to activate its cognate SRK specifically (Watanabe et al., 2003). Because the activity of the *S* allele is controlled sporophytically, dominance relationships influence the ultimate phenotype of both the stigma and pollen. Molecular analysis has demonstrated that the dominance relationships between *S* alleles in the stigma were determined by SRK itself, but not by the relative expression level. In contrast, in the pollen, the expression of *SCR/SP11* from the recessive *S* allele was specifically suppressed in the *S* heterozygote, suggesting that the dominance relationships in pollen were determined by the expression level of *SCR/SP11* (Watanabe et al., 2003).

Because the *S* locus consists of genes encoding both female and male *S* determinants within one segregational unit, and each gene comes in many versions, or alleles produces a different version of the same protein, different *S* allele combination of these genes are referred to as *S* haplotypes. *S* haplotypes have been classified into two groups, class I, which has been described as having high activity, showed phenotypically strong self-incompatibility characteristics in both the stigma and pollen, while class II demonstrated weak activity and was recessive to other alleles in the pollen (Thompson and Taylor, 1966). Most *B. napus* cultivars contain a class I *S* haplotype (similar to S^{47} of *B. rapa*) on the A genome and a class II (similar to S^{15} of *B. oleracea*) on the C genome (Okamoto et al., 2007; Zhang et al., 2008). Self-compatibility in *B. napus* is caused by independent mutations in dominant *S* haplotypes, which result in suppressing the functional recessive *S* haplotype on different chromosomes (Okamoto et al., 2007). As was described previously, the key determinants of SI system are the pollen-specific SCR/SP11 protein and its receptor, the pistil-specific SRK protein. Only proteins encoded by matching alleles from each *S* haplotype can interact (for example, $SCR/SP11_1$ can bind only to SRK_1), thus avoiding unnecessary pollen rejection. Normally, either *S* haplotype can cause pollen rejection if it is shared by the anther and the pistil; however, there are exceptions to the dominance of one *S* haplotype, masking the recessive *S* haplotype (Goring et al., 2010). This dominant-recessive relationship between *S* haplotypes is supposed to be due to the effect of fourth gene, *SP11 methylation inducer* (*SMI*), which is tightly linked to the gene trio mediating SI and is responsible for this altered trait. The suppression of the pollen-recessive *SP11/SCR* gene correlates with methylation of the *SCR/SP11* promoter sequence through the action of the *SMI*, small non-coding RNA (sRNA) specifically produced in the anther, causing gene silencing. Genetic changes in polyploid (amfidiploid) *B. napus*, due to the possible chromosomal rearrangements (Udall et al., 2005), epigenetic phenomena (Gaeta et al., 2007; Lukens et al., 2006) or by presence of putative suppressor locus controlling of SI via unknown mechanism, altering gene expression and phenotype should be also considered (Ekuere et al., 2004).

The aim of this study was to improve molecular tools for selection of SI/SC plants in early developmental stages and to use this approach based on application of molecular selection in hybrid breeding of oil seed rape.

MATERIAL AND METHODS

Plant material

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 glucosinolate content at the Research Institute of Crop Production in Prague, Czech Republic.

The segregating population of DH lines was derived from three crosses between SI line AIK 6 and self-compatible (SC) cultivar Rasmus, SI line AIK3 and SC line OP-571/00 and finally SI line AIK6 and SC line OP BN-03. AIK3 and AIK6 are DH lines originated from crosses between a donor of recessive self-incompatibility (naturally self-incompatible discovered plant of line Tandem) and donor of 00-quality (OP-1051) (Figure 1). 120 plants of segregating population were analyzed for SI/SC phenotype by seed test followed by a comparative analysis using molecular markers for the purpose of genotyping. The major inflorescence and two branches were bagged 3 days before flowering. The bags were tapped gently every two days to ensure sufficient self-pollination. The bags were removed after 3 weeks in order to allow the seeds to develop in a more natural environment. After the seedpods matured, the seeds and flowers produced from each bag were counted for phenotypic expression (number of seeds per siliqua so-called "seed test"; Kučera et al., 1999; Vyvadilová et al., 2008). The seed set by selfing was checked when siliquae were ripen. Plants were considered as SI when average number of seeds per siliqua was ranging from 1 to 10, as partially SI with average from 10 to 30 and completely SC with more than 30 seeds per siliqua.

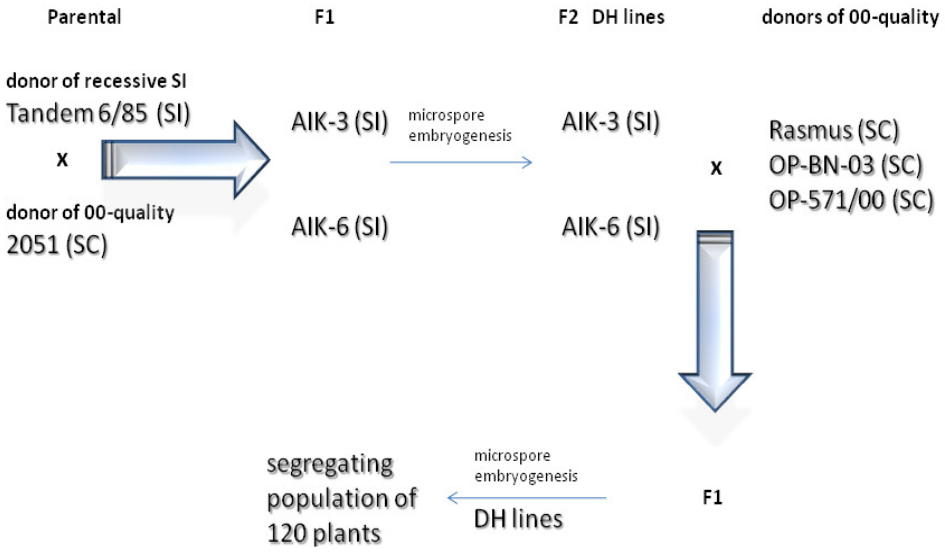

Figure 1. Production of *Brassica napus* doubled haploid lines

DNA extraction, PCR amplification DNA isolation and PCR analyses

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).

Amplification of specific fragments of *SLG, SCR* and *SRK* genes was as following: PCR was performed with 40 ng of genomic

DNA as template, mixed with 0.25 µl (50 µM) of each primer (see Table 1), 12.5 µl SYBR MM (qPCR 2x SYBR Master Mix, TopBio) and distilled water to make a final volume of 25 µl.

Table 1. Sequences of the *S* locus specific primers

Primer	Nucleotide sequence	Source
SLG		
PS5	5'-ATGAAAGGCGTAAGAAAAACCTA-3'	Nishio et al., 1996
PS15	5'-CCGTGTTTTATTTTAAGAGAAAGAGCT-3'	Nishio et al., 1996
PS3	5'-ATGAAAGGGGTACAGAACAT-3'	Nishio et al., 1996
PS21	5'-CTCAAGTCCCACTGCTGCGGG-3'	Nishio et al., 1996
SCR		
2a	5'-TTGGACTTTGACATATGTTC-3'	Žaludová et al., 2007
2b	5'-CTCTGAAGTGGGTTTTACAG-3'	Žaludová et al., 2007
F	5'-GCGAAAATCTTATATACTCATAAG-3'	Shiba et al., 2002
R	5'-TTCGTTGATCAATTATGATT-3'	Shiba et al., 2002
SRK		
PK1	5'-CTGCTGATCATGTTCTGCCTCTGG-3'	Nishio et al., 1997
PK4	5'-CAATCCCAAAATCCGAGATCT-3'	Nishio et al., 1997

PCR reaction with class I *SLG*-specific primers PS5 and PS15 (Nishio et al., 1996) involved pre-denaturation for 5 min at 94°C, 35 cycles of 1 min at 93°C, 2 min at 58°C and 3 min at 72°C, finally, 10 min at 72°C. The PCR condition for *SLG* II gene was performed as described Nishio et al. (1996).The genomic structures of *SCR* gene class II were determined by using a set of primers designed from the 5' and 3' ends of the coding region of SRK_{60} (Shiba et al., 2002). The PCR conditions were 45 cycles of 30 s at 94°C, 30 s at 55°C and 1 min at 72°C. These primers were also used for amplification of cDNA under following RT-PCR conditions: 42 cycles of 93°C for 30 s, 45°C for 30 s, 72°C for 1 min and one cycle of 72°C for 5 min resulting in ~350 bp fragment which was cloned, sequenced and used for primer designing (2a and 2b primers) as described below. Targeted allele specific *SCR* II gene primers 2a and 2b for functional allele originating from SI line Tandem (SCRII-Tandem 2CB allele; Žaludová, 2007) were designed. PCR involved 35 cycles of 30 s at 94°C, 30 s 60°C and 1 min at 72°C.

SRK gene was amplified with class I *SRK*-specific primers; forward primer PK1 having the nucleotide sequence of the second exon of S^6 *SRK* and a reverse primer PK4 having the nucleotide sequence complementary to the fifth exon of S^6 *SRK* and PCR was performed as described by Nishio et al. (1997). All of PCR products were subjected to electrophoresis on 1.5% agarose gel in 1 × TBE buffer, and detected by staining with ethidium bromide.

mRNA isolation and cDNA synthesis

Anthers of the doubled haploid SI lines were collected from buds at 2 to 3 days before opening. Total RNA was isolated using RNasy Plant Mini Kit (Qiagen). Isolation included DNA degradation step using DNase. The mRNA was isolated from total RNA using Oligotex mRNA Kit (Qiagen). Approximately 20 µg of RNA was subjected first-strand cDNA synthesis using Omniscript (Qiagen) with an oligo$(dT)_{18}$ primer. Second strand was amplified with a set of *SCR* II specific primers (Shiba et al., 2002).

Cloning and sequencing of class II *SCR* gene

The fragments obtained by RT-PCR with *SCR* II specific primers (Shiba et al., 2002) were extracted from gel with QIAquick Gel Extraction kit (Qiagen) and ligated using TOPO TA Cloning kit (Invitrogen). For plasmid DNA extraction from bacterial cell

suspension was Plasmid Miniprep kit (Qiagen) used. Plasmid were digested with *Eco*R I to confirm inserts size and individual target clones were subsequently sequenced by using a ABI PRISM 310 sequencer.

RESULTS

Three genes were used as molecular markers for selection of self-incompatible plants. Each gene was used for a different strategy aiming to detect SI/SC plant. PCR with class I *SLG*-specific primers has resulted in approximately expected 1300 bp fragment (Figure 2). The nucleotide sequence of PS5 was taken from the 5' terminal region of the open reading frame of the S^8 *SLG* in *B. rapa* (Dwyer et al., 1991). The nucleotide sequence of PS15 was taken from 3' untranslated region of the S^6 *SLG* in *B. oleracea* and shows high similarity to the S^8 *SLG* in *B. rapa* in the corresponding region with only one nucleotide difference at the point near the 5' end (Nishio et al., 1996). Molecular marker was detected in all SC donors of 00 quality, whereas it was not present in SI donors, suggesting that AIK3 and AIK6 contained a class II *S* haplotypes and no class I *S* haplotype. The data confirmed that this fragment was specifically present in plants considered to be self-compatible. *SLG* I gene was then subsequently used for screening of 120 DH lines and its expression was compared with expression of phenotype which was characterised by seed test. 82 (78.8%) of 104 plants with a clear phenotypic expression for SC/SI trait corresponded with *SLG* I gene molecular marker and for the rest of 22 (21.2%) plants detection by this marker was unsuccessful (Figure 2). However these results support the possibility of using *SLG* I gene as useful selective marker, which can be used for first-step based selection of potential breeding material, with the aim to reduce number of plants for following experiments. Screening with specific primers to the class-II SLGs resulted in amplification of single DNA fragment having expected size approximately 1.0 kb. The fragment was detected in the SI homozygous lines negative to class-I *SLG* primers, confirming their expected class II/II type. It is assumed, that self-incompatibility in *B. napus* is caused by independent mutations in dominant *S* haplotypes, which result in suppressing the functional recessive *S* haplotype on different chromosomes (Okamoto et al., 2007). Amplicons of *SLG* II gene detected in donor plants of 00-quality used for second cycle of crossing indicating, that these donors have both class I and class II *S* haplotypes except of one plant (2051), used in first cycle of crossing, where ~1.3 kb DNA fragment was amplified. As in the case of Nishio et al. (1996), this plant was inferred to be *SLR*2. These results are in accordance with deduction that in most SC *B. napus* cultivars, the class I *S* haplotype is derived from *B. rapa* S^{47}, and the class II *S* haplotype from *B. oleracea* S^{15}.

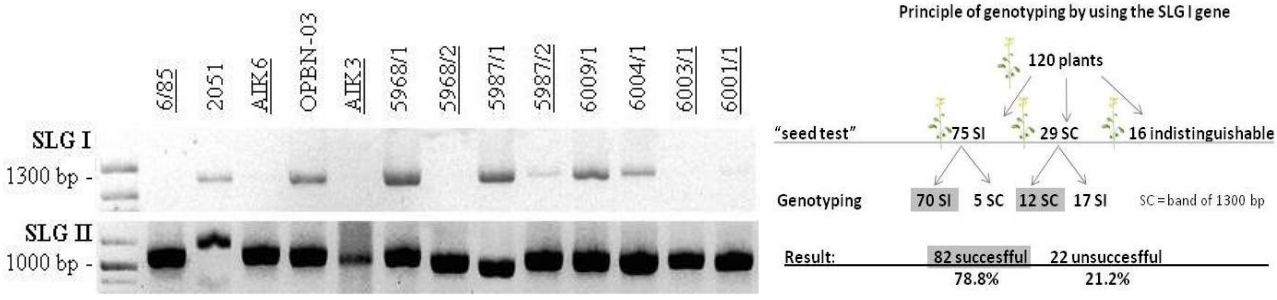

Figure 2. Amplification of class I *SLG* gene. PCR with class I *SLG*-specific primers (PS5 and PS15) has resulted in approximately 1300 bp fragment which are specifically present in plants considered to be self-compatible (SC). This molecular marker reported greater than 78% of successful detection of SI/SC genotype in 104 tested DH lines. PCR with specific primers to the class-II SLGs resulted in amplification of single DNA fragment having expected size approximately 1.0 kb in all plants (except of the samples 2051), detecting the presence of recessive *S* haplotype and causing inability to use this marker system for SI/SC detection. Self-incompatible plants are underlined.

Second marker system was based on the use of functional allele of essential gene in SI reaction and this approach was specifically targeted on alleles of class II *SCR* gene. PCR using *SCR* II primers (Shiba et al., 2002) designed from coding region of *SRK60* resulted in 450bp amplicons which were present in all tested plants and did not lead to successful differentiation of SC/SI plants. These primers were subsequently used for RT-PCR analysis from anthers of plants with clear SI/SC genotype. This analysis revealed, that this genomic region is expressed as 326 bp fragment in anther tissue and produces a secreted cysteine-rich small basic protein, as shown in Figure 3. Alignment of obtained sequences led to the discovery of the presence of two different specific alleles for SC and SI genotype of *SCR* II gene. The sequence similarity between these two alleles was 95% and comparison with database NCBI showed for both SC/SI sequences 99 and 95% identity with *Brassica rapa* S^{60}-SP11 haplotype respectively, but in case of SC sequence 99% identity was also observed for *B. oleracea* S^{15}-SP11 haplotype. This could suggest that recessive class II *S* haplotype of SI plants originates from *A* genome of *B. rapa* and for SC plants due to the large similarity especially in sequence for primers may come from C genome of *B. oleracea*.

According to the evolutionary model of *Brassica* SI proposed by Uyenoyma (2000) a low survival rate of a newly arisen mutation and less divergence among alleles in class II SCR/SP11 than in class I was predicted. The amino acid sequence of the S^{60}-SP11 (the GenBank accession number S^{60}-SP11: AB067446) and SC sequence were found to be almost similar (F amino acid instead of I), whereas SI sequence showed some highly variable amino acid residues, suggesting that these sites are targets of the selection and are probably functionally important for SI determination. Unlike SC sequence, where was the change in one amino acid present in the *SCR* coding region, for SI sequence these changes cover also signal peptide and transmembrane region (Figure 3). Across all the differences in amino acids of both sequences (SC and SI), they still contain eight cysteine residues, a glycine residue between C1 and C2 and an aromatic residue between C3 and C4 that are conserved. However for both SI/SC sequences unlike of the present signal peptide and transmembrane region no matches for the present *SCR* gene were found in the InterPro Scan database.

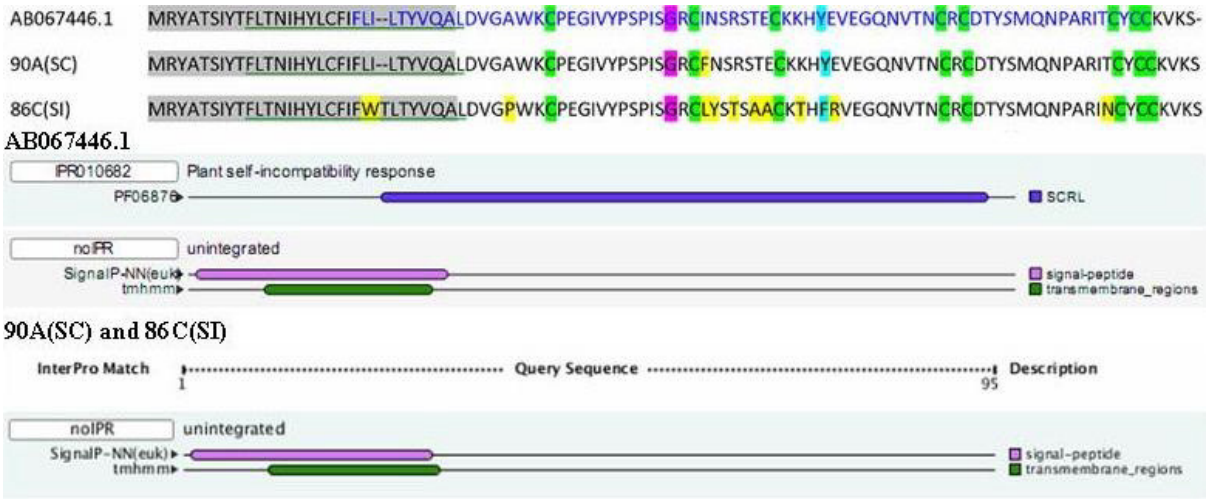

Figure 3. Alignment of the predicted amino acid sequences of three allelic variants of class II *SP11* composed of signal peptide (gray boxes), transmembrane domain (underlined letters) and *SCR* gene region (blue letters). Gaps (hyphens) were introduced to optimize the alignment. Conserved amino acid residues, eight cysteine residues (C1 to C8 in green boxes), a glycine residue (purple boxes), and aromatic amino acid residue (blue boxes) are marked. Mutations of single alleles are highlighted with yellow boxes (InterProScan, version 4.8; European Bioinformatic Institute).

From this two particular alleles obtained from SI/SC plants, allele-specific primers in their variable region have been derived (2a and 2b, Žaludová, 2007).

These primers were used for screening of segregating population and a single ~ 280 bp band was obtained in SI plants only (Figure 4). The amplicon has a 149 bp long intron between the transmembrane domain and a protein kinase catalytic center. A sequence analysis of the RT-PCR product revealed that the intron is precisely spliced out at the same position as it is in other *SCR* alleles, such as in the case of 90 bp intron of S_{60}-SP11 presented in study of Shiba (2002).

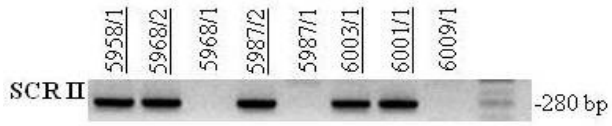

Figure 4. PCR screening of segregating population with allele-specific primers (2a and 2b, Žaludová, 2007) led to amplification of a single ~ 280 bp band present in SI plants only. Self-incompatible plants are underlined.

Third marker system was focused on analysis of *SRK* gene. Primer pair PK1 and PK4 was used for amplification of polymerphic DNA fragments from *SRK* gene of many *S* haplotypes class I. PCR reaction using this primer pair gave a single DNA fragment approximately 950 bp detecting all SI plants (Figure 5), as in the case of class I *SLG* specific primers.

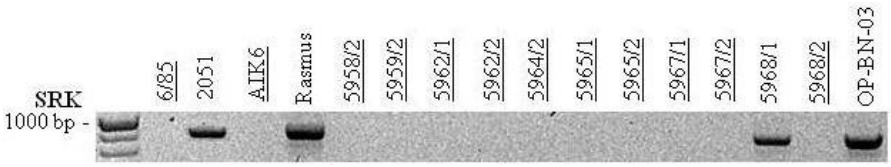

Figure 5. Amplification of *SRK* gene by using PK1 and PK4 primers. DNA fragments ~ 950 bp detected self-compatible plants. Self-incompatible plants are underlined.

The inability to amplify class-II *SRK* may be due to either homology with the primer sequences at the corresponding sites, or to the long first or the third intron (Tantikanjana et al., 1993; Zhang et al., 2008). This marker detected plants with dominant *S* haplotype which suppresses the functional recessive *S* haplotype.

Comparison of selection success of these three molecular markers and summary of length and presence of amplified bands are given in table 2.

Table 2. Three molecular markers in (Tandem × 2051) AIK3 and AIK6 derived F_2 DH population and (AIK3 × OP-771-00; AIK6 × Rasmus; AIK6 × OP-BN-03) F_2 DH population. The numbers represent amount of the plants discriminated by using these primers

Length of PCR product					genotyping of segregating population		
			F_2		F_2		
	Tandem	2051	AIK3 AIK6	Rasmus OP-BN-O3 OP-571/00			
phenotype	SI	SC	SI	SC	SC	SI	indifferent
SLGI (PS5/PS15)	-	1300 bp	-	1300 bp	12 (29*)	5 (75*)	(16*)
SCRII (2a/2b)	280 bp	-	280 bp	-	- (29*)	75 (75*)	(16*)
SRK I (PK1/PK4)		950 bp		950 bp	12 (29*)	5 (75*)	(16*)

*The number plants classified by seed test

DISCUSSION

Specific primers PS5/PS15, described as a class I *SLG* (Nishio et al., 1996), co-segregated with SC phenotype, whereas no amplicon was obtained in recessive type of SI plants. These results corresponded with the results of some other studies (Dolanská and

Čurn, 2004; Zhang et al., 2008). Even if the *SLG* gene is believed to enhance the SI phenotype by stabilizing SRK (Dixit et al., 2000; Takasaki et al., 2000), it was proved, that SLG is estimated not to be necessary for self-recognition (Kusaba and Nishio 1999; Suzuki et al., 2000). Several reports show that *SLG* was mutated or deleted in some *S* haplotypes of self-incompatible *B. oleracea* (Okazaki et al., 1999; Suzuki et al., 2000) or *B. rapa* (Suzuki et al., 2003). However, the *S* haplotypes lacking *SLG* are a minority in *Brassica*.

If SLG functions as a stabilizer of the SRK-SP11 complex, one of the possible explanations of the normal SI phenotype of SI plants is replacement of *SLG* I gene in form of observed recessive form of *SLG* II gene with its proper function, or the existence of a substitute for the SLG protein such as eSRK. The regulation of the stable SI phenotype in the SLG-less *S* haplotypes is one of the most interesting unsolved questions that should be clarified in the near future.

The SCR allelic specific marker was developed after analyses of SI line derived from Tandem. This approach was supposed to be more accurate than application of *SLG* marker gene. The amplified fragment corresponding to recessive allele of *SCR/SP11* II gene (280 bp) detected all SI DH plants derived after second cycle of crossing (see Figure 4 and Table 2). For such a selective detection of SI plants allele-specific primers were used and SI plants with "*Tandem SI alleles*" were specifically detected. Due to the finding, that the dominant/recessive relationship between class I and class II *S*-haplotypes in the determination of pollen phenotype is generated at the level of *SCR/SP11* transcription, it is likely, that dominant class I *SCR/SP11* gene does not cause silence of class II *SCR/SP11* gene in studied population probably due to its malfunction, or due to some changes in its regulation. In any case when using SCR allelic specific marker system one should take into account that the allele specific primers were designed on the basis of sequences obtained from specific plant material and, given the *SCR* polymorphic nature, it is evident that they cannot easily amplify single alleles in different cultivars.

For the specific amplification of *SRK* DNA, primer pair PK1 and PK4 was used. This molecular marker showed the same degree of success as marker for class I *SLG* gene detection. These results confirm the assumption of Nishio et al. (1997) that the tight linkage between *SLG* and *SRK* is hardly ever, or never, broken at meiosis. The low possibility of recombination between *SLG* and *SRK* indicates that selection of SC plants with either *SLG* or *SRK* is adequate for the identification, but genotyping with the second marker system can be used as an additional control of efficiency of selection.

It can be concluded, that the used molecular markers, based on analysis of *SLG* I, II, *SCR* I and *SRK* I, can be successfully used for selection of SI plants and undesirable SC plants can be eliminated at early ontogenetic stage. Additionally comparative analysis of all of these molecular markers for individual genes can achieve successful selection of functional carriers of recessive *S* alleles during breeding programmes. However, further molecular analyses on the function of *S*-locus-related genes are necessary, because several reports show that other unknown factors might be involved in SI response (Tarutani et al., 2010).

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REFERENCES

Bateman, A.J., 1955. *Self-incompatibility systems in angiosperms*: III. *Cruciferae*. Heredity, 9: 53-68. DOI: 10.1038/hdy.1955.2.

Cabrillac, D., Delorme, V., Garin, J., Ruffio-Chåble, Giranton, J.-L., Dumas, C., Gaude, T., Cock, J.M., 1999. *The S15 self-incompatibility haplotype in Brassica oleracea includes three S gene family members expressed in stigmas*. Plant Cell, 11: 971-986. DOI: 10.1105/tpc.11.5.971.

Dixit, R., Nasrallah, M.E., Nasrallah, J.B., 2000. *Post-transcriptional maturation of the S receptor kinase*

of Brassica correlates with co-expression of the S-locus glycoprotein in the stigmas of two Brassica strains and in transgenic tobacco plants. Plant Physiol., 124: 297-311. DOI: 10.1104/pp.124.1.297.

Dolanská, L., Čurn, V., 2004. *Analysis of SLG gene – the molecular marker in hybrid breeding of oil seed rape*. Journal Central European Agriculture, 5: 23-28.

Dwyer, K.G., Balent, M.A., Nasrallah, J.B., Nasrallah, M.E., 1991. *DNA sequences of self-incompatibility genes from Brassica campestris and B. oleracea: polymorphism predating speciation.* Plant Mol. Biol., 16: 481-486. DOI: 10.1007/BF00024000.

Ekuere U.U., Parkin I.A., Bowman C., Marshall D., Lydiate D.J., 2004. *Latent S alelles are widespread in cultivated self-compatible Brassica napus.* Genome, 47: 257-265. DOI: 10.1139/g03-120.

Fu, T.D., Liu, H.L., 1975. *Preliminary report on the breeding of self-incompatible lines of Brassica napus.* Oil Crops China, 4: 77-85.

Gaeta R.T., Pires J.C., Iniguez-Luy F., Leon E., Osborn T.C., 2007. *Genomic changes in resynthesized Brassica napus and their effect on gene expression and phenotype.* Plant Cell, 19: 3403-3417. DOI: 10.1105/tpc.107.054346.

Goring, D.R., Galvin, T.L., Schafer, U., Rothstein, S.J., 1993. *An S receptore kinase gene in self-compatible Brassica napus, has a 1-bp deletion.* Plant Cell, 5: 531-539. DOI: 10.1105/tpc.5.5.531.

Goring, D., Indriolo, E., 2010. *Gene expression: How plants avoid incest*. Nature, 466: 926-928. DOI: 10.1038/466926a.

Kishi-Nishizawa, N., Isogai, A., Watanabe, M., Hinata, K., Yamakawa, S., Shojima, S., Suzuki, A., 1990. *Ultrastructure of papilla cells in Brassica campestris revealed by liquid helium rapid freezing and substitution-fixation method*. Plant Cell Physiol., 31: 1207-1219.

Nasrallah, J.B., 1997. *Evolution of the Brassica self-incompatibility locus: A look into S-locus gene polymorphisms.* Proc. Natl. Acad. Sci. USA, 94: 9516 -9519. DOI: 10.1073/pnas.94.18.9516.

Kučera, V., Vyvadilová, M., Tomášková, D., 1999. *Development of self-incompatible double low winter oilseed rape lines by means of double haploid system*. Proceeding of 10th International Rapeseed Congress. University Canberra, p. 183.

Kusaba, M., Nishio, T., 1999. *Comparative analysis of S haplotypes with very similar SLG alleles in Brassica rapa and Brassica oleracea*. Plant J., 17: 83-91. DOI: 10.1046/j.1365-313X.1999.00355.x

Lukens, L., Pires J.C., Leon E., Vogelzang R.D., Oslach L., Osborn T., 2006. *Patterns of sequence loss and cytosine methylation within a population of newly resynthesized Brassica napus allopolyploids.* Plant Physiology, 140: 336-348. DOI: 10.1104/pp.105.066308.

Nasrallah, J.B., Nasrallah, M.E., 1993. *Pollen-stigma signaling in the sporophytic self-incompatibility response.* Plant Cell, 5: 1325-1335. DOI: 10.1105/tpc.5.10.1325.

Nishio, T., Kusaba, M., Sakamato, K., Ockendon, D.J., 1997. *Polymorphism of the kinase domain of the S-locus receptor kinase gene (SRK) in Brassica oleracea L.* Theor Appl Genet, 95: 335-342. DOI: 10.1007/s001220050568.

Nishio, T., Kusaba, M., Watanabe, M., Hinata, K., 1996. *Registration of S alleles in Brassica campestris L by the restriction fragment sizes of SLGs.* Theor. Appl. Genet., 92: 388-394. DOI: 10.1007/BF00223684.

Okamoto, S., Odashima, M., Fujimoto, R., Sato, Y., Kitashiba, H., Nishio, T., 2007. *Self-compatibility in Brassica napus is caused by independent mutations in S-locus genes.* Plant J., 50: 391-400. DOI: 10.1111/j.1365-313X.2007.03058.x.

Okazaki, K., Kusaba, M., Ockendon, D.J., Nishio, T., 1999. *Characterization of S tester lines in Brassica oleracea: polymorphism of restriction fragment length of SLG homologues and isoelectric points of S locus glycoproteins.* Theor. Appl. Genet., 98: 1329-1334. DOI: 10.1007/s001220051199.

Sato, K., Nishio, T., Kimura, R., Kusaba, M., Suzuki, T., Hatakeyama, K., Ockendon, D.J., Satta, Y., 2002. *Coevolution of the S-locus genes SRK, SLG and SP11/SCR in Brassica oleracea and B. rapa*. Genetics, 162: 931-940.

Suzuki, T., Kusaba, M,. Matsushita, M., Okazaki, K., Nishio, T., 2000. *Characterization of Brassica S haplotypes lacking S-locus glycoprotein.* FEBS Lett, 482: 102-108. DOI: 10.1016/S0014-5793(00)02048-2.

Suzuki, G., Kakizaki, T., Takada, Y., Shiba, H., Takayama, S., Isogai, A., Watanabe, M., 2003. *The S haplotypes lacking SLG in the genome of Brassica rapa.* Plant Cell Rep., 21: 911-915. DOI: 10.1007/s00299-003-0598-5.

Shiba, H., Iwano, M., Entani, T., Ishimoto, K., Shimosato, H., Che, F-S., Satta, Y., Ito, A., Takada, Y., Watanabe, M., Isogai, A., Takayamaa, S., 2002. *The Dominance of Alleles Controlling Self-Incompatibility in Brassica Pollen Is Regulated at the RNA Level.* The Plant Cell, 14: 491-504. DOI: 10.1105/tpc.010378.

Stein, J. C., Howlett, B., Boyes, D. C., Nasrallah, M. E., Nasrallah, J. B., 1991. *Molecular cloning of a putative receptor protein kinase gene encoded at the self-incompatibility locus of Brassica oleracea.* Proc. Natl. Acad. Sci. USA, 88: 8816-8820. DOI: 10.1073/pnas.88.19.8816.

Takasaki, T., Hatakeyama, K., Suzuki, G., Watanabe, M., Isogai, A., Hinata, K., 2000. *The S receptor kinase determines self-incompatibility in Brassica stigma.* Nature, 403: 913-916. DOI: 10.1038/35002628.

Tantikanjana, T., Nasrallah, M.E., Stein, J.C., Chen, C-H., Nasrallah, J.B., 1993. *An alternative transcript of the S-locus glycoprotein gene in a class-II pollen-recessive self-incompatibility haplotype of*

Brassica oleracea encodes a membrane-anchored protein. Plant Cell, 5: 657-666. DOI: 10.1105/tpc.5.6.657.

Tarutani, Y., Shiba, H., Iwano, M., Kakizaki, T., Suzuki, G., Watanabe, M., Isogai, A., Takayama, S., 2010. *Trans-acting small RNA determines dominance relationships in Brassica self-incompatibility*. Nature, 466: 983-986. DOI: 10.1038/nature09308.

Thompson, K.F., Taylor, J.P., 1966. *Non-linear dominance relationships between S alleles*. Heredity, 21: 345-362. DOI: 10.1038/hdy.1966.36.

Udall, J.A., Quijada, P.A., Osborn, T.C., 2005. *Detection of chromosomal rearrangements derived from homeologous recombination in four mapping populations of Brassica napus L*. Genetics, 169: 967-979. DOI: 10.1534/genetics.104.033209.

Uyenoyama, M.K., 2000. *Evolutionary dynamics of self-incompatibility alleles in Brassica*. Genetics, 156: 351-359.

Vyvadilová, M., Klíma, M., Kučera, V., 2008. *Metodika produkce dihaploidních linií pro šlechtění řepky ozimé*. VURV, V.V.I., Praha – Ruzyně: 1-27.

Watanabe, M., Takayama, S., Isogai, A., Hinata, K., 2003. *Recent progresses on self-incompatibility research in Brassica species*. Breeding Science, 53: 199-208. DOI: 10.1270/jsbbs.53.199.

Watanabe, M., Takasaki, T., Toriyama, K., Yamakawa, S., Isogai, A., Suzuki, A., Hinata, K., 1994. *A high degree of homology exists between the protein encoded by SLG and the S receptor domain encoded by SRK impatible Vrassica campestris L*. Plant Cell Physiol., 35: 1221-1229.

Zhang, X., Ma, C., Fu, T., Li, Y., Wang, T., Chen, O., Tu, J., Shen, J., 2008. *Development of SCAR markers linked to self-incompatibility in Brassica napus L*. Mol. Breeding, 21: 305-315. DOI: 10.1007/s11032-007-9130-x.

Žaludová, J., 2007. *The structure and the function of the S-locus in oilseed rape (Brassica napus L.)*. Ph.D thesis. JU ZF, České Budějovice.