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DECLARATION

This research work reported in this Ph.D. thesis was done by me under guidance and supervision of doc. Ing. Vladislav Čurn, Ph.D., Biotechnological Centre, Faculty of Agriculture, University of South Bohemia in Ceske Budejovice as a one of the requirements for the degree of Doctor of Philosophy in Specialized Plant Production.

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Jana Žaludová

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1. INTRODUCTION

Oilseed rape breeding in Czech Republic currently has several problems. The lack of financial funds limit the application and development of new and modern breeding methods. So the all breeding station in the Czech Republic dealing with oilseed rape breeding have unified into association termed 'The Czech Oilseed Rape' and this association is in close cooperation with Research Institute of Crop Production in Prague-Ruzyně and Biotechnological Centre of Faculty of Agriculture of University of South Bohemia. This association take advantage of different area of application and synthetisize all possibilities of single workplaces. As a result of 10 years common effort in 2006 new cultivar 'Oponent' was registred.

Nowdays, the promissing plant breeding firms are concerning on biotechnology methods, it means genetic engineering techniques, generating doublehaploids to obtain homozygote lines, plant genome mapping including comparative mapping, candidate gene searching, and finally the application in breeding process: selection of plants with appropriate qualitative trait according to breeding aims.

The main task of breeding is to reduce the costs. Creating of molecular marker or mapping agronomical traits is time and money-consuming comparing with traditional approaches. But it could reduce the costs required for selection performed by traditional techniques, which requires large amount of handwork and extra space for growing of the residual unsuitable plants.

Aim of this dissertation was to find a marker gene connected with SI reaction in order to select SI plant from segregating population after crossing SI line with SC donor of quality. Self-incompatibility is a system how to prevent self-pollination events. Productivity of hybrid cultivars predict them to be in great demand and hybrids on SI basis can be created. Present cultivars have lack of variability due to intensive selection. Considering that S-genes, genes required for SI, are very polymorphic, it should be contributing to detect S-alleles in common cultivar without regard to SI/SC phenotype in order to assess variability among cultivar. Collaboration with 'The Czech Oilseed Rape' especially with RICP and breeding station Opava enabled the possibility to study unique breeding material. Application of knowledge from molecular analysis in practical breeding would help to give us an idea how useful and profitable marker selection in Czech conditions could be.

2. AIMS OF THE Ph.D. THESIS

This work consists of six studies focused on self-incompatibility and its genetic disposition. In nineties, the S-locus, the locus determinig SI, was properly examined, especially its three genes, *SRK* female determinant, *SLG*, previously considered to be a pollen determinant and later proved to have not yet specified function enables SI reaction facilitate, and finaly *SCR* gene, the elusive pollen determinant that was as the latest found. The previous studies on SI, that have been done in ZF JU, were focused on *SLG* gene analysis. As soon as the pollen determinant was found, there was a possibility to obtain specific molecular marker based on a gene for essential protein in SI reaction.

Main aims of Ph.D. thesis:

1. To develop molecular marker for recessive type of SI

The hypothesis supposed the *SCR* gene, which is very important in SI recognition reaction, has in SC plants some defective allele. With the purpose to find out the defective allele, cloning and sequencing of *SCR* gene in *Brassica napus* was planned to carry out. The oligonucleotide structure of both alleles should be compared and the possible changes should serve as an information for marker development.

2. To compare *SLG* I marker that tags SC plants with developed SI marker

The positive function of *SLG* gene is up to now not known and furthemore was proven that SLG protein is not essential for SI reaction although. In contrast, the SCR is essentia in SI reaction. So the task is whether the *SCR* marker gene should not select SI genotypes more preciously than the *SLG* marker gene.

3. To apply markers in marker-assisted selection

The application of both marker genes in practical breeding will be realiesed in co-operation with 'The Czech Oilseed Rape' association. The efficiency and profit of selection using molecular markers would be disscused.

4. Assessment of variability in oilseed rape with aspect to SLG II polymorphism

The *SLG* gene, as well as the other genes on *S*-locus, is highly polymorphic. A huge amount of S-alleles was found in *B. oleracea* and *B. rapa* using the cross test and also molecular

techniques, such as PCR-RFLP of *SLG* gene, or *S*-genes sequencing. Cloning and sequencing of *SLG* II gene in different cultivars and SI lines would give us some knowledge about distribution of S-alleles in spectrum of common rapeseed cultivars. According to progressive evolution of S-alleles in relative species we can assess a variability of courent rapeseed cultivars and an influence of the specified agronomic trait orientated selection.

3. APPLICATION OF MOLECULAR MARKERS IN *BRASSICA NAPUS* BREEDING

Genetic maps: Brassica napus genome mapping and applications genetic maps in breeding

Genetic linkage map is essential tool for development of molecular markers linked with agronomic important traits in *B. napus.* High density maps can be created through different molecular markers which are evaluated together. Acquired data are used for physical maps construction. Multiple segregating populations enable mapping of a larger number of loci, thus providing a higher number of potencial useful markers in various genetic backgrounds (Piquemal et al., 2005).

A number of dense RFLP-based genetics linkage maps have been developed for *B. napus* (Landry et al. 1991; Ferreira et al. 1994; Uzunova et al. 1995). Over the past few years a number of new PCR-based marker technologies such as RAPDs, AFLPs and microsatellites have been developed and applied to crop plants, *B. napus* included. SSR markers are mostly derived from *B. rapa*, *B. nigra*, *B. oleracea* and *Arabidopsis thaliana.*

Other way to identify linked markers, discover gene and/or quatitative trait loci is comparative mapping in order to consensus genetic markers development. The well-known plant physiology and genetic backgroung of A. thaliana offers new opportunities for better understanding the *Brassicaceae*, the same taxonomic family. Six loci sequenced from *A. thaliana* were amplified using degenerated primers and direct sequencing PCR (Brunel et al. 1999). Comparative mapping of *Brassica* species have been carried out for *B. oleracea* with *B. rapa* and *B. napus* (Teutonico and Osborn 1994), for *B. oleracea* with *B. napus* (Cheung et al. 1997), and for synthetic *B. napus* and *B. oleracea* and *B. rapa* (Lyndiate et al. 1993).

Analysis of *B. napus* segregating populations using molecular markers shows homeologous exchanges, such as homeologous nonreciprocal translocations (Sharpe et al. 1995, Parkin et al. 1995) or a homeologous reciprocal transposition (Osborn et al. 2003). Chromosomal rearrangements caused by homeologous recombination seem to be widespread in *B. napus*. The effects of chromosomal rearrangements on allelic and phenotypic diversity are suggested (Udall et al. 2005).

In *B. napus*, many genetic maps based on single populations were reported (Landry et al. 1991; Ferreira et al. 1994; Parkin et al. 1995; Sharp et al. 1995; Uzunova et al. 1995;

Foisset et al. 1996; Cheung et al. 1997). Genetic maps based on multiple populations were generate to cover whole rapeseed genome. Parkin and Lydiate (1997) designated an integrated map based on RFLP markers using two populations with three different parents. Lombard and Delourme (2001) used three populations to construct a map using RFLP, AFLP or RAPD markers. In addition, Udall et al. (2005) aligned RFLP maps from four independent mapping populations. Genetic maps are usually used to localize genes and QTLs involved in various agronomic traits as well as transgenes (Baranger et al. 1997) or interspecific introgressions (Barret et al. 1998a; Delourme et al. 1998).

Candidate genes

An amplified consensus gene markers are developed by sequencing of homologous genes from various species of the same phylogenetic family and the detection of intragenic polymorphism. The constant progress in plant physiology and genetic knowledge from the *Arabidopsis thaliana* projects bring new opportunities to apply this knowledge to *Brassica* which belongs to the same taxonomic family: the *Brassicaceae*. Amplified consensus gene markers (ACGMs) that amplified gene sequences from *Arabidopsis thaliana* and *Brassica napus* (Brunel et al. 1999, Fourmann et al. 2002). To find appropriate candidate genes expressed sequence tags (ESTs) approach were used. *Arabidopsis thaliana* expressed sequence tags (ESTs) with homology to cloned plant genes were used for disease-resistance genes.

Application of molecular markers in hybridisation event

Hybrid cultivars of oilseed rape were successful used in agriculture for their significant yield increase. One of the most useful application of molecular markers in seed purity control of hybrid varieties (Marshal et al. 1994). Hybridisation and introgression frequency between domesticated *B. napus* and wild relatives is assessed partialy due to the potential risk of transgenes escaping from genetically modified rapeseed to wilde populations of related weeds. Introgression in a mixed weedy population of oilseed rape and its relative *B. rapa* was investigated using species-specific AFLP markers (Hansen et al. 2001). Further progress of introgression was studied on offspring from the maternal plants from the

introgressed population with the same AFLP markers and species-specific chloroplast marker (Hansen et al. 2003). Hybridisation rate was also judged using SSR-PCR and primers specific for either C or the A genomes in progeny from *B. napus* and *B. rapa* crosses (Pallett et al. 2006). Mikkelsen et al. (1996) verified interspecific hybridisation of progeny of *B. napus* crossed with B. campestris backcrossed to *B. campestris* using *B. napus*-specific RAPD markers. *B. napus* specific markers were also applied to determine hybridisation between *B. juncea* and *B. napus* hybrid with *B. juncea* as a female partner (Frello et al. 1995).

Genetic diversity analyses

Oilseed rape is one of the most important oilseed crop and intesive breeding has led to comparatively narrow genetic basis in current breeding material. The gene pool of elite oilseed rape breeding material has been considerably reduced by the emphasis on specific quality traits. As a consequence, genetic variability is restricted with regard to many characters of value for breeding puposes. Halldén et al. (1994) compared *B. napus* breeding lines with RFLPs and RAPDs, while Diers and Osborn (1994) compared RFLP paterns in 61 winter and spring rapeseed genotypes and concluded that the two forms constitute two genetically different groups. Molecular markers analyses confirmed that winter and spring rapeseed genotypes constitute two genetically different groups (Becker et al. 1995, Diers et al. 1996). Seyis et al. (2003) described genetic diversity in a large set of resynthesised rapeseed lines and spring rape varieties. Hasan et al. (2006) used publicly available mapped SSR markers from *B. napus* genome to characterise a set of 96 selected genotypes from *B. napus* core colection developed from European gene bank material. This material were devided into four general groups: spring oilseed and fodder, winter oilseed, winter fodder and vegetable genotypes. The molecular genetic information enables the identification of untapped genetic variability for rapeseed breeding and is potentially interesting with respect to increasing heterosis in oilseed rape hybrids.

Cultivar registration

The current testing system of new oilseed rape cultivars is based on the assessment of a range of standardised morphological characters, which is expensive and time-

consuming.Each candidate variety must be shown to be distinct from others of common knowledge, and to be sufficiently uniform and stable in diagnostic characteristics use to demonstrate distinctness. Halldén et al. (1994) used RFPL markers to compare three *B. napus* breeding lines and found that a minimum of 30 probe/enzyme combinations were required to distinguish reliably between them and to describe their relationship. This approach requires relatively large quantities of sample DNA and moreover, identifying of suitable probe/enzyme combinations is often time-consuming. In contrast, RAPD analysis is a simple, quick and convenient procedure requiring much smaller quantities of template DNA. RAPD markers have been successfuly applied in *B. napus* and *B. rapa* cultivars identification, however to find stable polymorhic markers generated with RAPD is difficult (Mailer et al. 1994). Among the avaible DNA molecular techniques, AFLP is powerful technique for cultivar identification (Powell et al. 1996). As compared with RFLP for which polymophism among cultivars is low (Diers and Osborn 1994), AFLPs have very attractive qualities for DUS testing in rapeseed. Lombard et al. (2000) evaluated discrimination power of 17 AFLP primer combinations tested on a colection of 83 spring and winter rapeseed cultivars. The use of only two primers combinations was sufficient to identify uniquely all the cultivars. Primers complementary to SSRs and with variable three-base 'anchors' at their 5' end, were used in PCR analyses to compare pooled DNA samples from various *B. napus* and *B. rapa* cultivars. Anchored SSR-PCR analysis is highly informative and reproducible methodfor fingerprinting oilseed rape populations, but there is intra-cultivar polymorphism considerably varied between cultivars. Intra-cultivar variation should be investigated before using banding profiles from pooled samples for the identification of individuals (Charters et al. 1996). Three multiplex SSR sets composed of five markers each were used measure the extent of diversity within and between a set of ten varieties. The significance of any corelation between SSRs, pedigree and five of the morphological characters currently used for statutory distinctness, uniformity and stability testing of rapeseed varieties. Varieties were separated in three groups corresponding to winter, spring and forage types. These results suggested that SSRs could be used for pre-screening or grouping of existing and candidate varieties, allowing the number of varieties that need to be grown for comparision to be reduced (Tommasini et al. 2003).

Molecular markers application in oilseed rape breeding

Male sterility

There are several ways avaible for hybrid breeding in *Brassica* species, including cytoplasmic male sterility (CMS), genic male sterility (GMS), self-incompatibility (SI), and chemical hybridizing agens (CHA).

There are several kinds of GMS, such as a dominant genic male sterility (DGMS) (Li et al. 1985, 1990) and recessive genic male sterility (RGMS) (Hou et al. 1990, Li et al. 1993, Tu et al. 1997). The sterility of DGMS line is conditioned by the interaction of two genes, i.e. dominant genic male sterility (*Ms*) and the suppressor gene (*Rf*) (Li et al. 1988). Lu et al. (2004) applied AFLP method to identify *Ms* locus. Also Song et al. (2006) found AFLP markers tightly linked with to the male sterility allele (*Ms*) and moreover developed SCAR marker specific to the restorer allele (*Rf*).

Ke et al. (2005) subjected to AFLP analysis a recessive epistatic genic male sterility two-type line, in which is sterility controlled by two pairs of recessive duplicate sterile genes (*ms1* and *ms2*) interacting with one pair of recessive epistatic inhibitor gene (*rf*). Seven markers tightly linked to one recessive genic male sterile gene (*ms*) were identified.

Cytoplasmic male sterility (CMS) is often considered to be a nuclear-mitochondrial, which can be suppressed with specific nuclear genes, termed restorers of fertility (Hanson 1991). There are four main sources of CMS in *B. napus*: the *nap*, *ogu*, *pol* and *tour* cytoplasms (Fu and Yang 1998). Jean et al. (1997) identified the linkage group containing the locus of *Rfp1* restorer for the *pol* cytoplasm from cultivar 'Polima' (Fu 1981).

The CMS originally found in radish (*Raphanus sativus* L.) by Ogura (1968) has been transferred to the *B. napus* by interspecific crosses (Bannerot et al. 1974). Delourme et al. (1994) employed bulked segregant analysis to RAPD markers linked to the restorer gene (*Rfo*) used in the 'Ogura' radish cytoplasmic male sterility. DNA marker was linked to the restorer gene with polymorphic DNA fragments being associated either with the fertility restorer allele or with sterility maintainer allele. Data from RAPD markers analysis of F_2 oilseed rape population originating form a single cross between a female parent homozygous for the restorer allele and carring the 'Ogura' male-sterile cytoplasm, and a male parent homozygous for non-restorer allele and having normal cytoplasm were applied to marker-assisted selection

(Hansen et al. 1997). Bulked segregant analysis and comparative mapping were also applied to map *Rfo* restorer gene used for the *Ogu*-INRA cytoplasmic male-sterility system and it was conluded that the introgression of the linkage group connected with CMS from radish had occurred through homoeologous recombination (Delourme et al. 1998). The *tournefourtii* cytoplasmic male-sterility requires two dominant genes, one major (*Rft1*) and another minor (*Rft2*) to achieveve complete fertility restoration.

Self-incompatibility

Self-incompatibility is an important mechanism of genes transfer due to cross-pollination (outbreeding) in many species of flowering plants. The most applied method of *S*-haplotype determination is a polymerase chain reaction restriction fragment length polymophism (PCR-RFLP), which is used to analyse polymorphism of three main *S* genes, *SLG*, *SRK*, and *SCR*, on the *S*-locus. Amplified PCR products of genomic DNA using *S*-locus sequences specific primers were digested with different restriction enzymes and separated by agarose gel electrophoresis to detect polymorphism (Brace et al., 1993; Brace et al., 1994; Nishio et al., 1996; Nishio et al., 1997; Park et al., 2001; Park et al., 2002). This type of marker has also been desribed as cleaved amplified polymorphic sequences (CAPS) (Koniecny and Ausubel, 1993). Nishio et al. (1996) classified 18 out of 27 test lines of *B. rapa* after digestion with *Afa*I of the PCR products amplified with the class I primer. *SLG*s showed high sequence homology with the *S*-locus related genes (*SLR*1 and *SLR*2), which is not linked linked with the *S*-locus (Watanabe, et al., 1992). This results suggest that *SLG* is a not good marker of *S*-haplotypes. PCR-CAPS analysis of *SRK* was successfully used for for identifying *S*-haplotypes (Park et al., 2002). Currently, the SCR gene is for its more polymorphic character than *SRK* gene frequently used. Self-incompatible plant in a segregatin F_2 population were distinguished by CAPS markers using *S*-locus specific primers (Möhring et al., 2005).

Disease resistence

Blackleg, caused by *Leptosphaeria maculans*, in one of the most demanding desease of oilseed rape. The introduction of resistence genes in the oilseed rape genome has been done by sexual crosses or by asymmetric protoplast fusion. The presence of resistance genes in interspecific hybrids was controlled by RAPD (Chevre et al., 1997) or RFLP (Plieske et al.,

1998). Pilet et al. (2001) derived genetic maps from two doublehaploid populations arising from crosses resistant and susceptible oilseed rape cultivars using RAPD and RFLP markers.Different genetic background and and different disease infestations levels afected the inconsistencies observed between populations. Plieske and Struss (2001) converted RFLP and RAPD markers linked with B-genome blackleg resistance into STS markers for use in marker-assisted backcrossing of the introgressed trait in *B. napus*. Resistance-associated B-genome-specific randomly amplified (RAPD) and resistance gene analog (RGA) DNA polymorphismus were converted into three specific markers in spring oilseed rape carrying B genome from *B. juncea* (Saal and Struss, 2005). The resistence genes originating from 'Darmor' cultivar were mapped into two genomic regions on LG10 and LG16 linkage groups (Delourme et al. 2004).

Turnip mosaic virus (TuMV) is major virus infecting *Brassica* crops. A *TuRB01* gene is a dominant gene, that confers extreme resistance to some isolates of TuMV on *Brassica napus.* RFLP linkage map were constructed and *TuRB01* were located on linkage group N6 of the *B. napus* A-genome indicating that the gene probably originated from *B. rapa* (Walsh et al. 1999). One AFLP and six microsatellite markers were associated with the resistence locus, named *TuRB03*, and these mapped to the same region on the chromosome N6 as a previously mapped TuMV resistance gene *TuRB01* (Hughes et al. 2003).

Genes of turnip yellow virus (TuYV), which is responsible for a recognizable loss of yield, were mapped using AFLP markers and one major quatitative locus was found, explaining up to 50% of phenotypic variaton (Dreyer et al. 2001).

Oil content/quality

In oilseed rape, genetic control of linolenic acid has been reported as a three additive loci (Chen and Beversdorf 1990), three loci with cytoplasmic effects (Rajcan et al. 2002) and five QTL based on molecular mapping information (Burns et al. 2003). Genetic control of oleic acid in *B. napus* in *B. napus* has been reported to be determined by four QTL (Burns et al. 2003).

Ecke et al. (1995) used a F_1 microspore-derived DH population for analysis of distribution of erucic acid and seed oil content. Three different linkage groups were located representing three QTLs for seed oil content. Two of the QTLs for oil content showed a close association to the two erucic genes. Rajcan et al. (1999) found RAPD marker significantly associated with the erucic acid level and oil control. Using degenerate PCR primers designed

on the *Arabidopsis* and rapeseed *FAE1* sequences, two B. napus genes *BN-FAE1.1* and *BN-FAE1.2*, corresponding to parental species B. rapa and B. oleracea *FAE1* genes, were amplified. These two genes are considered to be co-segregating with *E1* and *E2* loci controlling erucic acid content (Fourmann et al. 1998). Fatty acid elongase genes were cloned from B. campestris and B. oleracea and their effects on erucic acid levels were described (Das et al. 2003).

Tanhuanpää et al. (1995) discovered one RAPD marker tagging gene affecting linolenic acid concentration as well as Hu et al. (1995). Jourdren et al. (1996) identified twolinkage groups of 6 RAPD markers, which corresponded to two QTL loci connected with low linolenic acid content. Somers et al. (1998) identified RAPD markers associated with loci controlling linoleic acid desaturation and found *fad3* allele near one of the QTLs identified for this locus. Hu et al. (1999) also found two RAPD markers to be associated with oleic/linoleic content. Futhermore developed codomonant SCAR marker for low and high linolenic content converted from RAPD marker. Schierholt et al. (2000) mapped three AFLP markers linked to high oleic mutation, which were localised near the locus of one copy of the *fad2* gene. Hu et al. (2006) designed two single nucleotid polymorphism (SNP) markers corresponding to the *fad2* and *fad3c* gene mutations. Javidfar et al. (2006) investigated RAPD and ISSR techniques for development of molecular marker for genes controlling oleic and/or linolenic acid content. Markers that were identified were converted into SCAR markers for use in breeding.

Morphological traits

All presently grown cultivars of *B. napus* are black-seeded, and the development of yellowseed forms has been proposed as a mean to improve canola-meal quality by reducing fibre content and increasing protein content (Shirzadegan and Röbbelen 1985). Compared with black seeds, yellow seeds of *Brassica* have a significantly thinner seed coat, thereby leading to lower hull proportion in the seed and, consequently, a higher oil content (Stringam et al. 1974). Some other advantages of yellow seeds include a moretransparent oil and a higher protein and lower fibre content. a better feeding value for livestock (Tang et al. 1997; Meng et al. 1998). Somers et al. (2001) analysed a double-haploid population derived from F_1 generacion of the cross 'Apollo' (black-seeded) × YN90-1016 (yellow-seeded) *Brassica napus*. A single major gene (*pigment* 1) flanked by eight RAPD markers co-segregating with the yellow seed coat colour trait in the population explained over 72% of the phenotypic variation in seed coat colour. In addition, two additional genes were revealed suggestin there

is a dominant, epistatic interaction between the *pigment 1* locus and the two additional genes. Zhi-wen et al. (2005) found seed colour to be under control of the maternal genotype and the yellow seed trait to be partially dominant over the black seed trait. Segregation analysis revealed a single gene locus for the partial dominance over the black seed trait. Two RAPD markers and five AFLP markers were revealed to be linked to the allele for yellow seed while the three AFLP markers were linked to the allele for the black seed.

Pod lengh is an imporatnt parameter invoved in total yield in *Brassica napus*. Hawkins et al. (2005) found that long pods are controlled by two independent genes in a dominant relationship with additive effects. Two linkage groups that segregate with the long pod trait were identified.

A dwarf trait is interesting with regard to the level of resistence to lodging and yield performance. Genetic analysis of dwarf trait revealed that it is under control of a single mutated gene (*Bzh*) with an additive effect. Difficulties appeared in the accucurate determination of homozygous (dwarf; *bzh/bzh*) and heterozygous (semidwarf; *Bzh/bzh*) plants in segregating progenies. In order to overcome these difficulties, molecular mapping of the dwarf locus was initiated. A linkage map was constructed around *Bzh* gene. The nearest flanking marker, named OPM07-730, was linked at 0.8 ± 0.7 cM (Foisset et al. 1995). In order to obtain a specific co-dominant PCR-based marker, Barret et al. (1998b) transformed OPM07-730 into a PCR-based SCAR marker.

Abiotic stress

Winter survival is an important characteristic of oilseed rape and it is supposed to be affected by genetic variation for other cold-regulated traits, such as freezing tolerance and vernalization flowering time. Corespondence in the map positions of the QTL controlling different traits within species provided evidence that some alleles causing greater (Kole et al. 2002). The other mapped trait is boron efficiency. A major gene controlling the boron-efficiency trait was mapped in the ninth linkage group of *Brassica napus*. At the same time, three minor loci at three linkage groups were also detected (Xu et al. 2001).

Tabular summary

Table bellow summarize utilization of molecular markers of important agronomical traits in *Brassica napus*.

Tab. 1. Examples of some important traits in *Brassica* species where genetic markers to map loci or clone genes have been used.

Character	Specific trait	Molecular marker	Selected references
Morphological traits	Pod length	RAPD	Hawkins et al. 2005
	Plant height (dwarf locus)	SCAR	Barret et al. 1998
			Foisset et al. 1995
	Seed colour	RAPD	Somers et al. 2001
		RAPD, AFLP	Zhi-wen et al. 2005
	Flowering time		Ferreira et al. 1995c
	Petal-less flower		Fray et al. 1997
Disease resistance	Blackleg (*Leptosphaeria maculans*)	RAPD	Delourme et al. 2004
		RAPD	Chevre et al. 1997
		RAPD, RFLP	Plieske et al. 1998
		SCAR, RGA (resistance gene analog)	Saal and Struss 2005
		STS	Plieske and Struss 2001
			Dion et al. 1995
			Ferreira et al. 1995a
		RAPD, RFLP	Pilet et al. 1998a,b, 2001
	White rust (*Albugo candida*)		Ferreira et al. 1995b
			Kole et al. 2002b
	Turnip mosaic virus (TuMV)	AFLP, SSR	Hughes et al. 2003
		RFLP	Walsh et al. 1999
	Turnip yellows virus (TuYV)	AFLP	Dreyer et al. 2001
	Sclerotinia sclerotiorum		Zhao and Meng 2003
Male sterility	'Ogura' fertility restorer	RFLP, RAPD	Delourme et al. 1998
		RAPD	Hansen et al. 1997
		RAPD	Delourme et al. 1994
	'Polima' fertility restorer	RFLP, RAPD	Jean et al. 1997, 1998
	tournefortii fertility restorer	AFLP	Janeja et al. 2003
	CMS from *A. thaliana*	RFLP	Leino et al. 2003
	Recessive genic male sterility	AFLP	Ke et al. 2004, 2005
	Dominant genic male sterility	AFLP	Lu et al. 2004, Song et al. 2006
Self-incompatibility			
Abiotic stress	Cold tolerence, winter hardiness	RFLP, AFLP	Kole et al. 2002a
Oil content / quality	Erucic acid content	RFLP	Ecke et al. 1995
		RAPD	Rajcan et al. 1999
	Glucosinolate content	RFLP	Uzunova et al. 1995
	Oleic acid content	SCAR, RAPD	Hu et al. 1999
		AFLP	Schierholt et al. 2000
		RAPD, ISSR, SCAR	Javidfar et al. 2006
	Linoleic acid content	SCAR, RAPD	Hu et al. 1999
	Linolenic acid content	RAPD	Hu et al. 1995
		RFLP, RAPD	Tanhuanpää et al. 1995
		RAPD	Jourdren et al. 1996
		RAPD	Somers et al. 1998
	Seed glucosinulates	RFLP	Uzunova et al. 1995
			Howell et al. 2003

4. SELF-INCOMPATIBILITY IN *BRASSICA NAPUS*

Self-incompatibility is a mechanism which can prevent self-pollination. Oilseed rape is important crop and current oilseed rape breeding is focused on hybrid breeding in order to increase the yield. Self-incompatibility could be used successfully for this purpose due to use of two different self-incompatible lines. The advantage is an easy propagation of self-incompatible lines after spraying with weak salt solution to overcome self-incompatibility.

Hybrid breeding of oilseed rape

Brassica napus is one of the most important source of edible oil. At present, hybrid cultivars have higher productivity than convenctional ones and their seed quality (contents of erucic acid and glucosinulates) has also been graetly improved (Fu, 2000). Therefore, the breaders are interested in development of commercial F_1 hybrids of *Brassica* species. There are several ways avaible for hybrid breeding in *Brassica* species, including cytoplasmic male sterility (CMS), genic male sterility (GMS), self-incompatibility (SI), and chemical hybridizing agens (CHA). In comparison with other ways for hybrid breeding, SI is regarded as one of the most valuable strategies for the following reasons: (1) it does not have any of the negative effects that exist in male sterility system; (2) it can be easy overcome; and (3) it can be selected easily in a breeding programme.

Sporophytic and gametophytic self-incompatibility

Self-incompatibility is an important mechanism of genes transfer due to cross-pollination (outbreeding) in many species of flowering plants. According to SI recognition there are two forms: gametophytic (GSI) and sporophytic (SSI). In GSI type the self-incompatible reaction is controled by haploid pollen genotype, whereas in SSI type it is controled by anther diploid genotype of parental plant. So basic difference is that pollen of SSI type carries two S alleles instead of one. This enables SSI type various dominance interactions among S-alleles independently occuring in pollen as well as in stigma. Molecular mechanism of GSI type is based at least on two different pathways, while in SSI it is known only one signaling pathway (Franflin-Tong and Franklin, 2003).

In evolutionary-advanced families such as the *Brassicaceae*, *Poaceae* and *Asteraceae,* establishment of pollen compatibility occurs in stigma surface where incompatible grains will either fail to germinate or, if the pollen tubes are produced, these will fail to penetrate (de Nettancourt, 1977). These species representing SSI tend to have 'dry' stigmas, in contrast to species with GSI such as *Nicotiana* and *Petunia* where the stigma surface is covered with sticky secretions, ideal for capture and subsequent development of pollen grain. In these latter species, incompatible pollen is not inhibited at the stigmatic surface but in the stylar transmitting tract following pollen germination (Dodds et al., 1996). Apart from SI, inter-species incompatibility, preventing gene flow among species to restrict outbreeding, usually occurs unilaterally, and is known as unilateral incompatibility (UI) (Hiscock and Dickinson, 1993). In some plants of *Brassica rapa,* the pollen factor of UI was not linked to the *S*-locus although UI phenotype closely resembled that of SI (Takada et al., 2005).

Difference between GSI and SSI type could be in timing of *S*-genes activation: in sporofytic system, SI-related genes are activated before meiosis and in gametofytic system after meiosis (Pandey,1958). In GSI, S-glycoproteines are present in style and shows RNase activity which represents 40 to 80 % of RNase activity of total extracts in *Nicotiana alata* (Jahnen et al., 1989). This phenomenon is not unversal, for example S-glycoprotein have no RNase activity in poppy (Franklin-Tong et al., 1991). *S*-glycoproteins are present on the surface of the stigmatic papillae and the extracellular matrix that separates the files of transmitting tract cells of the style. *S*-RNases are also present in ovary where are in single cell layer of the inner epidermis of the placenta (Anderson et al., 1989). Polen tube is in direct contact with RNase throughout its growth through the stigma and style to the ovules (Jahnen et al., 1989).

After genetic background investigation of *Brassica* species SI was found out that SI is under control of S-locus with many *S*-alleles (Ockendon, 1974; de Nettancourt, 1977). Single alleles of S-locus were devided according their phenotype effects into two classes, class I and class II supposed to be dominant and recesive, respectively (Nasrallah et al., 1991). Recently many review articles focused on molecular mecanismus of sporofytic SI in *Brassica* species were published (Watanabe et al., 1999; Watanabe and Hinata, 1999; Watanabe et al., 2000;Watanabe et al., 2001; Takayama and Isogai, 2003; Isogai and Hinata, 2002; Kachroo et al., 2002; Takayama and Sakagami, 2002; Watanabe et al., 2003).

Male determinant

Even though the female determinant was known, the pollen determinant was always elusive. Candidate for the pollen determinant was predicted to have several characteristics. As well as *SLG* and *SRK* gene it should be located at the *S*-locus. The pollen grain would have showed doubled haploid character in S-haplotype of male determinant as it is required in sporophytic form of SI. To fulfill this condition pollen determinant should be expressed bebore meiosis in pollen mother cells or expressed later in the tapetum cells. (Watanabe et al., 2003).

Characterization of the coating has revealed the presence of several families of gametophytically expressed small cysteine-rich proteins (PCPs-pollen coat proteins). PCP-A class proteins have specific affinities for stigmatic and S- and S-related proteins (Doughty et al., 2000).

Two independent studies have revealed a gene for pollen determinant at the same time. SCR (S-locus cysteinerich protein; SCHOPFER ET AL., 1999), syn. SP11 (SUZUKI ET AL., 1999) is expressed in anthers only. Anther tapetum cells produce SCR protein that bind with a high affinity to SLG irrespestive of S-genotype.(Doughty et al., 1998; Takayama et al., 2000). Putative pollen determinant fulfilled all requirements such as highly polymorphic character, anther-specific expression, physical linkage with SLG and SRK. *SCR* is invariably located between *SLG* and *SRK* in upstream orientation to SLG, closer to SRK than to SLG (Takayama et al., 2000). SCR encodes a small (<8 kDa) hydrophobic and positively charged peptide that exists as a monomer (Takayama et al., 2001). Based on immunostaining is suggested that S_8 SP11 was secreted from the tapetal cell into anther locule as a cluster and translocated to the pollen surface at the early developmental stadge of the anther. During the pollination proces, SP11 was translocated from the pollen surface to the papilla cell, and then penetrated the cuticle layer of the papilla cell to diffuse across the pectine cellulose layer (IWANO ET AL., 2003)

Eight conserved cysteine residues, a glycine residue, and an aromatic amino acid residue are characteristic for amino acid arragement of *SCR* gene product. *SCR* gene consist of two ORF, the first ORF for putative signal protein is higly conservative among *Brassica* species, while the second is highly polymorphic. L1 loop, disignated as the hypervariable (HV) region is the most variable region of the SCR, suggesting to be responsible for imparting the allele-specific interaction with receptors. It folds into an α/β sandwich that

resembles those of plant defensins (Mishima et al., 2003). As well as S-haplotypes the SCR gene is divided into two classes. The mRNA of class II was predominantly expressed in homozygotes, while the expression in heterozygotes was suppressed (SHIBA ET AL., 2002). The 522 bp 5' upstream region support the correct function of SCR gene (SHIBA ET AL., 2001). It was demonstrated that SCR alleles determined S-haplotype (SHIBA ET AL., 2001). Duplicated SCR found in *Brassica oleracea* S15 haplotype produced two different sizes of transkripts (Shiba et al., 2004).

SCR alleles are higly divergent (Watanabe et al., 2000). In four *Brassica rapa* class-II *S*-haplotypes, linear dominance relationship were observed. Using RNA gel blot analysis, linear dominance relationship regulation by expression of *SP11* was found (Kakizaki et al., 2003).

Female determinant

Two genes localised on the *S*-locus (*S*-locus glycoprotein - *SLG* and *S*-locus receptor kinase – SRK) were almost exclusively found in stigmas. Both genes exhibit allelic sequence diversity among *S*-haplotypes.

Molecular and biochemical studies revealed the structure of *SLG*, consisting of a cleavable signal peptide, several *N*-glycosylation sites, three hypervariable regions, and 12 conserved cysteine residues located towards the C-terminus (Takayama et al., 1987; Nasrallah et al., 1987). SLG is not essential for this response but may enhanse the self-incompatibility response for some *S* haplotypes (Takasaki et al., 2000; Suzuki et al., 2000; Silva et al., 2001).

SRK consists of a signal peptide, an SLG-like predicted extracellular domain (*S*-domain), a transmembrane domain, and an intracellular serine/threonine kinase domain (Stein et al., 1991). Takasaki et al. (2000) reported that transgenic plants expressing the SRK^9-transgene acquired the S^9 haplotype specificity in the stigma, but not in the pollen. In contrast, the transgenic plants expressing the SLG^9-transgene did not acquire the S^9 haplotype specificity in the stigma nor in the pollen. These data clearly show that *SRK* is the sole determinant of the *S* haplotype specificity of the stigma (Takasaki et al., 2000). By analogy with animal receptor kinases, SRK is generally thought ton function as a dimer, although this has not been shown experimentally (McCubbin and Kao, 2000). Other possible explanation could be that SRK is active as a homodimer but it is also active as a heterodimer in combination with SLG molecule (Takasaki et al., 2000).

Structural organization of the *S*-locus

The *Brassica S*-locus is composed of all components that specify an *S*-haplotype. For SI to be maintained, these compponents must remain as a tightly linked genetic union. Physical maps have been constructed of the *S*-locus region of several *S*-haplotypes from *B. oleracea* (Boyes et al., 1997), *B. rapa* (Boyes et al., 1997; Suzuki et al., 1999), and *B. napus* (Cui et al., 1999). Using different techniques (direct sequencing, cDNA selection techniques, and RNA differential display), a number of genes have been identified in the *S*-locus region (Cui et al., 1999; Suzuki et al., 1999; Casselman et al., 2000).

Comparison of two *S*-regions revealed that the gene organization downstream of *SLG* in both *S*-haplotypes is highly co-linear. Distance between *SLG* and *SRK* genes in different *S*-haplotypes is highly variable. Intervening region is filled with retroelements and haplotype-specific genes (Cui et al., 1999). Cui et al. (1999) also suggested that the *SLG* downstream region might be one border of the *S*-locus and that the accumulation of heteromorphic sequences may act as a mechanism to suppress recombination between *SLG* and *SRK*. This have been confirmed by comprehensive recombinational and physical mapping analyses of the *S*-locus of the S_8-haplotype of *Brassica campestris* (Casselman et al., 2000).

Self-incompatibility signaling pathway

*SP11*s is predominantly expressed in the tapetum cells of anther locules, and accumulated on the pollen surface during pollen maturation. Upon self-pollination, SP11 molecules penetrate the papilla cell wall and interact with the receptor complex containing SRK molecules. The ligand-receptor interaction and receptor activation occur in an *S* haplotype-specific manner.

To date, SI downstream regulation is not clearly understood. There are three different molecules interacting with the kinase domain (SRK-KD) and/or transmebrane domain of SRK (SRK-TM). Two kinds of proteins (THL1/THL2 and ARC1) were isolated and characterized using a yeast two hybrid system with SRK-KD as a bait (BOWER ET AL., 1996; GU ET AL., 1998). This interaction induces the autophosphorylation of the SRK molecule. The ARC1 (arm-repeat-containing protein 1) protein bound to the SRK-KD with phosphorylation-dependent manner (GU ET AL., 1998). Suppression of ARC1 production in the stigma of

transgenic plants caused breakdown of SI (STONE ET AL., 1999). After the subsequent signal transduction, which is not defined, the rejection of the self-pollen occurs.

Ikeda et al. (1997) studied a self-compatible line of *B. campestris*, which has mutaions in both the *S*-locus (Nasrallah et al., 1994) and an unlinked locus, called *MOD*. The authors suggest that aquaporine like protein is encoded by the *MOD* gene propose that it regulates the availability of water at the stigma surface. In the SI response, SRK activation would lead to activation of this aquaporin-like protein and an increase in the water flow away from the pollen, thus preventing pollen hydration. However, recent molecular analysis of the candidate gene for *MOD* in several self-incompatible lines demonstrated that *MOD* locus is not real *MOD* gene (Fukai et al., 2001).

Distribution of different *S*-haplotypes

Number of *S*-alleles are estimated at 34 in *Raphanus* (Sampson, 1959), 30 in *Brassica rapa* (Nou et al., 1993), and more than 50 in *Brassica oleracea* (Brace et al., 1994), respectively. The alleles in specific *Brassica oleracea* cultivar can be indentified through test-crosses with *S*-allele tester set (Thompson, 1968; Ockendon, 1975). Generally, *S*-alleles in *B. oleracea* that are low in dominance series, are more subject to modification than alleles high in dominance series (Ockendon 1974, Smith 1977). Self-incompatibility recognition represents allele-specific interaction between highly polymorphic stigmatic *S*-locus receptor kinase (SRK) and its pollen ligand, the *S*-locus cysteine-rich protein (SCR). The specific function was shown to be determine by four contiguous amino acids in one variant, indicating that the minimum sequence requirement for gaining a new specificity can be low (Chookajorn, et al., 2004). Random mutations of the SCR persist as long as they retain capacity to bind and activate appropriate allele of SRK and thus would lead to rapid sequence diversification of SCRs within each functional allelic class. This could be a possible way how the new SI specificities were evolved (Chookajorn, et al., 2004).

In the pair of *S*-haplotypes BrS^{46} in *B. rapa* and BoS^{7} in *B. olerecea* the *SP11* alleles were found to be similar, with 96,1% identity in deduced amino acid sequence. It is considered that BrS^{46} and BoS^{7} have maintained the same recognition specificity since the divergence of these two species has occured. Amino acid substitutions bettween both the SRKs and the SP11s of BrS^{46} and BoS^{7} have no influence on recogniton specificity (Kimura et al., 2002).

Highly similar *S*-haplotypes between *B. oleracea* and *B. rapa* have been identified by Kusaba and Nishio (1999), and commonality of self-recognition specificity of the similar *S*-haplotypes between these species has been demonstrated by pollinaton test usinginterspecific hybrids, transgenic plants, and recombined SP11 proteins (Kimura et al., 2002; Sato et al., 2003).

Highly similar sequences of SP11 to those in *Brassica* were found in *Raphanus sativus* suggesting the presence of the same or similar *S*-haplotypes within different *Brassica* species and in different genera within the Brassicaceae (Okamoto et al., 2004). It has been suggested that the divergence between *B. oleracea* and *B. rapa* occurred more recently than the divergence between *R. sativus* and the two species in *Brassica* (Sakamoto et al., 1998).

Dominant / recessive allele interactions

As above, The *S*-alleles have been divided into two groups: strong dominant and week pollen-recessive alleles. The alleles in dominant group are usually dominat to all alleles in week group, while allelic interactions within each group are complex (Thompson and Taylor, 1966). When *S*-alleles are combined in an *S*-allele heterozygote, one of four different types of interaction may result. Type I interaction occurs when allele *a* is dominant over allele *b* (*a* > *b*) in both the pollen and the stigma. Type II occurs when allele *a* is dominant over allele *b* (*a* > *b*) in stigma, and *b* is codominant (*a* = *b*) in the pollen. Type III interaction occurs when *a* = *b* in the stigma and *a* > *b* in the pollen. Type IV when *a* = *b* in the stigma and the pollen (Wallace, 1979).

More complicated situacion is in resynthesised *Brassica napus* where *B. oleracea* and *B. rapa* *S*-alleles were tested. A general feature of *B. oleracea* *S*-alleles tested in *B. napus*is is an apparent weakening of *S*-alleles of *S*-alleles in the SI/SC heterozygous condition. A weakening of the *B. rapa* alleles in the SI/SC heterozygote has been also reported (Ripley and Beversdorf, 2003).

Self-incompatibility in *Brassica* breeding

In *Brassica* vegetables, the most common *S*-haplotypes found in open-pollinated populations and commercial cultivars are the pollen recessive ones (Thompson and Taylor,

1966). Thompson and Taylor (1966) suggested that intense selection for uniformity could lead to the loss of *S*-haplotypes and accumulation of recessive *S*-haplotypes. The weak self-incompatibility is undesirable in the F_1 hybrid breeding of vegetable because it results in the frequent contamination of selfed seeds in F_1 seeds. Replacement of class II haplotypes with class I is recquired to improve the quality of vegetable F_1 seeds. In contrast, this reduction in SI expression could be advantage in *B. napus*, especially if a modified three-way cross were used (Govers, 1975). Self- compatible plants can be maintained through application of an NaCl solution or use of elevated CO_2 levels at the time of self pollination (Guohua and Rui, 1986).

Oilseed rape *Brassica napus* is naturally self-compatible in contrast to its parental species that are generally self-incompatible. In general, the three diploid *Brassica* species *B. rapa* (AA, 2n = 20), *B. oleracea* (CC, 2n = 18) and *B. nigra* (BB, 2n = 16) are self-incompatible, while three amphidiploids, *B. napus* (AACC, 2n = 38), *B. juncea* (AABB, 2n = 36) and *B. carinata* (BBCC, 2n = 34) are self compatible. However, in diploid species self-compatible form occur in a few instances (citace). Similarly, in amphidiploid species, self-incompatible forms were found in oilseed rape (*B. napus* ssp. *oleifera*) (Ollson 1960, Havel, 1996) and swedes (*B. napus* ssp. *rapifera*) (Gowers, 1974). Two possibilities of self-incompatible *B. napus* achievement exist. It is either selection of SI plant from rapeseed breeding material or introduction of *S* alleles from its parental species into oilseed rape by resynthesis.

Ekuere et al. (2004) suggests that latent *S*-alleles in oilseed rape may be masked by a suppressor system common to oilseed rape. According to phenotype segregation the existence of suppressor loci, which are not linked to the *S* locus, is suggested.

Molecular markers for self-incompatility

Based on molecular markers differentiation of single *S*-alleles to determine *S*-haplotype in Brassicaceae was utilised. In *Brassica napus*, a segregating F_2 population of from a cross of self-incompatible and self-compatible parents using molecular marker for self-incompatibility should be analyzed. The most applied method of *S*-haplotype determination is a polymerase chain reaction restriction fragment length polymophism (PCR-RFLP), which is used to analyse polymorphism of three main *S* genes, *SLG*, *SRK*, and *SCR*, on the *S*-locus Amplified PCR products of genomic DNA using *S*-locus sequences specific primers were

digested with different restriction enzymes and separated by agarose gel electrophoresis to detect polymorphism (Brace et al., 1993; Brace et al., 1994; Nishio et al., 1996; Nishio et al., 1997; Park et al., 2001; Park et al., 2002). This type of marker has also been desribed as cleaved amplified polymophic sequences (CAPS) (Koniecny and Ausubel, 1993). Nishio et al. (1996) classified 18 out of 27 test lines of *B. rapa* after digestion with *Afa*I of the PCR products amplified with the class I primer. *SLG*s showed high sequence homology with the *S*-locus related genes (*SLR*1 and *SLR*2), which is not linked linked with the *S*-locus (Watanabe, et al., 1992). This results suggest that *SLG* is a not good marker of *S*-haplotypes. PCR-CAPS analysis of *SRK* was successfully used for for identifying *S*-haplotypes (PARK ET AL., 2002). Currently, the SCR gene is for its more polymorphic character than *SRK* gene frequently used. Self-incompatible plant in a segregatin F_2 population were distinguished by CAPS markers using *S*-locus specific primers (Möhring et al., 2005)

In order to determine SI recognition specificity of Brassicaceae vegetables two methods of dot-blot analysis of *SP11* alleles were developed. One is dot blotting of DNA samples, i.e. plant genomic DNA probed withlabeled $SP11^m$, and the other is dot blotting of $SP11^m$ DNA fragments probed with labeled DNA samples, i.e. the *SP11* coding region labeled by PCR using a template of plant genomic DNA. The former is useful for testing many plant materials. The later is suitable, if there is no previous information on the *S*-haplotypes of plant materials (Fujimoto and Nishio, 2003).

5. DEVELOPMENT OF SPECIFIC MOLECULAR MARKER FOR SELF-INCOMPATIBILITY IN *BRASSICA NAPUS*

Abstract

Recently the pollen determinant of Brassiceae, *SP11/SCR* gene, was revealed in *Brassica oleracea, B. rapa* and as well in *Raphanus sativus*. This sole protein confers S-haplotype specificity and it is responsible for self-incompatible reaction in pollen. As a determinant of SI, this gene was cloned and sequenced in *Brassica napus* in order to develop molecular marker specificaly distinguishing SI plants. From genomic DNA was amplified *SP11/SCR* gene and subsequently cloned and sequenced. Several clones were sequenced and all obtained sequences were identical. According to restriction sites in hypervariable areas three restriction enzymes were used for PCR-RFLP analysis. No polymorphism has been detected between SI and SC plants. Further analysis were performed on the cDNA level. PCR-RFLP with cDNAs of *SCR* gene cloned into plasmid vector showed two different patterns. After sequencing of different clones two sequences of *SCR* gene alleles, allele 1 and allele 2, were acquired and allele-specific primers from these sequences were derived. One of these alleles correlated with SI segregating ratio. This molecular marker was exclusively functional only in SI plants originating from SI line from whitch was developed and nonfunctional in different S-haplotypes.

Introduction

Brassica species have mechanisms to avoid self-pollination, self-incompatibility (SI), which is system where self-pollen is rejected at the surface of the papilla cells due to recognition of self and non-self pollen. SI in *Brasica* is sporofically controlled by a single S locus with multiple alleles (Bateman 1955). S locus is highly polymorphic region where thhe three main genes are located: SRK (S receptor kinase gene), SLG (S locus glycoprotein gene), and SP11/SCR (S locus protein 11 gene/cysteine-rich protein gene) (Schopfer et al. 1999).

SP11/SCR encodes small protein (8,5 kDa) which is specifically expressed in anther tapetum and immature pollen (Takayama et al. 2000). SCR belongs to a family of cysteine-

rich proteins (PCP-A: Pollen Coat Proteins-class A), known for an ability to bind stigmatically-expressed components of the S-locus in *Brassica* (Stephenson et al. 1997). Some members of PCP-A family showed interaction with SLG (Doughty et al. 1993). SP11 shows an S haplotype-specific sequence polymorphism (Takayama et al. 2000, Watanabe et al. 2000) but in *B. oleracea* and *B.rapa* (Sato et al. 2003) and even in different genera, *Raphanus sativus* and *Brassica*, similar S haplotypes remain (Okamoto et al. 2004). SP11s have two classes which common to general feature of S-locus. Class II *SP11*s originating from B. olerecea and *B. rapa* form a distinct group separated from class I *SP11*s (Shiba et al. 2002). Putative aminoacid SCR sequence revealed 8 conserved cysteines, one glycine residue and one aromatic amino residue (Schopfer et al. 1999). Highly conserved residues are important for maintaining the structural integrity of SP11s among the allelic variations. According to model of alignment of primary and secondary structure hyper-variable region with the most length variability was designed (Mishima et al. 2003). SCR consists of signale peptide which is very conservative and have alternative cleavage sites resulting in two alternative transcriptional forms (class II) (Shiba et al. 2002). In the self-incompatible S^{15} haplotype of *B.oleracea* duplicated *SP11* genes , S^{15}-*SP11a*, S^{15}-*SP11b*, and S^{15}-*SP11b'* (Shiba et al. 2004). Binding ability SCR to eSRK is not consistent with SI activation (Chookajorn et al. 2004). Kachroo et al. (2001) describes $eSRK_6$ bound poorly to SCR_{13} chimeric protein in contrast to 10-fold stronger binding of $eSRK_6$ to $selfSCR_6$ than to non-self SCR_{13}. Despite extensive amino acid changes, SCR can maintain the same predicted overall structure and moreover a minimum of alternation is required for gaining a new specificity (Chookajorn et al. 2004).

The mRNA of class II SP11 was detected predominantly in the anther tapetum in homozygotes and was not detected in the heterozygotes of class I and class II S-haplotypes, suggesting that the dominant/recessive relationships of pollen are regulated at the mRNA level of SP11 (Shiba et al. 2002). The linear dominance relationship of SI phenotype on pollen side in class II SP11 is regulated by the expression of *SP11*(Kakizaki et al. 2003). In A self-compatible B. rapa of class I S-haplotype insertion of retrotransposon-like sequence in first introne of *SRK* and 89-bp deletion in the SP11 promoter was revealed (Fujimoto et al. 2006). Transcription of functional *SP11-60* allele of *B. rapa* was suppressed in heterozygotes with S-f2 allele originating from class I self-compatible *B. rapa* (Fujimoto et al. 2006).

Materials and Methods

Plant materials

A segregating F2 populations 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 6/85'. This population was consisted of 118 plants and phenotype was characterised by seed test. 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. Further 15 cultivars ('Odila', 'Navajo', 'Lirajet', 'Later', 'Pilot', 'Ramiro', 'Cando', 'Catonic', 'Orkan', 'Jesper', 'Global', 'Topas', 'Regent', 'Sonata' and 'Solida') a additional 4 SI lines ('Start (86/1)', 'WRG 15', 'Tandem 6/85' and 'Tandem 1/85') were tested.

Methods

DNA extraction

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

SCR gene was amplified with class II *SCR*-specific oligonucleotide primers for genomic DNA (Shiba et al. 2002). Allele specific amplification were performed with sets of primers (allele 1: 5'-TTTGATTTTGACATATGTTC-3' and 5'-CCCCTCAACTTCATAGTGTT-3'; allele 2: 5'-TTGGACTTTGACATATGTTC-3' and 5'-CTCTGAAGTGGGTTTTACAG-3') designed according to highly variable segments between two found *SCR* alleles. 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 *Taq* polymerase (TaKaRa) in a final volume of 25 μl. The PCR conditions were 45 cycles of 30 s at 94°C, 30

s at 55°C and 1 min at 72°C. PCR fragments were analysed using agarose and polyacrylamide gel electrophoresis and stained with ethidium bromide.

mRNA isolation and cDNA synthesis

Anthers of the doublehaploid SI lines and rapeseed cultivars 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. 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 (5'-GCGAAAATCTTATATACTCATAAG-3' and 5'-TTCGTTGATCAATTATGATT-3' Shiba et al. 2002). RT-PCR was performed under these 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 was cloned and sequenced.

DNA Cloning and Sequencing

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 (*Mnl* I, *Hha* I) 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).

Results

With class II specific *SCR* primers a 450 bp band from genomic DNA was amplified (fig.1.). Subsequently was this product cloned and sequenced. Resulting sequence had all characteristic of SCRs, e.d. eight conserved cysteines, one glycine, and one tyrosine (fig 2.). Only one single allele was found. A cDNA synthesis of class II *SCR* from anthers was performed and 350 bp long fragment was obtained and subsequently again cloned and sequenced. In order to find other allele, screening of clones was performed by PCR-RFLP method (fig. 3). According to known sequence that was obtained, the restriction enzymes were chosen in such a way to have their recognition sites in putative variable segments. Two different PCR-RFLP pattern were revealed corresponding to two clas II *SCR* alleles (fig. 3.)

The two class II *SCR* gene alleles were sequenced and marked as allele 1 and allele 2. The sequence similarity between these two alleles was 85 % (fig. 4). Comparison with database NBCI (http://www.ncbi.nlm.nih.gov) showed that one allele was identical with S^{15} allele from *Brassica oleracea*. Using PCR-RFLP of single cloned cDNAs we have found out that both alleles were present in SC plants as well as SI plants (fig. 3). It seemed that allele 2 expression is often much higher in both phenotypes. Occurrence of single nucleotide mutations in both alleles was quite frequent. Furthermore, we found that S^{15} allele was duplicated. Similarly we observed that two forms of transcrips exist (fig. 2). We performed screening of segregating doublehaploid population with allele specific primers. A single approximately 280 bp band was present in SI plants only (fig. 5). Amplification of allele 1 carried out at temperature of annealing 55°C but did not carry out at temperature of annealing 60°C. Also presence of allele 1 and 2 in spectrum of cultivars (fig. 6) and in several SI lines (fig. 7) was tested. Amplification of allele 1 in spectrum of cultivars resulted in about 700 bp long fragment in cultivar 'Jesper' and about 550 bp long fragment in cultivar 'Regent' despite these specific primers are designed to amplify approximately 300 bp long fragment. Allele 2 specific primers amplified 280 bp long fragment in cultivar 'Lirajet' and cultivar 'Solida'. Aproximately 150 bp long fragment is often non-specificaly amplified. In SI lines two fragments about 480 and 520 bp long of allele 1 in SI line 'WRG 15' and 'AIK 6' and one single fragment about 500 bp long in SI line 'AIK 3' were amplified. Allele 2 was detected in SI lines 'Start (86/1)' (1), 'Tandem 6/85' (3), 'AIK 3' (5) and 'AIK 6' (6).

Fig. 1. Amplification *SCR* class II gene from genomic DNA. After amplification a 450 bp and approximately 1000 bp long products were obtained in samples 1 to 5. In sample 6 and 7 only single 450 bp product was obtained. Lane 1- cultivar 'Stela', 2 - cultivar 'Westar', 3 - cultivar 'Global', 4 – SI line 'Tandem', 5 - *Brassica oleracea* var. *Capitata* L., 6 - *Brassica rapa* L. f. *praecox*, 7 – *Brassica rapa* var. *Rapifera*.

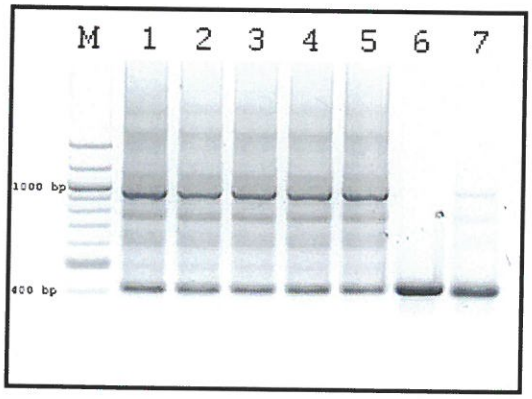


Fig. 2. Comparison of all sequenced class II *SCR* alleles. The first amino acid sequences belong to allele 2 and the rest to allele 1. Clones 99-B to T3 are from genomic DNA. Conserved cysteines are marked with blue colour. Red colour marks amino acids which can be missing due to alternative splicing. Mutation of single alleles are highlighted with yellow boxes.

Fig. 3. PCR-RFLP of *SCR* class II cDNA clones. Cloned cDNAs were amplified by PCR and then restricted with *Mnl* II. Allele 1 had three restriction sites (sample 84SC A to 91SC D, 20SC A to 20SC B). Allele 2 had two restriction sites (sample 86SI F to 74SI B, 6SI 5 to 4SI C, 20SC C to 20SC D). Clone 86SI H had 1 bp mutation in one recognition site. Putative phenotype was marked with letter SI or SC.

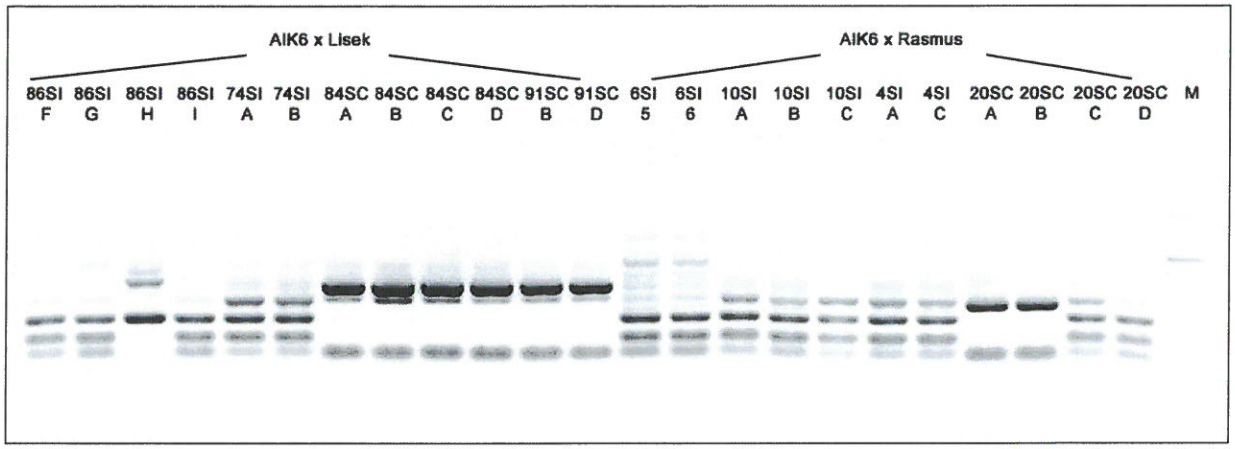


Fig. 4. Comparison of cDNA sequences of two revealed *SCR* class II alleles in *Brassica napus*. The sequence similarity between these two alleles was 85 %.

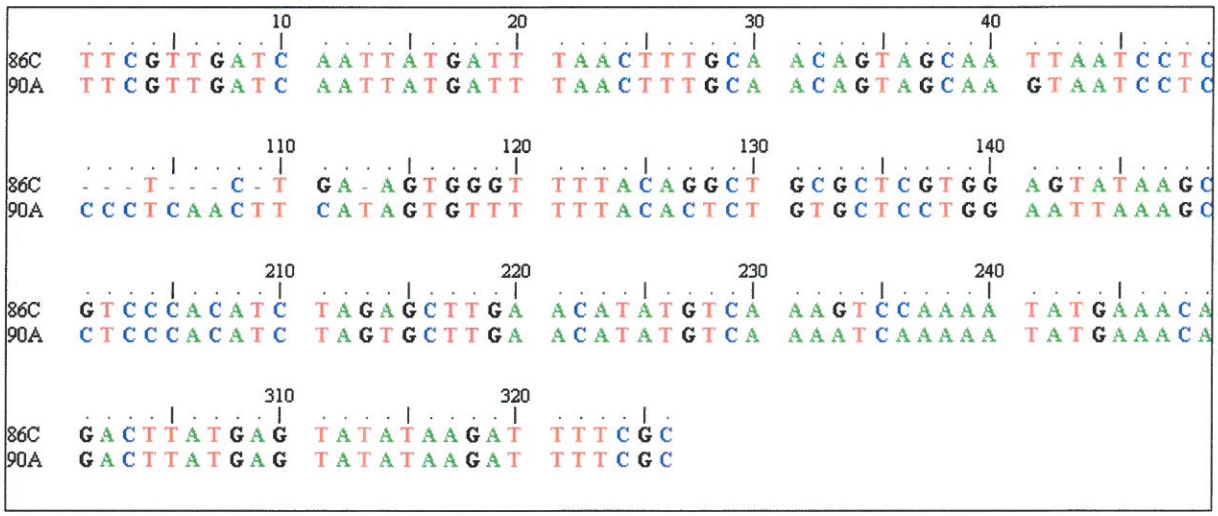


Fig. 5. Screening of segregating doubled hapliod population with allele 2 specific primers. A single approximately 280 bp fragment was amplified in putative SI plants.

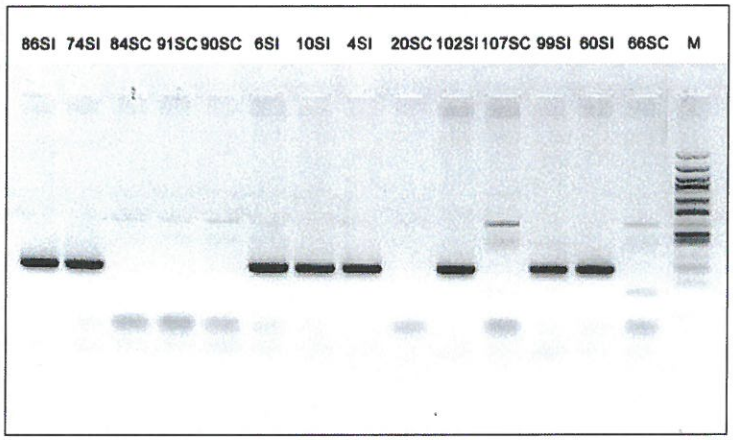
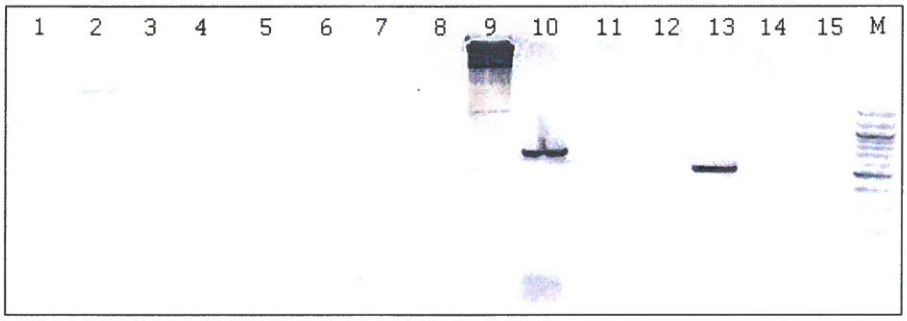


Fig. 6. Amplification of allele 1 and allele 2 of class II *SCR* gene in different *Brassica napus* cultivars.

Lane 1 – cultivar 'Odila', 2 - cultivar 'Navajo', 3 - cultivar 'Lirajet', 4 - cultivar 'Later', 5 - cultivar 'Pilot', 6 - cultivar 'Ramiro', 7 - cultivar 'Cando', 8 - cultivar 'Catonic', 9 - cultivar 'Orkan', 10 - cultivar 'Jesper', 11 - cultivar 'Global', 12 - cultivar 'Topas', 13 - cultivar 'Regent', 14 - cultivar 'Sonata', 15 - cultivar 'Solida'. **A) Amplification of allele 1.** Allele 1 specific primers amplified about 700 bp long fragment in cultivar 'Jesper' (10) and about 550 bp long fragment in cultivar 'Regent' (13) despite these specific primers are designed to amplify approximately 300 bp long fragment. **B) Amplification of allele 2.** Allele 2 specific primers amplified 280 bp long fragment in cultivar 'Lirajet' (3) and cultivar 'Solida' (15). Aproximately 150 bp long fragment is often non-specificaly amplified.

A)



B)

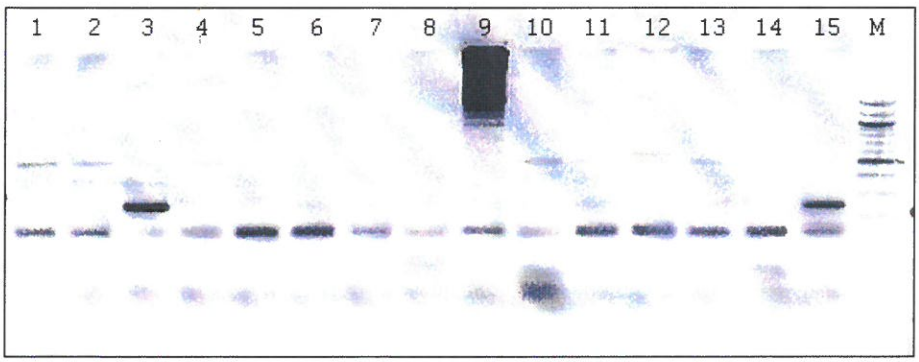
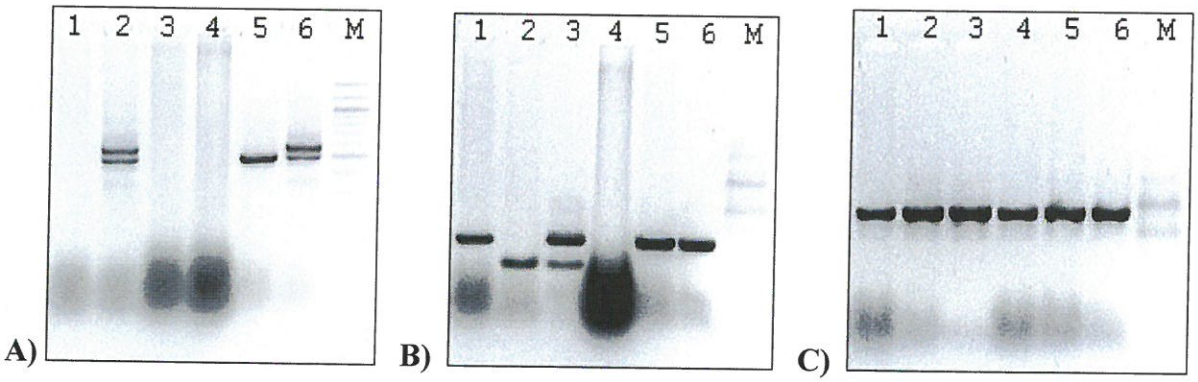


Fig. 7. Amplification of class II *SCR* alleles in different SI lines.
Line 1 - 'Start (86/1)', 2 - 'WRG 15', 3 - 'Tandem 6/85', 4 - 'Tandem 1/85', 5 – 'AIK 3', 6 - 'AIK 6'. **A) Amplification of allele 1.** Allele 1 specific primers amplified two fragments about 480 and 520 bp long in SI line 'WRG 15' (2) and 'AIK 6' (6) and one single fragment about 500 bp long in SI line 'AIK 3' (5). **B) Amplification of allele 2.** Allele 2 specific primers amplified 280 bp long fragment in SI lines 'Start (86/1)' (1), 'Tandem 6/85' (3), 'AIK 3' (5) and 'AIK 6' (6). The lower band in sample 2 and 3 is non-specificaly amplified. **C) Control amplification of class II *SCR* gene.** A 450 bp single fragment of class II *SCR* gene was amplified with universal class-II *SCR*-specific primers in all samples.



Discussion

SCR is sole pollen determinant of self-incompatibility (Schopfer et al., 1999; Suzuki et al., 1999). It has been confirmed that *SCR* gene is duplicated in *Brassica oleracea* S^{15} haplotype (Shiba 2004). Cabrillac et al. (1999) has found duplicated *SLG* gene in SC *Brassica oleracea*, line S^{15}. We found duplicated allele of *SCR*, which was 100% similar to S^{15} alele from *Brassica oleracea*. This could suggest that this allele originates from *B. oleracea*. The duplication could lead to the producing of incorrect lengths of transcripts responsible for dysfunction. The second allele was specifically occured in SI lines. Two types of transcrips, AL+ and AL-, were observed in *Brassica oleracea* and it is supposed that AL- type of transcript is the later form (Shiba et al., 2002). This finding is in consistent with our results. Both types of transcripts were expressed in SI and SC DH plants. Considering that the allele specific primers were designed on the basis of sequences obtained from specific plant material and the *SCR* polymophic nature, it is evident that they cannot easily amplify single alleles in different cultivars. Moreover, there is a big sequence similarity among genes at the *S*-locus and for instance SCR binding domain area of *SRK* gene can be amplified as well. Previously we do not succeed in amplification of allele 2 with class II specific *SCR* primers from genomic DNA. The difference between allele 2 amplification from cDNA and genomic DNA

may be caused by different numbers of copies of which the expression might be affected in a certain way.

Conclusion

Two alleles of *SCR* gene were revealed. One of them was identical with S^{15} allele from *Brassica oleracea* genome and it seemed to be dysfunctional and commonly occurred in rapeseed cultivars. The second allele was present only in SI lines and in some cultivars. It could serve as a molecular marker of self-incompatibility in specific plant material from which it was obtained.

6. MARKER-ASSISTED SELECTION OF SELF-INCOMPATIBLE OILSEED RAPE PLANTS.

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'). . 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 *Taq* polymerase (TaKaRa) in a final volume of 25 μl. The PCR conditions were 45 cycles of 1 min at 94°C, 2 min at 58°C and 3 min at 72°C. PCR fragments were analysed using agarose and polyacrylamide gel electrophoresis and stained with ethidium bromide.

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 (sample 1-3, 5, 11, 13-14, 17, 19-20) of segregating doubled haploid population.

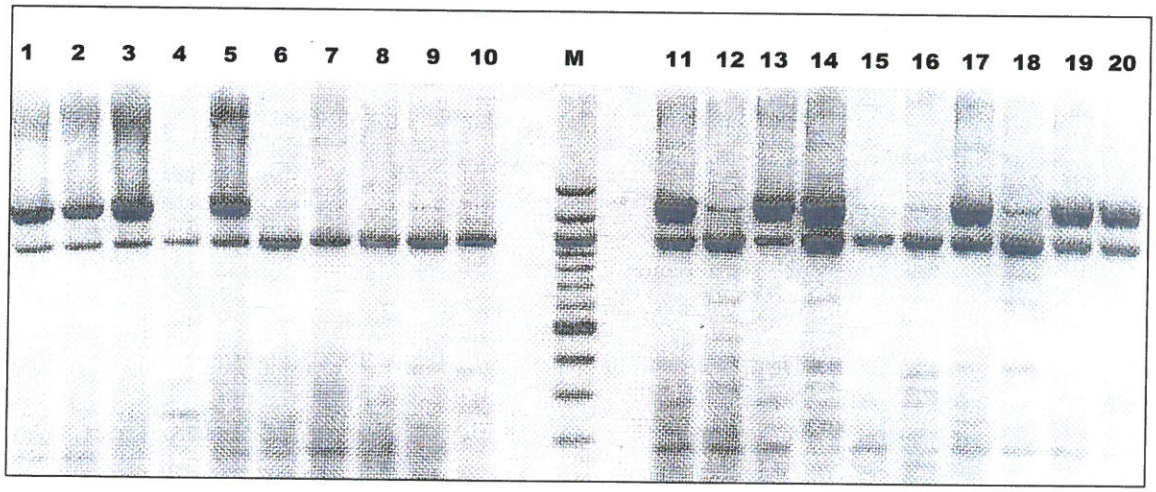
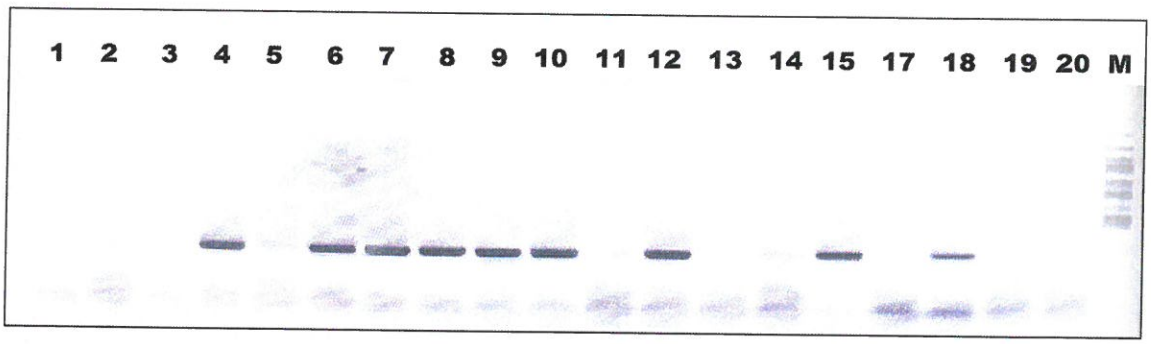


Fig. 2. Amplification of class-II *SCR* gene. An 280 bp band was amplified in putative self-incompatible oilseed rape plants (sample 4, 6-10, 12, 15, 18) of segregating doubled haploid population. Sample 16 is missing.



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.

7. GENETIC DETERMINATION OF SELF-INCOMPATIBILITY IN *BRASSICA NAPUS* AND MOLECULAR MARKER SELECTION.

Abstract

Self-incompatibility (SI) could be used as a pollination control system for *Brassica napus* hybrid production. 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. Amplification class I *SLG* gene screened dominant gene in all analysed *Brassica napus* plants originated from doubled haploid population derived from crosses between lines with recessive self-incompatibility and self-compatible donors of quality. *SLG I* gene was specificaly amplified in SC *Brassica napus* plants with no respect to *S*-haplotype of different self-incompatibility donor lines. Results of seed test and marker selection were compared and clear difference between marker and seed test segregation have been observed. Segregation ration 1:1 of marker class I *SLG* gene corresponded to segregation ratio of single locus in doubled haploid population while segregation ratio 1:3 of seed test corresponded to segregation ratio of two loci. These results can suggest two different loci genetic disposition of self-incompatibility in oilseed rape. However, class I *SLG* gene can exclude more than 90% self-compatible plants.

Introduction

Self-incompatibility (SI) is a natural mechanisms 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).

Cultivar development in *Brassica* napus has traditionally been based on open pollinated line development. However, development of hybrid cultivars is recantly a priority in rapeseed breeding for reasons of presence of significant heterosis, an effective pollination control system, and adequate pollen transfer between parental lines (Grand and Beversdorf 1985).

SI can be reintroduced into *B. napus* by backcrossing (Goring et al. 1992) or by resynthesis of the ancestors *B. oleracea* and *B. campestris* (Hodgkin 1986, Sundberg et al. 1987, Ozminkowski and Jourdan 1993). SI also occurs naturally in rapeseed cultivars and it is

possible to select SI plants from rapeseed breeding material (Rudloff 1991, Esch 1994, Kucera 1995), However is very low, about 0,1 % (Havel 1996).

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. 1988).

S haplotypes have been classified into two classes based on the nucleotide sequence similarity of *SLG* alleles, *SRK* alleles (Nasrallah et al. 1991), and *SP11* (Schopfer et al. 1999) Class-I *S*-haplotypes are known to be generally dominant to class-II *S*-haplotypes in the pollen (Nasrallah et al. 1991, Nasrallah et al. 1993). This recessiva phenotype in the pollen iscosidered to be controlled at the mRNA level (Kusaba et al. 2002; Shiba et al. 2002). Alleles can manifest dominance, independence, interaction, or mutual weakening. The SLGs i class I shows high activity and strong dominance allowing 0-10 pollens to germinate on the stigma surface, whereas the SLGs in class II show a very leaky incompatibility reaction in which 10-30 pollen can developed the tubes. However, within a class, alleles are usually codominant (Nasrallah et al 1991).

Materials and Methods

Plant materials

Double haploid regenerants were derived from four F_1 hybrids of SI line 'Start' and donor quality '2051', SI line 'Liropa' and donor of quality '2051', SI line 'WRG 15' and donor of quality '2051' and finally from SI line 'Tandem 1/85' and donor of quality '2051'. This plant material was kindly provided by the Research Institute of Crop Production (RICP), Prague- Ruzyne. SI in lines were recessively inherited.

Methods

A seed test was performed 3 days before flowering using bag-isolation. The average number of seeds per developed pod after self-pollination in flowers were counted. The seed set by selfing was checked when seedpods were ripe. As SI were considered plants with average of seeds per pod ranging from 1,0 to 4,6 (6,4), as partially SI with average from 5,1 to 6,8, and the completely SC with average from 6,5 to 34,3.

Genomic DNA was extracted from leaves of regenerated plants after 4 weeks of vernalization by the Dnasy Plant Mini kit (QIAGEN). The PCR reaction was performed with class-I *SLG*-specific primers PS5 and PS15 (Nishio et al.,1996). Approximatelly 50 ng genomic DNA was used as template in a final volume of PCR reaction 25 µl under following conditions: 10× buffer (10 mM Tris HCl pH 8.3, 50 mM KCl, 3 mM $MgCl_2$, 1% Triton X-10), 100 µM dNTP, a pair of primers (25 pM), and 1U Taq polymerase (TaKaRa). PCR conditions were 30 cycles of 1 min at 94°C, 2 min at 58°C and 3 min at 72°C.

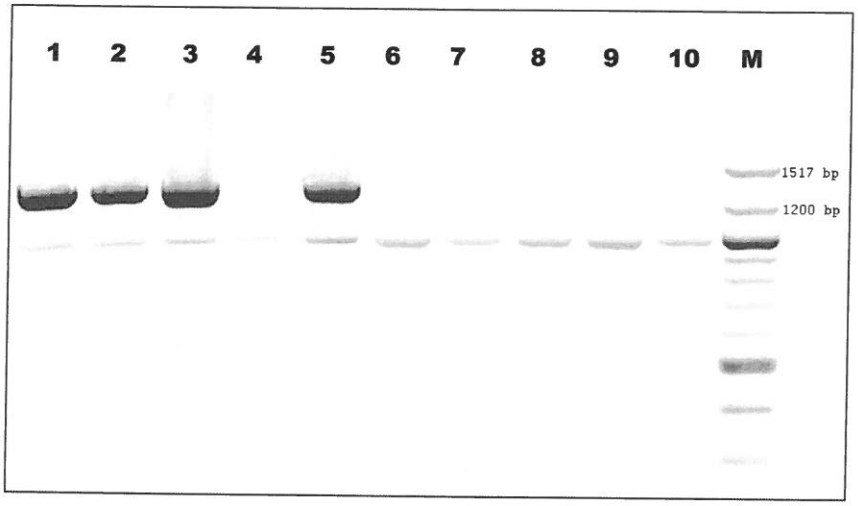
The amplified primers were resolved using electrophoresis on 1,5% agarose gel.

Results

PCR with class I *SLG*-specific primers has resulted in approximately 1300 bp fragment together with cca 1000 bp long and probably nonspecific fragment (fig. 1.). The *SLG* I specific band was amplified only in some plants suggesting that present exclusively in SC plants and not in plants with recessive type of SI.Segregating ratio of molecular marker was 1:1,1 (SI:SC). Class II *SCR* gene was not used because its allele specifically expressed in SI plants taggs Tandem derived SI only and not other S haplotypes. Simultaneously the seed test was performed. Segregatin ratio was assessed only for plants demonstrating full SI or SC. Expected segregation ratio 1:1 corresponding to doublehaploid segregating population in seed test was modified into 1:3,1.

Class I *SLG* molecular marker gene tagged 80% SC plants excluding SI/SC phenotype. If the SI/SC plants are considered as SC unsuitable in selection, efficiency of selection is higher than 90%.

Fig. 1. Amplification of class I *SLG* gene. The upper band of 1300 bp was amplified in putative self-compatible oilseed rape plants (sample 1-3, 5) of segregating doubled haploid population.



Discussion

Phenotype segregating ratio did not correspond with molecular marker determined genotype due to some modifications. On the basis of phenotype segregation ratio two different loci coding SI should exist. One of them is the S locus with two classses of alleles. Class I *S* locus was present only in SC plant and moreover in all SC cultivars (data not shown). It could suggest that class I S locus is in some way affected and in is plants replaced with functional class II locus. The second locus is repressor locus (R-locus), which is commonly present in SC oilseed rape cultivars and not present in SI lines. Ekuere te la. (2004) describes a suppressor loci with suppressing alleles in oilseed rape and non-suppressing alleles in diploid parents of resynthesized *B. napus* lines that are not linked to the *S* loci. Both of these loci could independently affect SI. As a lot of intermediate SI/SC phenotypes were observed, we should suggest that both S locus and R locus have alleles with different recessive or dominant interaction. Below you can see deduced segregation diagram that simply presents segregation of SI in doubled haploid population of *B. napus.* Data about class II *SCR* gene alleles were used from chapter 6.

Segregation diagram

Initial information:

Parents:

cultivar

- detected presence of class-I *SLG* gene, in comparison with known *S*-alleles from database NBCI this *S*-allele could considered to be A10 from *Brassica oleracea*
- detected presence of class-II *SLG* gene and class-II *SCR* gene, in comparison with known *S*-alleles from database NBCI this *S*-allele is identical with S^{15} allele
- possible presence of other class-II *S*-allele

SI line

- presence of class-I *S*-allele (*SLG*) was not detected
- detected presence of class-II *SLG* gene and class-II *SCR* gene
- two *S*-alleles of class-II *SCR* gene were found:
  - allele 1 – 100% identity with S^{15} allele of class-II *SCR* gene
  - allele 2 – not found in genebank

Doubled haploid population:

- SI phenotype – class-I *S*-locus is not present, both class-II *S*-alleles (*SCR* gene) detected
- SC phenotype - class-I *S*-locus is not present, both class-II *S*-alleles (*SCR* gene)
- SC phenotype - presence of class-I *S*-allele (*SLG*), S^{15} allele of class-II (*SCR* gene)

P:	SI line	×	SC Cultivar
	$S_{IIO}2$ $S_{IIR}15$ $S_{IIO}2$ $S_{IIR}15$ rr		**$S_{IO}A10$ $S_{IIR}X$ $S_{IO}A10$ $S_{IIR}15$ RR**

Comments: $S_{IIO}2$ class-II *S*-locus allele 2 originating from *B. oleracea* (class-II *SCR* gene)

$S_{IIR}15$ class-II *S*-locus allele 15 originating from *B. rapa* (class-II *SCR* gene)

$S_{IO}A10$ class-I *S*-locus allele A10 originating from *B. oleracea* (class-I *SLG* gene)

$S_{IIR}X$ undefined class-II *S*-locus allele X originating from *B. rapa* (class-II *SCR* gene)

rr repressor of the *R*-locus is not present

RR repressor of the *R*-locus is present

Gametes: **$S_{IIO}2$ $S_{IIR}15$ r**

$S_{IO}A10$ $S_{IIR}X$ R

$S_{IO}A10$ $S_{IIR}15$ R

F_1: **$S_{IIO}2$ $S_{IIR}15$ $S_{IO}A10$ $S_{IIR}15$ Rr**

$S_{IIO}2$ $S_{IIR}15$ $S_{IO}X$ $S_{IIR}15$ Rr

Presence of class-I S-locus causes SC phenotype.

Gametes: **$S_{IIO}2$ $S_{IIR}15$ r**

$S_{IIO}2$ $S_{IIR}15$ R

$S_{IO}A10$ $S_{IIR}15$ r

$S_{IO}A10$ $S_{IIR}15$ R

$S_{IO}A10$ $S_{IIR}X$ r

$S_{IO}A10$ $S_{IIR}X$ R

Regenerated doubled haploids:

genotype	**phenotype**
2× **$S_{IIO}2$ $S_{IIR}15$ $S_{IIO}2$ $S_{IIR}15$ rr**	**SI** - class-I *S*-locus not present
2× **$S_{IIO}2$ $S_{IIR}15$ $S_{IIO}2$ $S_{IIR}15$ RR**	**SC**- class-I *S*-locus not present, repressor present
1× **$S_{IO}A10$ $S_{IIR}15$ $S_{IO}A10$ $S_{IIR}15$ rr**	**SC**- class-I *S*-locus present
1× **$S_{IO}A10$ $S_{IIR}15$ $S_{IO}A10$ $S_{IIR}15$ RR**	**SC**- class-I *S*-locus present, repressor present
1× **$S_{IO}A10$ $S_{IIR}X$ $S_{IO}A10$ $S_{IIR}X$ rr**	**SC**- class-I *S*-locus present
1× **$S_{IO}A10$ $S_{IIR}X$ $S_{IO}A10$ $S_{IIR}X$ RR**	**SC**- class-I *S*-locus present, repressor present

Segregation ratio of phenotype: SI : SC = 1:3

Segregation ratio of genotype:

Confirmed segregation ratio of *S*-locus: class-I present : class-I not present = 1:1

Putative segregation ratio of *R*-locus: repressor present : represor not present = 1:1

Putative segregation ratio of *S*-locus and *R*-locus: class-I *S*-locus not present and repressor not present : class-I *S*-locus present and/or repressor present = 1:3

Conclusion

Class I *SLG* marker gene was used to screen doubled haploid population for self-compatible oilseed rape plants. Seed test did not correspond with doublehaploid theoretic segregation ratio and the real segregation ration was 1:3 (SI:SC). The real segregation ratio of SI could correspond to two independent loci. Besides S-locus, SI is most probably controled with other repressor locus. However class I S*LG* gene tagged 90% of self-compatible plants and could serve as an efficient molecular marker .

8. DIFFERENT DISTRIBUTION OF *S*-ALLELES IN BRASSICA NAPUS COMMERCIAL CULTIVARS AND SI LINES

Abstract

SI is a system how to prevent self-pollinaton in population and enable gene-flow and diversification. SI determs one polymorphic locus S-locus which contains several genes responsible for SI reaction. One of genes lying on the S-locus is the *SLG* gene and according sequence similarity SLGs are devided into two groups. First group is considered to be domminant and the second recessive. incompatible lines and five cultivars, different *SLG* alleles were cloned and sequenced. Thirty-one sequences of different *SLG* clones were obtained and phylogenetic tree deduced.Three main groups of *SLG* sequences are obvious and it seems to have different allelic specificity. Two groups included majority of sequences from SI lines and cultivars suggest two *S*-alleles very common in Brassica napus.

Introduction

Self-incompatibility is under is the mechanism of sexual reproduction control in Brassica species that prevents inbreeding and promotes outcrossing. In diploid Brassica species, this mechanismus is controlled by single S-locus, the S-locus, with more than 50 alleles (Ockendon 1974). Self-incompatibility research in Brassica species has reviewed (for example Watanabe et al. 2003)

Many S-alleles have been identified by diallel pollination (B. campestris Nou et al. 1993) or by pollination with tester line (B. oleracea Ockendon 1974,1982). Many of S-alleles have been characterised by their S-glycoproteins (SLG) (Umbach et al. 1990, Gaude et al. 1993) and their respective DNA sequences (Nasrallah et al. 1987, Chen and Nasrallah 1990). To distiguish single S-alleles and determine S-haplotype, molecular genetic methods were utilised (Nishio et al. 1997).

The SLG gene is consisted of a cleavable signal peptide, several *N*-glycosilation sites, three hypervariable regions, and twelve conserved cysteine residues towards the C terminus (Takayama et al. 1987, Nasrallah et al. 1987). *SLG* gene has great sequence similarity to the region encoding the S domain of SRK gene, that is female determinant of SI and co-operate SLG in pollen recognition reaction although its function is not clear (Takasaki et al. 2000).

SLGs of different S-alleles show extensive sequence divergence (Nasrallah et al. 1988, Ebert et al. 1989, Yamakawa et al. 1994). S- haplotypes have been devided according to criteria based on polymorphism among SLG sequences into two classes that refer to dominance and recessive interaction occuring between haplotypes in heterozygous plants (Nasrallah 1989, Nasrallah et al.1991).

Material and methods

Plants material

Three *Brassica napus* cultivars ('Global', 'Ramiro'and 'Rasmus') and nine self-incompatible lines ('Start', 'Liropa', 'Cathy', 'Local', 'Tandem 6/85', 'Tandem 1/85', 'AIK 6' and 'AIK 3') were analysed.

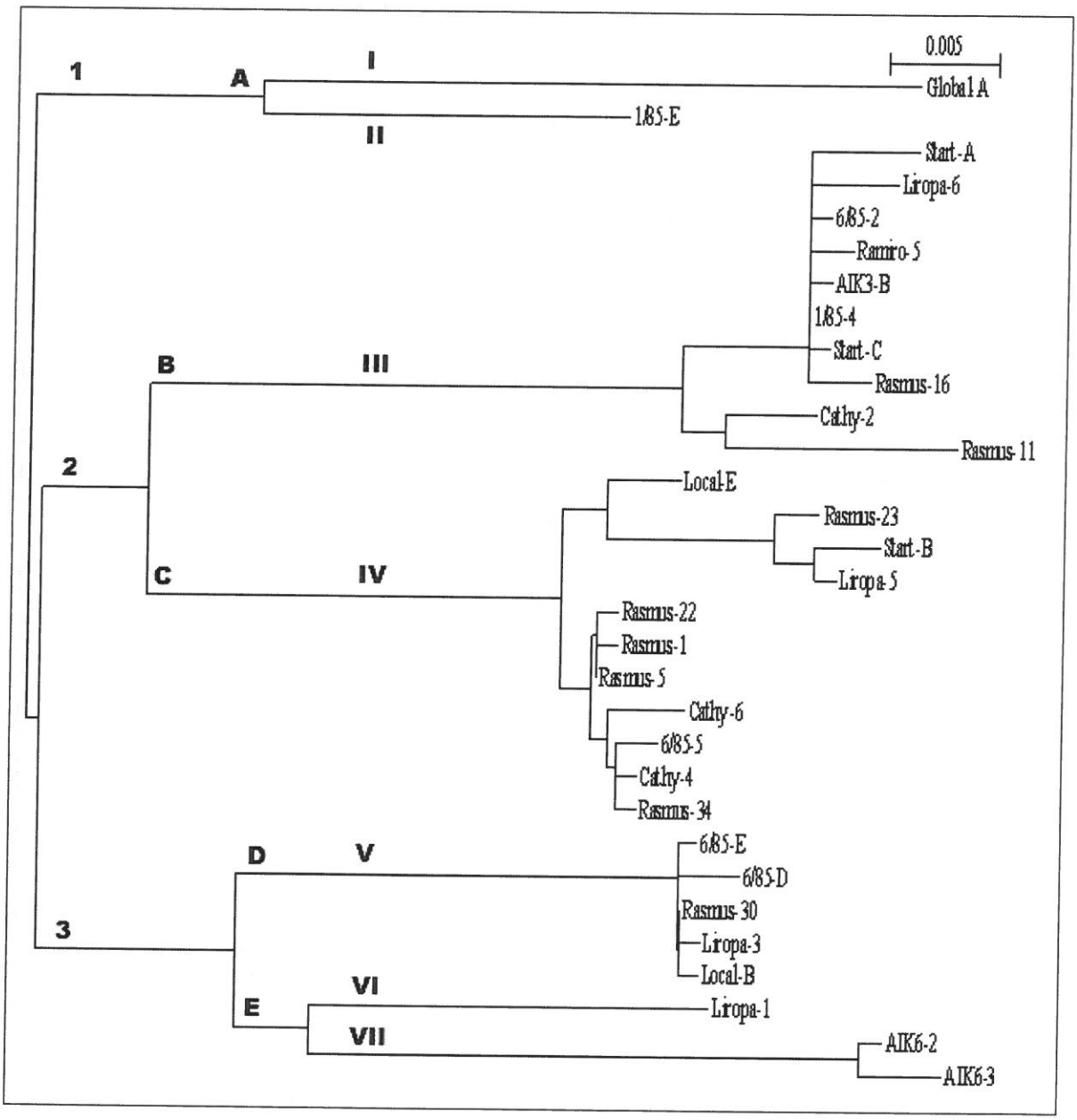
Methods

Genomic DNA were extracted from cotyledons of 7 AI lines and four cultivars of rapeseed. PCR bas performed with class II specific *SLG* primers PS 3 and PS 21 (Nishio et al., 1996). Aproximatly 1100 bp fragment were cloned (TOPO TA cloning kit). Clones were restricted by endonuclease EcoRI and AfaI. According to restriction fragments analysis different clones of SLG gene were sequenced (Perkin Elmer). Sequences were analysed by Bioedit. 31 sequences were align by Clustal and phylogenetitic tree by Neighbor Joining method was created. Obtained class II *SLG* sequences were also compared with *SLG*sequences from NBCI database (http://www.ncbi.nlm.nih.gov).

Results

Thirty-one sequences were obtained. After Clustal alignment sequences showed high level of polymorphism. Considerably extensive nucleotide insertion (about 50 bp) were in area of primer detected in 3 sequences. They are duplicated segments of primer area. In addition to these big insertion occurrence of short, 2-4 bp, substitution is very common. There is 33 bp area which is higly polymorphic in nucleotide sequences as well as length. Similarly conservative areas are present. Thirty-one clones of SLG II gene were divided into seven groups (fig. 1) and five subgroups (A-E). All sequences were devided into three classes (1-3). But most sequences were included in group III, IV and V.

Fig. 1. Cladogram of class II *SLG* gene sequences. Thirty-one clones of SLG II gene were divided into seven groups (fig. 1) and five subgroups (A-E). All sequences were devided into three classes (1-3).



Obtained class II *SLG* gene sequences of *Brassica napus* were compared with class II *SLG* gene as well as with class I *SLG* gene of *Brassica* species sequences from National Center for Biotechnology Information(http://www.ncbi.nlm.nih.gov). A phylogram (fig. 2) was devided into five groups.

Description of the groups

Group 1 – The most diverse group of all obtained *SLG* gene sequences. It consists of one sequence from study about diversity and distribution of S haplotypes in commercial cultivars Brassica vegetables from China (Wang et al. 2006, unpublished) available at NBCI (http://www.ncbi.nlm.nih.gov). S-haplotype class is not known.

Group 2 – This group contains five markedly similar Brassicas and includes SI as well as SC lines. All sequences come from one study which deals with comparison of S locus genes between SI and SC Brassica (He et al. 2003, unpublished). S-haplotype class is not known.

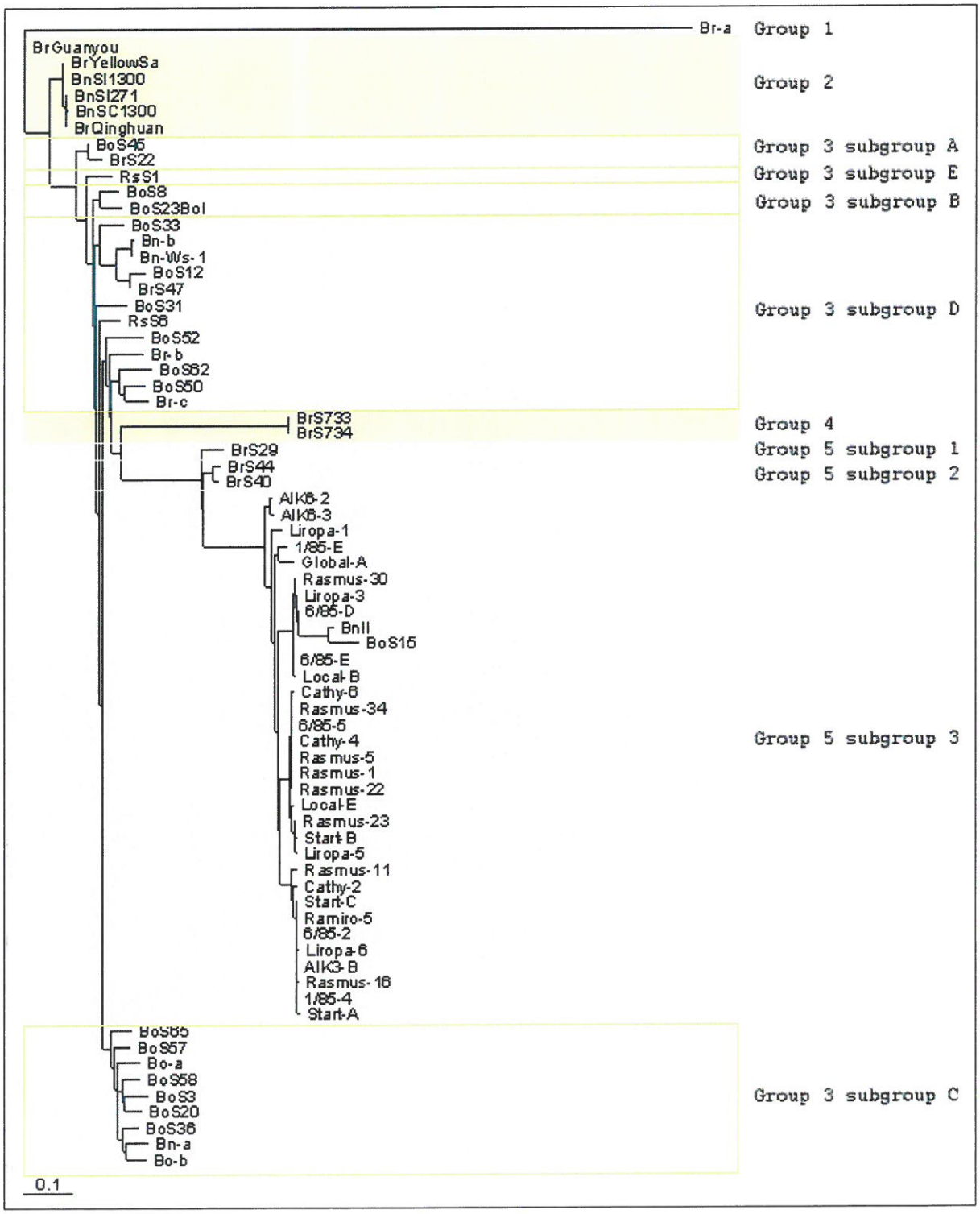
Group 3 – This group is the biggest one. It includes various *Brassica* species including *Raphanus sativus*. Thit group was devided into five subgroups (A-E). Absolute majority of SLG sequences were of class II *S*-haplotype, information about the rest of sequences is not published. Subgroup A consists of *B. oleracea* and *B. rapa SLG* sequences of different *S*-haplotype, subgroup B consists of two different S-haplotypes of *B. oleracea*, subgroup C consists of different S-haplotype of *B. oleracea* and one *B. napus* S-haplotype, subgroup D consists of different S-haplotypes of *B. oleracea*, *B. rapa*, *B. napus* and *Raphanus sativus.* Subgroup E is *Raphanus sativus.*

Group 4 – Two very distinct sequences of *Brassica rapa* with non-specified S-haplotype class (Gothandam et al. 2005).

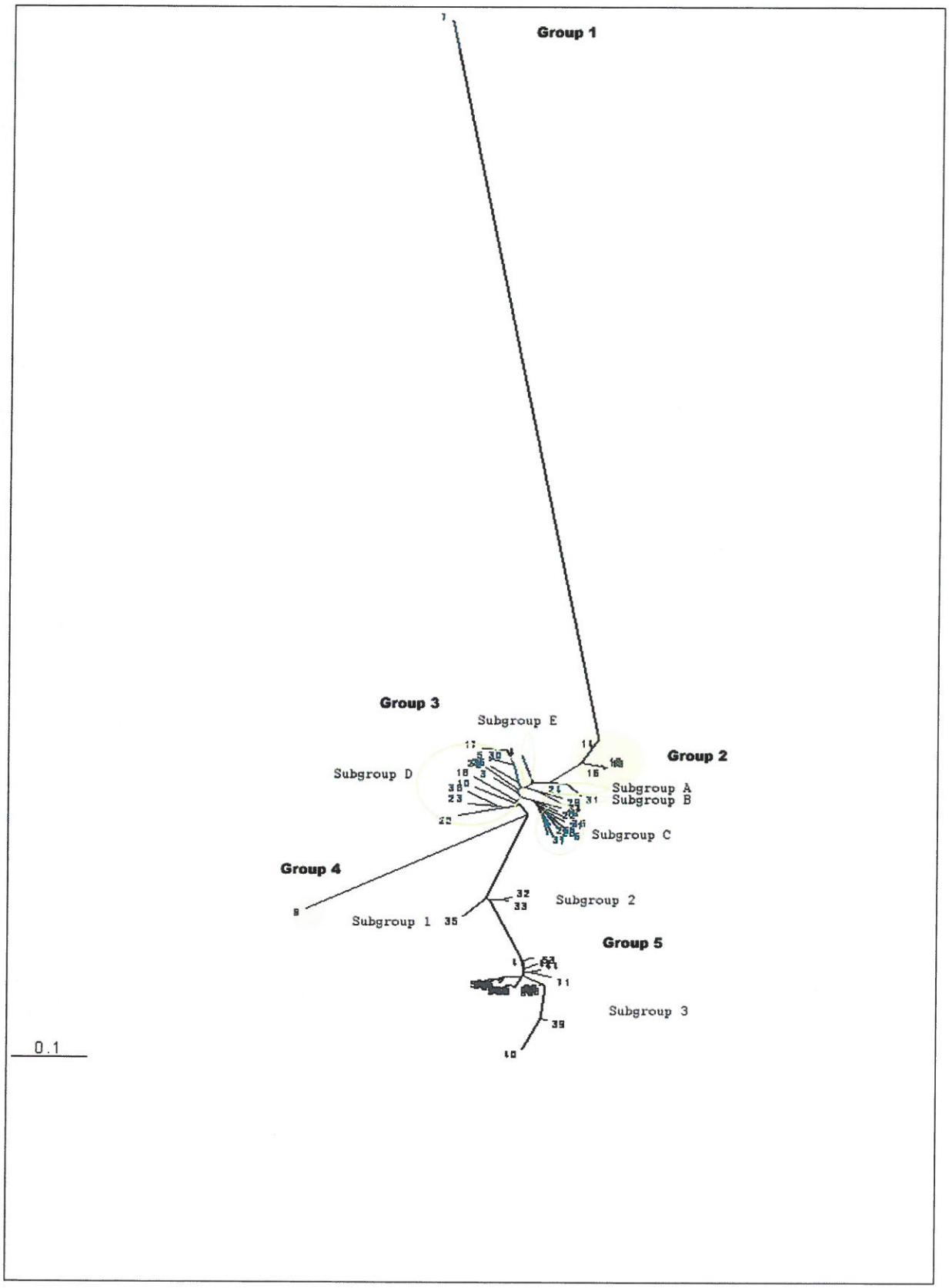
Group 5 – This group consists of class II *S*-haplotype only. Subgroup 1 and 2 are diffrent *S*-haplotypes of *Brassica rapa.* Subgroup 3 is compact group which includes analysed *Brassica napus* SI lines and cultivars and moreover B. oleracea *S*-haplotype 15 (Cabrillac et al. 1999) and B. napus closely non-specified *S*-haplotype (Robert et al. 1994)

Fig. 2. Phylogram of class II and I *SLG* alleles from different species. A) Phylogram is devide into 5 groups and represents a genetic distance among different alleles, species and *SLG* classes. **B)** This unrooted cladogram shows better the distancea among *SLG* sequences, especially of group 1. Annotations are under diagrams.

A)



B)


Group 1
Group 3
Subgroup E
Group 2
Subgroup D
Subgroup A
Subgroup B
Subgroup C
Group 4
Subgroup 2
Subgroup 1
Group 5
Subgroup 3
0.1

1:Z11725: B.napus mRNA for S-locus glycoprotein. (Goring et al. 1992) --> Bn-a
2:L08608: Brassica napus S-locus glycoprotein mRNA, complete cds. (Goring et al.1993, cultivar Westar) --> Bn-b
3:AB009682: Raphanus sativus SLG(S6) gene for S glycoprotein, partial cds.(Sakamoto et al. 1998) --> RsS6
4:AB009677: Raphanus sativus SLG(S1) gene for S glycoprotein, partial cds. (Sakamoto et al. 1998) --> RsS1
5:Z21608: B.napus mRNA for endogenous S-locus glycoprotein (clone SLG-Ws-1).(Robert et al. 1994) --> Bn-Ws-1
6:X79431: B.oleracea SLG3 mRNA. (Delorme et al. 1995) --> BoS3
7:DQ372708: Brassica rapa S-locus glycoprotein gene, complete cds. (Wang et al.2006) --> Br-a
8:DQ317532: Brassica rapa haplotype 733 S locus glycoprotein (SLG) mRNA,(Gothandam et al.2005) --> BrS733
9:DQ317531: Brassica rapa haplotype 734 S locus glycoprotein (SLG) mRNA, ,(Gothandam et al.2005) --> BrS734
10:D30050: Brassica rapa mRNA for S-locus glycoprotein of Brassica campestris (Watanabe et al.1994) --> Br-b
11:AY448036: Brassica napus cultivar SC1300 S-locus glycoprotein (SLG) gene, (HE et al.2003) --> BnSC1300
12:AY448034: Brassica napus cultivar SI271 S-locus glycoprotein (SLG) gene, (HE et al.2003) --> BnSI271
13:AY448032: Brassica napus cultivar SI1300 S-locus glycoprotein (SLG) gene, (HE et al.2003) --> BnSI1300
14:AY448030: Brassica rapa cultivar Guanyou S-locus glycoprotein (SLG) gene, (HE et al.2003) --> BrGuanyou
15:AY448028: Brassica rapa cultivar Yellow sarson S-locus glycoprotein (SLG) (HE et al.2003) --> BrYellowSarson
16:AY448026: Brassica rapa cultivar Qinghuang S-locus glycoprotein (SLG) gene, (HE et al.2003) --> BrQinghuang
17:AB180902: Brassica oleracea S-12 SLG gene for S-locus glycoprotein, complete (Fujimoto et al. 2006) --> BoS12
18:AB054815: Brassica rapa SLG-52 mRNA for S-locus glycoprotein, partial cds.(Takasaki et al.1999) --> BoS52
19:AB054737: Brassica oleracea SLG-65 mRNA for S-locus glycoprotein, partial (Sato et al. 2002) --> BoS65
20:AB054736: Brassica oleracea SLG-62 mRNA for S-locus glycoprotein, partial (Sato et al. 2002) --> BoS62
21:AB054735: Brassica oleracea SLG-58 mRNA for S-locus glycoprotein, partial (Sato et al. 2002) --> BoS58
22:AB054734: Brassica oleracea SLG-57 mRNA for S-locus glycoprotein, partial (Sato et al. 2002) --> BoS57
23:AB054733: Brassica oleracea SLG-50 mRNA for S-locus glycoprotein, partial (Sato et al. 2002) --> BoS50
24:AB054732: Brassica oleracea SLG-45 mRNA for S-locus glycoprotein, partial (Sato et al. 2002) --> BoS45
25:AB054731: Brassica oleracea SLG-36 mRNA for S-locus glycoprotein, partial (Sato et al. 2002) --> BoS36
26:AB054730: Brassica oleracea SLG-33 mRNA for S-locus glycoprotein, partial (Sato et al. 2002) --> BoS33
27:AB054729: Brassica oleracea SLG-31 mRNA for S-locus glycoprotein, partial (Sato et al. 2002) --> BoS31
28:AB054728: Brassica oleracea SLG-20 mRNA for S-locus glycoprotein, partial (Sato et al. 2002) --> BoS20
29:AB054727: Brassica oleracea SLG-8 mRNA for S-locus glycoprotein, partial cds. (Sato et al. 2002) --> BoS8
30:AB054705: Brassica rapa SLG-47 mRNA for S-locus glycoprotein, partial cds. (Sato et al. 2002) --> BrS47
31:AB054060: Brassica rapa SLG22 mRNA for S locus glycoprotein, complete cds. (Hatakeyama et al. 2001) --> BrS22
32:AB054059: Brassica rapa SLG44 mRNA for S locus glycoprotein, partial cds. (Hatakeyama et al. 1998) --> BrS44
33:AB054058: Brassica rapa SLG40 mRNA for S locus glycoprotein, partial cds. (Hatakeyama et al. 1998) --> BrS40

34:AB013719: Brassica oleracea mRNA for SLG23Bol, complete cds. (Kusaba and Nishio 1999) --> BoS23Bol
35:AB008190: Brassica rapa gene for SLG29, complete cds. (Hatakeyama et al. 1998) --> BrS29
36:D85228: Brassica oleracea DNA for S glycoprotein, partial cds.(Kusaba et al.1997)
--> Bo-a
37:D85229: Brassica oleracea DNA for S glycoprotein, partial cds. (Kusaba et al.1997) --> Bo-b
38:D85227: Brassica campestris DNA for S glycoprotein, partial cds. (Kusaba et al.1997) --> Br-c
39:Z19548: B.napus mRNA for S-locus glycoprotein type II (Robert et al. 1994) --> BnII
40:Y18261: Brassica oleracea SLGB gene, partial (Cabrillac et al. 1999) --> BoS15
41:AIK6-2
42:AIK6-3
43:AIK3-B
44:1/85-E
45:1/85-2
46:6/85-2
47:6/85-5
48:8/85-D
49:6/85-E
50: Start-A
51: Start-B
52: Start-C
53: Liropa-1
54: Liropa-3
55: Liropa-5
56: Liropa-6
57: Cathy-2
58: Cathy-4
59: Cathy-6
60: Local-B
61: Local-E
62: Rasmus-1
63: Rasmus-5
64: Rasmus-11
65: Rasmus-16
66: Rasmus-22
67: Rasmus-23
68: Rasmus-30
69: Rasmus-34
70: Ramiro-5
71: Global-A

Discussion

Aminoacid sequence of *SLG* gene is highly polymorphic but there are always 12 cysteine in similar position (Kusaba et al., 1997). Two very similar *S*-haplotypes were found in *Brassica oleracea* and *Brassica rapa* (Kusaba and Nishio, 1999). The common pollen recognition specificity was proven between these two species interspecific hybridization or through transgenosis (Kimura et al., 2002; Sato et al., 2003). Kimura et al. (2002) suggests that the same recognition specificity was preserved after these two specie diversication. In Brassica napus are most probably three conserved S-alleles (group III, IV, V) in population. Although *SLG* gene is highly polymorphic repesentation of single alleles in Brassica napus

population is low. In general there was no significant difference between sequences of class II *SLG* gene of SI lines and SC rapeseed cultivars.

According to phylogenetic tree which compares class II *SLG* gene sequences of *B. napus* with class I and class II *SLG* gene sequences of different *Brassica napus* or its relatives such as *B. oleracea*, B. rapa and *Raphanus sativus*, it seems there is a bigger difference between S-haplotypes classes than different species. Group 5 and group 2 show there is no difference in *SLG* gene sequences between SI and SC plants of *Brassica napus.* We can conclude from this information that *SLG* gene class II is not essential in self-incompatibility reaction.

Conclusion

According to analyse of 31 clones of *SLG* gene from different SI lines and cultivars three significantly different main group were suggest to be three most common alleles in *Brassica napus* population. No significant divergence between SI lines and cultivars of rapeseed was observed. Moreover, tested *SLG* alleles in *Brassica napus* were compared with sequences from National Center for Biotechnology Information resulted in observation that *S*-haplotype class has more substantial influence on *SLG* gene alelles diversification than species from which comes.

9. THE FULFILMENT OF THE AIMS

1. To develop molecular marker for recessive type of SI

Two *SCR* gene alleles were successfully identified in *B. napus*, termed allele 1 and allele 2, and the structure corresponds to class II *SCR* gene from *B. oleracea* and *B. rapa*. Allele 1 was found in a tandem copie and showed 100% identity with *S15* allele from *B. rapa*. The second was still not identified. Furthemore, alternative splicing of both alleles was found. The sequences of both alleles were highly divergent and according to variable areas in alleles a set of allele specific primers was designed. Amplification of allele 2 specifically occurred only in a part of plants. Segregation ratio correlated with doublehaploid segregation.

2. Compare SLG I marker that tags SC plants with developed SCR marker gene.

Amplified *SLG* I fragment was found in plants where the fragment of class II *SCR* allele 2 was not found. Which should mean that class I *SLG* marked SC plants and class II *SCR* SI plants. But there is one difference between both marker systems. Class I *SLG*I is not normally present in SI plants of recessive based self-incompatibility and requires not allele specific primers which makes this marker gene universal for different SI lines.Class II *SCR* gene is generally very polymorphic. Allele 2 of class II *SCR* gene was found in SI line 'Tandem 6/85' and it can serve as a marker gene only in SI lines derived from Tandem. I suppose that donor of SI, it means functional allele, come from *B. oleracea* genome, so the other SI markers would have been derived from single SI lines. It could increased the costs for marker development, if we want to select more lines recquired for hybrid development.

3. Applying markers in marker-assisted selection

Class I *SLG* marker used for SC plants screening tagged half of population of doubled haploids. Seed test did not correspond with doublehaploid theoretic segregation ratio and the real segregation ration was 1:3 (SI:SC). Self-incompatibility in *B. napus* is most probably controled with other locus (repressor locus) not linked to the *S* locus. 90% of tagged plants by class I *SLG* gene were SC, which means that this marker is highly efficient.

4. Assessment of variability in oilseed rape with aspect to SLG II polymorphism

A cluster tree was made from sequences of class II *SLG* alleles. *SLG* sequences were very divergent but the certain similarity devided them into 7 (3) main clusters which could be in accordance with 3 or seven alleles. Supposed variability was not proved in B. napus cultivars

due to narrowed base of oilseed rape genotypes caused long-time selection. The second finding was that *SLG* gene higher polymorphic between different classes of *S*-haplotypes than among relative species.

10. CONCLUSION

The *S*-locus structure study was focused especialy on structure class II *SCR* gene because recently was *SCR* gene proved to be pollen determinat in *B. rapa* and *B. oleracea*. The *SCR* gene was determined in *B. napus* in a short time by taking advantage of known oligonucleotide sequence of *SCR*s in related species. Two found *SCR* alleles corresponded to class II SCRs from B. rapa and B. oleracea and both expressed in SI and SC phenotype groups of plants. The first, termed allele 1, was identical to allele *S15* from *B. rapa* and occured in tandem copy and was presented in both SI and SC phenotype groups of plants. On the bases of published knowledge about *S15* allele disorders, we could assume *S15 S*-locus to be dysfunctional. In contrast, the second *SCR* allele was specifically amplified from genomic PCR only in SI plants. This selection using this marker gene was compared with other molecular marker gene, class I *SLG* gene, which is amplified in SC plants only. As an SI Segregating population used in this study were doublehaploids, 1:1 segregating ratio was expected. Suprisingly, the marker selection was almost uniform and corresponde to segregating ratio. However, appropriate phenotype determined using seed test was modified and final segregatin ratio was 1:3 (SI:SC). We can suppose on the basis of the seed test result that there is a repressor locus commonly present in *Brassica napus* cultivars which cause SC independently on *S*-locus. Furthermore this repressor locus is not participating in pollen-stigma surface recognition reaction but probably somewhere further in a signaling pathway responsible for SI reaction. Despite of this fact, using molecular markers is possible to select a majority of SC plants which can supposed as success.

To study representation of S-alleles in rapeseed population classification of the *SLG* gene class II alleles was performed. Previous PCR-RFLP analysis was not successful in S-alleles differentiation in different SI lines. Cloning and sequencing have revealed that class II *SLG* gene is very polymorphic but real number of alleles present at common cultivar and several SI lines is not so high. I have found three or seven groups corresponding to different *S*-allele specificity in cultivar and SI lines. Nothing suggested that SI lines have different *S*-alleles from common cultivars.

11. REFERENCES

Anderson, M.A., McFadden, G.I., Bernatzky, R., Atkinson, A., Orpin, T., Dedman, H., Tregear, G., Fernley, R., Clarke, A.R. (1989): Sequence variability of three alleles of the self-incompatibility gene of *Nicotiana alata*. *Plant Cell* **1**: 483-491.

Bannerot, H., Boulidard, L., Cauderon, Y., Tempe, J. (1874): Cytoplasmic male sterility transfer from *Raphanus* to *Brassica*. *Proc Eucarpia Meet Crop Set Cruciferae* **25**: 52-54.

Baranger A., Delourme R., Foisset. N., Barret, P., Dupuy, P., Renard, M., Chèvre, A. (1997): Wide mapping of a T-DNA insertion site in oilseed rape using bulked segregant analysis and comparative mapping. *Plant Breed* **116**: 553-560.

Barret, P.,Guérif, J., Reynoird, J., Delourme R., Eber, F., Renard, M., Chèvre, A.(1998a): Selection of stable *Brassica napus – Brassica juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans*) 2. A 'to and fro' strategy to localise and characterise interspecific introgression on the *B. napus* genome. *Theor Appl Genet* **96**: 1097-1103.

Barret, P., Delourme R., Foisset. N., Renard, M. (1998b): Development of a SCAR (sequence characterised amplified region) marker for molecular tagging of the dwarf BREIZH (*Bzh*) gene in *Brassica napus* L. *Theor Appl Genet* **97**: 828-833.

Bateman, A.,J. (1955) Self-incompatibility system in angiosperms. III. Cruciferae. Heredity 9: 52-68.

Becker, H.C., Engqvist, G.M., Karlssom, B. (1995): Comparison of rapeseed cultivars and resynthesized lines based on allozyme and RFLP markers. *Theor Appl Genet* **91**: 62-67.

Bower, M.S., Matias, D.D., Fernandes-Carvalho, E., Mazzurco, M., Gu, T., Rothstein, S., Goring, D.R. (1996): Two members of the thioredoxin-h family interact with the kinase domain of a *Brassica S* locus receptor kinase. Plant Cell 8: 1641-1650.

Boyes, D.C., Nasrallah, M.E., Vrebalov, J., Nasrallah, J.B. (1997): The self-incompatibility (S) haplotypes of Brassica contain highly divergent and rearranged sequences of ancient orgin. Plant Cell 9: 237-247.

Brace, J., Ockendon, D.J., King, G.J. (1993): Development of a method for identification of *S* alleles in *Brassica oleracea* based on digestion of PCR-amplified DNA with restriction endonucleases. Sex. Plan. Reprod. 6: 133-138.

Brace, J., King, G.J., Ockendon, D.J. (1994): A molecular approach to the identificationof S alleles in *Brassica oleracea*. Sex. Plant Reprod. 7: 203-208.

Brunel, D., Froger, N., Pelletier, G. (1999): Development of amplified consensus genetic markers (ACGM) in *Brassica napus* from *Arabidopsis thaliana* sequences of known biological function. *Genome* **42**: 387-402.

Burns, M.J., Barnes, S.R., Bowman, J.G., Clarke, M.H.E., Werner, C.P., Kearsey, M.J. (2003): QTL analysis of an intervarietal set substitution lines in *Brassica napus*: (i) seed oil content and fatty acid composition. *Heredity* **90**: 39-48.
Casselman, A.L., Vrebalov, J., Conner, J.A., Singhal, A., Giovannoni, J., Nasrallah, M.E., Nasrallah, J.B. (2000): Determining the physical limits of the *Brassica S* locus by recombinational analysis. Plant Cell 12: 23-33.

Cabrillac,D., Delorme,V., Garin,J., Ruffio-Chable,V., Giranton,J.L., Dumas,C., Gaude,T. and 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.

Charters, Y.M., Robertson, A., Wilkinson, M.J. (1996): PCR analysis of oilseed rape cultivars (*Brassica napus* L. ssp. *Oleifera*) using 5'-anchored simple sequence repeat (SSR) primers. *Theor Appl Genet* **92**: 442-447.

Chen, J.L., Beversdorf, W.D. (1990): Fatty acid inheritance in microspore-derived populations of spring rapeseed (*Brassica napus* L.). *Theor Appl Genet* **80**: 465-469.

Cheung, W.Y., Champagne, G., Hubert, N., Landry, B.S. (1997): Comparison of the genetic maps of *Brassica napus* and *Brassica oleracea*. *Theor Appl Genet* **94**: 569-582.

Chèvre, A.M., Barret, P., Eber, F., Dupuy, P., Brun, H., Tanguy, X., Renard, M. (1997): Selection of stable *Brassica napus-B. Juncea* recombinant lines resistant lines resistant to blackleg (*Leptosphaeria maculans*). 1. Identification of molecular markers, chromosomal and genomic orgin of the introgression. *Theor Appl Genet* **95**: 1104-1111.

Chookajorn, T., Kachroo, A., Ripoll, D.R., Clark, A.G., Nasrallah, J.B. (2004): Specificity determinants and divesification of the *Brassica* self-incompatibility pollen ligand. PNAS. **4**: 911-917.

Cui, Y., Brugiere, N., Jackman, L., Bi, Y.-M., Rothstein, S.J. (1999): Structural and transcriptional comparative analysis of the *S* locus regions in two self-incompatible *Brassica napus* lines. Plant Cell 11: 2217-1131.

Delourme, R., Bouchereau, A., Hubert, N., Renard, M., Landry, B.S. (1994): Identification of RAPD markers linked to fertility restorer gene for the *Ogura* radish cytoplasmic male sterility of rapeseed (*Brassica napus* L.). *Theor Appl Genet* **88**: 741-748.

Delorme,V., Giranton,J.L., Hatzfeld,Y., Friry,A., Heizmann,P., Ariza,M.J., Dumas,C., Gaude,T., Cock,J.M.(1995): Characterization of the S locus genes, SLG and SRK, of the Brassica S3 haplotype: identification of a membrane-localized protein encoded by the S locus receptor kinase gene. *Plant J.* **7**: 429-440.

Delourme, R., Foisset, N., Horvais, R., Barret, P., Champagne, G., Cheung, W.Y., Landry, B.S., Renard, M. (1998): Characterisation of the radish introgression carrying the *Rfo* restorer gene for the *Ogu*-INRA cytoplasmic male sterility in rapeseed (*Brassica napus* L.). *Theor Appl Genet* **97**: 129-134.

Delourme, R., Pilet-Nayel, M.L., Archipiano, M., Horvais, R., Tanguy, X., Rouxel, T., Brun, H., Renard, M., Balesdent, M.H. (2004): A Cluster of Major Specific Resistance Genes to *Leptosphaeria maculans* in *Brassica napus*. *Phytopathology* **94**: 578-583.

de Nettancourt, D. (1977): Incompatibility in angiosperms. Berlin: Springer.

Diers, B.W., Osborn, T.C. (1994): Genetic diversity of oilseed *Brassica napus* germplasm based on restriction fragment length polymorphisms. *Theor Appl Genet* **88**: 662-668.

Diers, B.W., McVetty, P.B.E., Osborn, T.C. (1996): Relationship between heterosis and genetic distance based on restriction fragment length polymorphism markers in oilseed rape (*Brassica napus*). *Crop Sci.* **36**: 79-83.

Dion, Y., Gugel, R.K., Rakow, G.F.W., Seguin-Swartz, G., Landry, B.S. (1995): RFLP mapping of resistance to the blackleg disease [causal agent, *Leptosphaeria maculans* (Desm.) Ces. et de Not. in canola (*Brassica napus* L). *Theor Appl Genet* **91**: 1190-1194.

Dodds, P.N., Clarke, A.E., Newbigin, E. (1996): A molecular perspective on pollination in flowering plants. Cell 85: 141-144.

Doughty, J., Hedderson, F., McCubbin, A., Dickinson, H. G. (1993): Interaction between a coating-borne peptide of the Brassica pollen grain and S (incompatibility)-locus linked stigmatic glycoproteins. *Proc. Natl. Sci. USA* **90**: 467-471.

Doughty, J., Dixon, S., Hiscock, J.S., Willis, A.C., Parkin, I.A.P., Dickinson, H.G. (1998): PCP-A1, a defensin-like, *Brassica* pollen coat protein that binds the *S* locus glycoprotein, is the product of gametophytic gene expression. Plant Cell 10: 1333-1347.

Doughty, J., Wong, H., Y., Dickinson,G. (2000): Cysteine-rich pollen coat proteins (PCPs) and their interactions with stigmatic *S* (incompatibility) and *S*-related proteins in *Brassica*: Putative roles in SI and pollination. Ann. Bot. 85 (Supplement A): 161-169.

Dreyer, F., Graichen, K., Jung, C. (2001): A major quantitative trait locus for resistance to Turnip Yellows Virus (TuYV, syn. Beet western yellows virus, BWYV) in rapeseed. *Plant Breeding* **120**: 457-462.

Ecke, W., Uzunova, M., Weißleder, K. (1995): Mapping the genome of rapeseed (*Brassica napus* L.). II. Localization of genes controlling erucic acid synthesis and seed oil content. *Theor Appl Genet* **91**: 972-977.

Ekuere, U.U., Parkin, I.A.P., Bowman, C., Marshall, D., Lydiate, D.J. (2004): Latent *S* alleles are widespread in cultivated self-compatible *Brasica napus*. Genome 42: 257-265.
Ollson, G. (1960): self-incompatibility and outcrossing in rape and white mustard. Hereditas 46: 241-252.

Ferreira, M.E., Williams, P.H., Osborn, T.C. (1994): RFLP mapping of *Brassica napus* using doubled haploid lines. *Theor Appl Genet* **89**: 615-621.

Ferreira, M.E., Rimmer, S.R., Williams, P.H., Osborn, T.C. (1995a): Mapping loci controlling *Brassica napus* resistance to *Leptosphaeria maculans* under different screening conditions. *Phytopathology* **85**: 213-217.

Ferreira, M.E., Williams, P.H., Osborn, T.C. (1995b): Mapping of a locus controlling resistance to *Albugo candida* in *Brassica napus* using molecular markers. *Phytopathology* **85**: 218-220.

Ferreira, M.E., Satagopan, J., Yandell, B.S., Williams, P.H., Osborn, T.C. (1995c): Mapping loci controling vernalisation requirement and flowering time in *Brassica napus*. *Theor Appl Genet* **90**: 727-732.

Foisset, N., Delourme, R., Barret, P., Renard, M. (1995): Molecular tagging of the dwarf BREIZH (Bzh) gene in *Brassica napus*. *Theor Appl Genet* **91**: 756-761.

Foisset, N., Delourme, R., Barret, P., Hubert, N., Landry, B.S., Renard, M. (1995): Molecular mapping analysis in *Brassica napus* using isozyme, RAPD and RFLP markers on doubled-haploid progeny. *Theor Appl Genet* **93**: 1017-1025.

Fourmann, M., Barret, P., Renard, M., Pelletier, G., Delourme, R., Brunel, D. (1998): The two genes homologous to *Arabidopsis FAE1* co-segregate with the two loci governing erucic acid content in *Brassica napus*. *Theor Appl Genet* **96**: 852-858.

Fourmann, M., Barret, P., Froger, N., Baron, C., Charlot, F., Delourme, R., Brunel, D. (2002): From *Arabidopsis thaliana* to *Brassica napus*: development of amplified consensus genetic markers (ACGM) for construction of a gene map. *Theor Appl Genet* **105**: 1196-1206.
Franklin-Tong, V.E., Atwal, K.K., Howell, E.C., Lawrence, M.J., Franklin, F.C.H. (1991): Self-incompatibility in *Papaver rhoeas*: There is no evidence for involvement of stigmatic ribonuclease activity. Plant Cell Environ. 14: 423-429.

Franklin-Tong, V.E., Franklin, F.C.H. (2003): Different mechanismus of gametophytic self-incompatibility. Phil. Trans. R. Soc. Lond. B 358: 1025-1032.

Fray, M.J., Puangsomlee, P., Goodrich, J., Coupland, G., Evans, E.J., Arthur, A.E., Lydiate, D.J. (1997): The genetics of stamenoid petal production in oilseed rape (*Brassica napus*) and equivalent variation in *Arabidopsis thaliana*. *Theor Appl Genet* **94**: 731-736.

Frello, S., Hansen, K.R., Jensen, J., Jørgensen, R.B. (1995): Inheritance of rapeseed (*Brassica napus*)-specific RAPD markers and a transgene in the cross *B. juncea* × (*B. juncea* × *B. napus*). *Theor Appl Genet* **91**: 236-241.

Fu, T.D. (1981): Production and research of rapeseed in the People's Republic of China. *Eucarpia Cruciferae Newsl* **6**: 6-7.

Fu, T.D., Yang, G.S. (1998): Rapeseed mustard. In: Banga, S.S., Banga, S.K. (eds) Hybrid cultivar development. Co-Publication Springer, Berlin Heidelberg New York and Narosa Publishing House, New Delhi, pp 402-431.

Fu, T.D. (2000): Breeding and utilization of rapeseed hybrid, 2nd edn. Hubei Science and Technology Press, Wuhan.

Fujimoto, R., Nishio, T. (2003): Identification of *S*-haplotypes in *Brassica* by dot blot analysis of *SP11* alleles. Theor Appl Genet 106: 1433-1437.

Fujimoto,R., Okazaki,K., Fukai,E., Kusaba,M., Nishio,T.(2006): Comparison of the genome structure of the self-incompatibility (s)locus in interspecific pairs of s haplotypes. *Genetics* **173**: 1157-1167.

Fukai, E., Nishio, T., Nasrallah, M.E. (2001): Molecular genetic analysis of candidate gene for *MOD*, a locus recquired for self-incompatibility in *Brassica rapa*. Mol. Genet. Genomics 265: 519-525.

Gu, T., Mazzurco, M., Sulaman, W., Matias, D.D., Goring, D.R. (1998): Binding of an arm repeat protein to the kinase domain of the *S* locus receptor kinase. Proc. Natl. Acad. Sci. USA 95: 382-387.

Gowers, S. (1974): The production of F_1 hybrid swedes (*B. napus* ssp. *rapifera*) by the utilisation of self-incompatibility. Euphytica 23: 205-208.
NISHIO, T., KUSABA, M., SAKAMOTO, T., 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.

Govers, S. (1975): Methods of producing F_1 hybrid swedes (*Brassica napus* ssp. *rapifera*). Euphytica 24: 537-541.

Grant, I., Beversdorf, W. D. (1985): Heterosis and combining ability estimates in spring planted oilseed rape (*Brassica napus* L.). *Can. J. Genet. Cytol.* **27**: 472-478.

Goring, D. R., Banks, P., Fallis, L., Baszczynski, C. L., Beversdorf, W. D., Rothstein, S. J.(1992): Identification of an S-locus glycoprotein allele introgressed from B. napus ssp. rapifera to B. napus ssp. Oleifera. *Plant J.* **2**: 983-989.

Goring, D. R., Glavin, T. L., Schafer, U., Rothstein, S .J.(1993): An S receptor kinase gene in self-compatible Brassica napus has a 1-bp deletion. *Plant Cell* **5**: 531-539.

Gothandam, K. M., Hyun, J., Chung,Y.-Y.: Dominance relationship between the two self-incompatible Brassica campestris plants in response to CO2 gas. Unpublished (submitted to genebank 2005, http://www.ncbi.nlm.nih.gov).

Guohua, T., Rui, Y. (1986): Use of CO_2 and salt solution to overcome self-incompatibility of chinese cabbage (*Brassica campestris* ssp. *pekinensis*). Cruc. Newsl. 11: 75-76.
Halldén, C., Nilsson, N.O., Rading, I.M., Säll, T. (1994): Evaluation of RFLP and RAPD markers in comparison of *Brassica napus* breeding lines. *Theor Appl Genet* **88**: 123-128.

Hansen, L.B., Siegismund, H.R., Jørgensen, R.B. (2001): Introgression between oilseed rape (*Brassica napus* l.) and its weedy relative *B. rapa* L. in a natural population. *Genetic Resources and Crop Evolution* **48**: 621-627.

Hansen, L.B., Siegismund, H.R., Jørgensen, R.B. (2003): Progressive introgression between *Brassica napus* (oilseed rape) and *B. rapa*. *Heredity* **91**: 276-283.

Hansen, M., Halldén, C., Nilsson, N.O., Säll, T. (1997): Marker-assisted selection of restored male-fertile *Brassica napus* plants using a set of dominant RAPD markers. *Molecular Breeding* **3**: 449-456.

Hanson, M.R. (1991): Plant mitochondrial mutations and cytoplasmic male sterility. *Annu Rev Genet* **25**: 461-486.

Hasan, M., Seyis, F., Badani, A.G., Pons-Kühnemann, J., Friedt, W., Lühs, W., Snowdon, R.J. (2005): Analysis of genetic diversity in the *Brassica napus* L. gene pool using SSR markers. *Genetic Resources and Crop Evolution* **53**: 793-802.

Hatakeyama, K., Takasaki, T., Watanabe, M., Hinata, K.(1998): Molecular characterization of S locus genes, SLG and SRK, in a pollen-recessive self-incompatibility haplotype of Brassica rapa L. *Genetics* **149**: 1587-1597.

Hatakeyama, K., Takasaki, T., Watanabe, M., Hinata, K.: Molecular characterization of SLG and SRK in an S22 haplotype of self-incompatible Brassica rapa L. Unpublished (submitted to genebank 2001, http://www.ncbi.nlm.nih.gov).

Havel, J. (1996): Získávání autoinkompatibilních linií řepky ozimé. *Genet. a šlecht.* **32**: 9-18.

Hawkins, G.P., Zhang, X., Thiagarjan, M.R., Corrigan, L.M., Stringam, G.R.(2005): Identification of RAPD markers linked to pod lengtht in *Brassica napus* L. canola. *Can. J. Plant Sci.* **85**: 803-808.

He, Y., Chen, B., Fu, T., Li, D., Tu, J.: Comparison of S-locus genes between SI and SC Brassica. Unpublished (submitted to genebank 2003, http://www.ncbi.nlm.nih.gov).

Hiscock, S. J., Dickinson, H.G. (1993): Unilateral incompatibility within the Brassicaceae. Further evidence for the involvement of the self-incompatibility (S)-locus. Theor Appl Genet 86: 744-753.

Howell, P.M., Sharpe, A.G., Lydiate, D.J. (2003): Homoeologous loci control the accumulation of seed glucosinolates in oilseed rape (*Brassica napus*). *Genome* **46**: 454-460.

Hu, J., Quiros, C., Arus, P., Struss, D., Robbelen, G. (1995): Mapping of a gene determining linolenic acid concentration in rapeseed with DNA-based markers. *Theor Appl Genet* **90**: 258-262.

Hu, J., Li, G., Struss, D., Quiros, C.F. (1999): SCAR and RAPD markers associated with 18-carbon acids in rapeseed *Brassica napus*. *Plant Breeding* **118**: 145-150.

Hu, X., Sullivan-Gilbert, M., Gupta, M., Thompson, S.A. (2006): Mapping of the loci controlling oleic and linolenic acid contents and development of *fad2* and *fad3* allele-specific markers in canola (*Brassica napus* L.). *Theor Appl Genet* **113**: 497-507.

Hughes, S.L., Hunter, P.J., Sharpe A.G., Kearsey M.J., Lydiate D.J., Walsh, J.A. (2003): Genetic mapping of the novel *Turnip mosaic virus* resistance gene *TuRB03* in *Brassica napus*. *Theor Appl Genet* **107**: 1169-1173.

Ikeda, S., Nasrallah, J.B., Dixit, R., Preiss, S., Nasrallah, M.E. (1997): An aquaporin-like gene required for the *Brassica* self-incompatibility response. Science 276: 1564-1566.
Stone, S.L., Arnoldo, M., Goring, D.R. (1999): A breakdown of *Brassica* self-incompatibility in ARC1 antisense transgenic plants. Science 286: 1729-1731.

Isogai, A., Hinata, K. (2002): Molecular mechanism for recognition reaction in self-incompatibilityof *Brassica* species. Proc. Jpn. Acad.78B: 241-249.

Iwano, M., Shiba, H., Funato, M., Shimosato, H., Takayama, S., Isogai, A. (2003): Immunohistochemical studies on translocation of pollen *S*-haplotype determinant in self-incompatibility of *Brassica rapa*. Plant Cell Physiol. 44: 428-436.

Jahnen, W., Batterham, M.P., Clarke, A.E., Moritz, R.L., Simpson, R.J. (1989). Identification, isolation and N-terminal sequencing of style glycoproteins associated with self-incompatibility in *Nicotiana alata*. Plan Cell 1: 493-499.

Janeja, H.S., Banga, S.S., Lakshmikumaran, M. (2003): Identification of AFLP markers linked to fertility restorer genes for tournefortii cytoplasmic male-sterility system in Brassica napus. *Theor Appl Genet* **107**: 148-154.

Javidfar, F., Ripley, V.L., Roslinsky, V., Zeinali, H., Abdmishani, C. (2006): Identification of molecular markers associated with oleic and linolenic acid in spring oilseed rape (*Brassica napus*). *Plant Breeding* **125**: 65-71.

Jean, M., Brown, G.G., Landry, B.S. (1997): Genetic mapping of nuclear fertility restorer genes for the 'Polima' cytoplasmic male sterility in canola (*Brassica napus* L.) using DNA markers. *Theor Appl Genet* **95**: 321-328.

Jean, M., Brown, G.G., Landry, B.S. (1998): Targeted mapping approaches to identify DNA markers linked to the Rfpl restorer gene for the 'Polima' CMS of canola (*Brassica napus* L.). *Theor Appl Genet* **97**: 431-438.

Jourdren, C., Barret, P., Horvais, R., Delourme, R., Renard, M. (1996): Identification of RAPD markers linked to linolenic acid genes in rapeseed. *Euphytica* **90**: 351-357.
Ke, L., Sun, Y., Liu, P., Yang, G. (2004): Identification of AFLP fragments linked to one recessive genic male sterility (RGMS) in rapeseed (*Brassica napus* L.) and conversion to SCAR markers for marker-aided selection. *Euphytica* **138**: 163-168.

Kachroo, A., Nasrallah, M.E., Nasrallah, J., B. (2002): Self-incompatibility in the Brassicaceae: receptor-ligand signaling and cell-to-cell communication. Plant Cell Sup.: S227-S238.

Kakizaki, T., Takada, Y., Ito, A., Suzuki, G., Shiba, H., Takayama, S., Isogai, A., Watanabe, M. (2003): Linear Dominance Relationship among Four Class-II *S* Haplotypes in Pollen is Determined by the Expression of *SP11* in *Brassica* Self-Incompatibility. *Plant Cell Physiol.* **44**: 70-75.

Ke, L.P., Sun, Y.Q., Hong, D.F., Liu, P.W., Yang, G.S. (2005): Identification of AFLP markers linked to one recessive genic male sterility gene in oilseed rape, *Brassica napus*. *Plant Breeding* **124**: 367-370.

Kimura, R., Sato, K., Fujimoto, R., Nishio, T. (2002): Recognition specificity of self-incompatibility maintained after the divergence of *Brassica oleracea* and *Brassica rapa*. Plant J. 29: 215-223.

Kole, C., Thormann, Karlsson, B.H., Palta, J.P., Gaffney, P., Yandell, B., Osborn, T.C. (2002): Comparative mapping of loci controlling winter survival and related traits in oilseed *Brassica rapa* and *B. napus*. *Molecular Breeding* **9**: 201-210.

Kole, C., Williams, P.H., Rimmer, S.R., Osborn, T.C. (2002b): Linkage mapping of genes controlling resistance to white rust (*Albugo candida*) in *Brassica rapa* (syn. *campestris*) and comparative mapping to *Brassica napus* and *Arabidopsis thaliana*. *Genome* **45**: 22-27.

Konieczny, A., Ausubel, F. (1993): A procedure for mapping *Arabidopsis* mutations using codominant ecotype specific PCR based markers. Plant J. 4: 403-410.

Kusaba, M., Nishio, T., Satt, Y., Hinata, K., Ockendon, D. (1997): Striking sequence similarity in inter- and intra-specific comparison of class I SLG alleles from Brassica oleracea and Brassica campestris: Implication for evolution and recognition mechanism. Proc. Natl. Acad. Sci. USA 94: 7673-7678.

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.

Landry, B.S., Hubert, N., Etoh, T., Harada, J.J., Lincoln, S.E. (1991): A genetic map for *Brassica napus* based on restriction fragment length polymorphisms detected with expressed DNA sequences. *Genome* **34:** 543-552.

Leino, M., Teixeira, R., Landgren, M., Glimelius, K. (2003): *Brassica napus* lines with rearranged *Arabidopsis* mitochondria display CMS and a range of developmental aberrations. *Theor Appl Genet* **106**: 1156-1163.

Li, S.L., Qian, Y.X., Wu, Z.H., Stefansson, B.R. (1988): Genetic male sterility in rape (*Brassica napus* L.) conditioned by interaction of genes at two loci. *Can J Plant Sci* **68**: 1115-1118.

Lombard, V., Baril, C.P., Dubreuil. P., Blouet, F., Zhang, D. (2000): Genetic relationship and fingerprinting of rapeseed cultivars by AFLP: Consequences for varietal registration. *Crop Sci* **40**: 1417-1425.

Lombard, V., Delourme, R. (2001): A consensus linkage map for rapeseed (*Brassica napus* L.): construction and integration of three individual maps from DH populations. *Theor Appl Genet* **103**: 491-507.

Lu, G.Y., Yang, G.S., Fu, T.D. (2004): Molecular mapping of dominant genic male sterility gene *Ms* in rapeseed (*Brassica napus*). *Plant Breeding* **123**: 262-265.

Lydiate, D., Sharpe, A., Lagercrantz, U., Parkin, I. (1993): Mapping the *Brassica* genome. *Outlook Agrik* **22**: 85-92.

Mailer, R.J., Scarth, R., Fristensky, B. (1994): Discrimination among cultivars of rapeseed (*Brassica napus* L.) using DNA polymorphisms amplified from arbitrary primers. *Theor Appl Genet* **87**: 697-704.

Marshall, P., Marchand, M.C., Lisieczko, Z., Landry, B.S. (1994): A simple method to estimate the percentage of hybridity in canola (*Brassica napus*) F1 hybrids. *Theor Appl Genet* **89**: 853-858.

McCubbin, A.G., Kao, T-h. (2000): Molecular recognition and response in pollen and pistil interactins. Annu. Rev. Dev. Biol. 16: 333-364.

Meng, J.L., Shi, S.W., Gan, L., Li, Z.Y., Qu, X.S. (1998): The production of yellow-seeded *Brassica napus* (AACC) through crossing interspecific hybrids of *B. campestris* (AA) and *Brassica carinata* (BBCC) with *B. napus*. *Euphytica* **103**: 329-333.

Mikkelsen, T.R., Jensen, J., Jørgensen, R.B. (1996): Inheritance of oilseed rape (*Brassica napus*) RAPD markers in a backcross progeny with *Brassica campestris*. *Theor Appl Genet* **92**: 492-497.

Mishima, M., Takayama, S., Sasaki, K.-I., Jee, J.-G., Kojima, C., Isogai, A., Shirakawa, M. (2003): Structure of the Male Determinant Factor for *Brassica* Self-incompatibility. *The Journal of Biological Chemistry* **278**: 36389-36395.

Möhring, S., Horstmann, V., Esch, E. (2005): Development of a molecular CAPS markers for the self-incompatibility locus in *Brassica napus* and identification of different *S* alleles. Plant Breeding 124: 105-110.

Nasrallah, J.B., Kao, T.-H., Chen, C.-H., Goldberg, M.L., Nasrallah, M.E. (1987): Amino acid sequences of glycoproteins encoded by three alleles of the *S* locus of *Brassica oleracea.* Nature 326, 617-619.

Nasrallah, J.B., Nishio, T., Nasrallah, M.E. (1991): The self-incompatibility genes of *Brassica*: Expression and use in genetic ablation of floral tissues. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 393-422.

Nasrallah, J.B., Nasrallah, M.E. (1993): Pollen-stigma signaling in the sporophytic self-incompatibility response. *Plant Cell* **5**:1325-1335.

Nasrallah, M.E., Rundle, S.J., Nasrallah, J.B. (1994): Genetic evidence for requirement of the *Brassica S*-locus receptor kinase gene in the self-incompatibility response. Plant J. 5: 373-384.

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:** 1329-1334.

Nishio, T., Kusaba, M., Sakamoto, K., Ockendon, D.J. (1997): Polymorphism of the kinase domain of the *S*-locus receptor kinase gene (SRK) in *Brassica napus* L. *Theor Appl Genet* **85**: 335-342.

Nou, I., Watanabe, M., Isogai, A., Hinata, K. (1993): Comparison of S-alleles and *S*-glycoproteins between two wild populations of *Brassica campestris* in Turkey and Japan. Sex. Plant. Reprod. 6: 79-86.

Ockendon, D.J. (1974): Distribution of self-incompatibility alleles and breeding structure of open pollinated cultivars of Brussels Sprouts. Heredity 33: 159-171.

Ockendon, D.J. (1975): The S allele collection of *Brassica oleracea.* Incomp. Newsl. 5: 82-84.

Ogura, H. (1968): Studies on the new male-sterility in Japanese radish, with special reference to the utilization of this sterility towards the practical raising of hybrid seeds. *Mem Fac Agric Kagashima Univ* **2**: 39-78.

Okamoto, S., Sato, Y., Sakamoto, T., Nishio, T. (2004): Distribution of similar self-incompatibility (*S*) haplotypes in different genera, *Raphanus* and *Brassica*. Sex Plant Reprod **17:** 33-39.

Okazaki, K., Kusaba, M., Ockendon, D., J., Nishio, T. (1999): Characterisation of *S* tester lines in *Brassica oleracea*: polymorphism of restriction fragment length of *SLG* homologous and isoelectric points of *S* locus glycoproteins. *Theor. Appl. Genet.* **98**: 1329-1334.

Olsson, G. (1960): Self-incompatibility and outcrossing in rape and white mustard. *Hereditas* **46**: 159-177.

Osborn, T.C., Butruille, D.V., Sharpe,A.G., Pickering, K.J., Parkin, I.A.P. et al. (2003): Detection and effects of a homeologous reciprocal transposition in *Brassica napus*. *Genetics* **165**:1569-1577.

Pandey, K.K. (1958) Time of *S*-allele action. *Nature* 181: 1220-1221.

Pallett, D.W., Huang, L., Cooper J.I., Wang, H. (2006): Within-population variation in hybridisation and transgene transfer between wilde *Brassica rapa* and *Brassica napus* in UK. *Ann Appl Biol* **148**: 147-155.

Park, J.I., Nou, I.S., Lee, S.S., Kang, K.K., Watanabe, M. (2001): Identification of S-genotypes by PCR-RFLP in breeding lines of *Brassica*. *Mol. Cells* 12: 227-232.

Park, J.I., Lee, S.S., Watanabe, M., Takahata, Y., Nou, I.S. (2002): Identification of *S*-alleles using polymerase chain reaction-cleaved amplified polymorphic sequence of the *S*-locus receptor kinase in inbreeding lines of *Brassica oleracea*. Plant Breeding 121: 192-197.

Parkin, I.A.P., Sharpe, A.G., Keith, D.J., Lydiate, D.J. (1995): Identification of the A and C genomes of amphidiploid *Brassica napus* (oilseed rape). *Genome* **38**: 1122-1131.

Parkin, I.A.P., Lydiate, D.J. (1997): Conserved patterns of chromosome pairing and recombination in *Brassica napus* crosses. *Genome* **40**: 496-504.

Piquemal, J., Cinquin, E., Couton, F., Rondeau, C., Seignoret, E., Doucet, I., Perret, D., Villeger, M.-J., Vincourt, P., Blanchard, P. (2005): Construction of an oilseed rape (*Brassica napus* L.) genetic map with SSR markers. . *Theor Appl Genet* **111**: 1514-1523.

Pilet, M.L., Delourme, R., Foiset, N., Renard, M. (1998a): Identification of loci contributing to quantitative resistance to blackleg disease, causial agent *Leptosphaeria maculans* (Desm.) Ces. et de Not., in winter rapeseed (*Brassica napus* L). *Theor Appl Genet* **96**: 23-30.

Pilet, M.L., Delourme, R., Foiset, N., Renard, M. (1998b): Identification of QTL involved in field resistance to light leaf spot (*Pyrenopeziza brassicae*) and blackleg resistance (*Leptosphaeria maculans*) in winterseed rape (*Brassica napus* L.). *Theor Appl Genet* **97**: 398-406.

Pilet, M.L., Duplan, G. Archipiano, Barret, P., Baron, C., Horvais, R., Tanguy, X., Lucas, O.M., Renard, M., Delourme, R. (2001): Stability of QTL for field resistance to blackleg across two genetic backgrounds in oil rape. *Crop Sci.* **41**: 197-205.

Plieske, J., Struss, D., Röbbelen, G. (1998): Inheritance of resistance derived from the B-genome of *Brassica* against *Phoma lingam* in rapeseed and the development of molecular markers. *Theor Appl Genet* **97**: 929-936.

Plieske, J., Struss, D. (2001): STS markers linked to *Phoma* resistance genes of the *Brassica* B-genome revealed sequence homology between *Brassica nigre* and *Brassica napus*. *Theor Appl Genet* **102**: 483-488.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., Rafalski, A. (1996): The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol Breed* **2**: 225-238.

Rajcan, I., Kasha, K.J., Kott, L.S., Beversdorf, W.D. (1999): Detection of molecular markers associated with linolenic and erucic acid levels in spring rapeseed (*Brassica napus* L.). *Euphytica* **105**: 173-181.

Rajcan, I., Kasha, K.J., Kott, L.S., Beversdorf, W.D. (2002): Evaluation of cytoplasmic effects on agronomic and seed quality traits in two doubled haploid populations of *Brassica napus* L. *Euphytica* **123**: 401-409.

Ripley, V., L., Beversdorf, W., D. (2003): Development of self-incompatible *Brassica napus*: (III) *B. napus* genotype effects on S-allele expression. *Plant Breeding* **122**: 12-18.
Wallace, D.H. (1979): Interactions of *S*-alleles in sporophytically controlled self-incompatibility of *Brassica.* Theor. Appl. Genet. 54: 193-201.

Robert, L. S., Allard, S., Franklin, T. M., Trick, M.(1994): Sequence and expression of endogenous S-locus glycoprotein genes in self-compatible Brassica napus. *Mol. Gen. Genet.* **242**: 209-216.

Saal, B., Struss, D. (2005): RGA- and RAPD-derived SCAR markers for a *Brassica* B-genome introgression conferring resistance to blackleg in oilseed rape. *Theor Appl Genet* **111**: 281-290.

Sakamoto, K., Kusaba, M., Nishio, T. (1998): Polymorphism of the *S*-locus glycoprotein gene (*SLG*) and *S*-locus related gene (*SLR1*) in *Raphanus sativus* L. and self-incompatible ornamental plants in the Brassicaceae. Mol Gen Genet 258: 397-403.

Sampson, D.R. (1957): The genetics of self-incompatibility in the radish. J. Heredity 48: 26-29.

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

Sato, K., Fujimoto, R., Toriyama, K., Nishio, T. (2003): Commonality of self-recognition specificity of *S* haplotypes between *Brassica oleracea* and *Brassica rapa*. Plant Mol Biol 52: 617-626.

Sharpe, A.G., Parkin, I.A.P., Keith, D.J., Lydiate, D.J. (1995): Frequent nonreciprocal translocations in the amphidiploid genome of oilseed rape (*Brassica napus*). *Genome* **38**: 1112-1121.

Shiba, H., Takayama, S., Iwano, M., Shimohato, H., Funato, M., Nakagawa, T., Che, F.-S., Suzuki, G., Watanabe, M., Hinata, K., Isogai, A. (2001): A Pollen Coat Protein, SP11/SCR, Determines the Pollen *S*-Specificity in the Self-Incompatibility of *Brassica* Species. *Plant Physiology* **125**: 2095-2103.

Shiba, H., Iwano, M., Entani, T., Ishimto, K., Shimohato, H., Che, F.-S., Satta, Y., Ito, A., Takada, Y., Watanabe, M., Isogai, A., Takayama, S. (2002): The Dominance of Alleles Controlling Self-Incompatibility in *Brassica* Pollen Is Regulated at the RNA Level. *The Plant Cell* **14**: 491-504.

Shiba, H., Park, J.-I., Suzuki, G., Matsushita, M., Nou, I.-S., Isogai, A., Takayama, S., Watanabe, M. (2004): Duplicated *SP11*genes produce alternative transkripts in the S^{15} haplotype of *Brassica oleracea*. *Genes. Gent. Syst.* **79**: 87-93.

Shierholt, A., Becker, H.C., Ecke, W. (2000): Mapping a high oleic acid mutation in winter oilseed rape (*Brassica napus* L.). *Theor Appl Genet* **101**: 897-901.

Shirzadegan, M., Röbbelen, G. (1985): Influence of seed coulour and hull proportions on quality properties of seeds in *Brassica napus* L. *Fette Seifen Anstrichm* **87**: 235-237.

Schopfer, C.R., Nasrallah, M.E., Nasrallah, J.B. (1999): The male determinant of self-incompatibility in *Brassica*. *Science* **286**: 1697-1700.

Seyis, F., Snowdown, R.J., Lühs, W., Friedt, W. (2003): Molecular characterization of novel resynthesized rapeseed (*Brassica napus*) lines ad analysis of their genetic diversity in comparison with spring rapeseed cultivars. *Plant Breed* **122**: 473-478.

Silva, N.F., Stone, S.L., Christie, L.N., Sulaman, W., Nazarian, K.A., Burnett, L.A., Arnoldo, M.A., Rothstein, S.J., Goring, D.R. (2001): Expression of the *S* receptor kinase in self-compatible *Brassica napus* cv. Westar leads to the allele-specific rejection of self-incompatible *Brassica napus* pollen. Mol. Genet. Genomics 265: 552-559.

Smith, B.M. (1977): The improvement of self-incompatibility in Brussels Sprouts by incorporation of dominant *S*-alleles from kale. Incomp. Newsl. 8: 10-14.
Song, L.Q., Fu, T.D., Tu, J.X., Ma, C.Z., Zang, G.S. (2006): Molecular validation of allele inheritance for dominant genic male sterility gene in *Brassica napus* L. *Theor Appl Genet* **113**: 55-62.

Somers, D.J., Friesen, K.R.D., Rakow, G. (1998): Identification of molecular markers associsted with linoleic acid desaturation in *Brassica napus*. *Theor Appl Genet* **96**: 897-903.

Somers, D.J., Rakow, G., Prabhu, V.K., Friesen, K.R.D. (2001): Identification of a major gene and RAPD markers for yellow seed coat colour in *Brassica napus*. *Genome* **44**: 1077-1082.

Song, L.-Q., Fu, T.-D., Tu, J.-X., Ma, C.-Z., Yang, G.-S. (2006): Molecular validation of multiple allele inheritance for dominant genic male sterility gene in *Brassica napus* L. *Theor Appl Genet* **113**: 55-62.

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.* Proceedings of the National Academy of Sciences, USA 88: 8816-8820.

Stephenson, A. G., Doughty, J., Dixon, S., Elleman, C., Hiscock, S., Dickinson, H. G.(1997): The male determinant of self-incompatibility in *Brassica oleracea* is located in the pollen coating.

Stone, S., Arnoldo, M., Goring, D. (1999): A breakdown of *Brassica* self-incompatibility in ARC1 antisense transgenic plants. Science 286: 1729-1731.

Stringam, G.R., McGregor, D.I., Pawlowski, S.H. (1974): Chemical and morphological characteristics associated with seed coat colour in rapeseed. In: Proc 4th Int Rapeseed Conf. Giessen, pp 99-108.

Suzuki, G., Kai, N., Hirose, T., Fukui, K., Nishio, T., Takayama, S., Isogai, A., Watanabe, M., Hinata, K. (1999): Genomic organization of the S locus: identification and characterization of genes in SLG/SRK region of S^9 haplotype of *Brassica campestris* (syn. *rapa*). *Genetics* **153**: 391-400.

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.

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.

Tang, Z.L., Li, J.N., Zhang, X.K., Chen, L., Wang, R.(1994): Genetic variation of yellow-seeded rapeseed lines (*Brassica napus* L.) from different genetic sources. *Plant Breed* **116**: 471-474.

Takada, Y., Nakanowatari, T., Sato, J., Hatakeyama, K., Kakizaki, T., Ito, A., Suzuki, G., Shiba, H., Takayama, S., Isogai, A., Watanabe, M. (2005): Genetic analysis of novel intra-species unilateral incompatibility in *Brassica rapa* (syn. *Campestris*) L. Sex Plant Reprod 17: 211-217.

Takasaki, T., Hatakeyama, K., Watanabe, M., Toriyama, K., Isogai, A., Hinata, K. (1999): Introduction of SLG (S locus glycoprotein) alters the phenotype of endogenous S haplotype, but confers no new S haplotype specificity in Brassica rapa L. *Plant Mol. Biol.* **40**: 659-668.

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.

Takayama, S., Isogai, A., Tsukamoto, C., Ueda, Y., Hinata, K., Okazaki, K., Suzuki, A. (1987): Sequences of glycoproteins, products of *Brassica campestris* self-incompatibility locus. Nature 326: 102-105.

Takayama, S., Shiba, H., Iwano, M., Shimosato, H., Che, F.-S., Kai, N., Watanabe, M., Suzuki, G., Hinata, H., Isogai, A. (2000): The pollen determinant of self-incompatibility in *Brassica campestris*. Proc. Natl. Acad. Sci. U.S.A. 97: 1920-1925.

Takayama, S., Shimosato, H., Shiba, H., Funato, M., Che, F.-S., Watanabe, M., Iwano, M., Isogai, A. (2001): Direct ligand-receptor complex interaction controls *Brassica* self-incompatibility. Nature 413: 534-538.

Takayama, S., Sakagami, Y. (2002): Peptide signaling in plants. Cur. Opin. Plant Biol. 5: 382-387.

Takayama, S., Isogai, A.(2003): Molecular mechanism of self-recognition in *Brassica* self-incompatibility. J. Exp. Bot. 54: 149-156.

Tanhuanpää, P.K., Vilkki, J.P., Vilkki, H.J. (1995): Association of a RAPD marker with linolenic acid concentration in the seed oil of rapeseed (*Brassica napus* L.). *Genome* **38**: 414-416.

Teutonico, R.A., Osborn, T.C. (1994): Mapping of RFLP and quantitative trait loci in *Brassica rapa* and comparison to the linkage maps of *B. napus*, *B. oleracea*, and *Arabidopsis thaliana*. *Theor Appl Genet* **86**: 885-894.

Thompson, K.F. (1968): Classified S-Alleles for Brassica Breeders. In: G.E. Dixon (ed.), Brassica meeting of Eucarpia 1968, Wellesbourne, Warwick, 25-28.

Thompson, K.F., Taylor, J.P. (1966): Non-linear dominance relationships between *S*-alleles. Heredity 21: 345-362.

Tommasini, L., Batley, J., Arnold, G.M., Cooke,R.J., Donini, P., Lee, D., Law, J.R., Lowe, C., Moule, C., Trick, M., Edwards, K.J. (2003): The development of multiplex simple

sequence repeat (SSR) markers to complement distinctness, uniformity and stability testing of rape (*Brassica napus* L.) varieties. *Theor Appl Genet* **106**: 1091-1101.

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.

Uzunova, M., Ecke, W., Weißleder, K., Röbbelen, G. (1995): Mapping the genome of rapeseed (*Brassica napus* L.). I. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. *Theor Appl Genet* **90**: 194-204.

Wallace, D.H. (1979): Interactions of S-alleles in sporophytically controlled self-incompatibility of Brassica. Theor. Appl. Genet. 54: 193-201.

Walsh, J.A., Sharpe A.G., Jenner, C.E., Lydiate D.J. (1999): Characterisation of resistance to turnip mosaic virus in oilseed rape (*Brassica napus*) and genetic mapping of *TuRB01*. *Theor Appl Genet* **99**: 1149-1154.

Wang, T. T., Li, H. X., Lu, Y.E., Zhang, J. H., Ye, Z. B.: Diversity and distribution of S haplotypes in commercial cultivars Brassica vegetables from China. Unpublished (submitted to genebank 2006, http://www.ncbi.nlm.nih.gov).

Watanabe, M., Nou, I.S., Takayama, S., Yamakawa, S., Isogai, A., Takeuchi, T., Hinata, K. (1992): Variations in and inheritance of glycoprotein in self-incompatible *Brassica campestris* L. Plant Cell Physiol. 33: 343-351.

Watanabe, M., Nou, I.S., Takayama, S., Yamakawa, S., Isogai, A., Takeuchi, T., Hinata, K. (1992): Variations in and inheritance of glycoprotein in self-incompatible *Brassica campestris* L. Plant Cell Physiol. 33: 343-351.

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 in self-incompatible Brassica campestris L. *Plant Cell Physiol.* **35**: 1221-1229.

Watanabe, M., Suzuki, G., Hatakeyama, K., Isogai, A., Hinata, K. (1999): Molecular biology of self-incompatibility in *Brassica* species. Plant Biotech. 16: 263-272.

Watanabe, M., Hinata, K. (1999): Self-incompatibility. *In* „Biology of *Brassica* coenospecies". Gomez-Campo, C. (ed.), Elsevier, Amsterdam. p. 149-183.

Watanabe, M., Suzuki, G., Takayama,S., Isogai, A., Hinata, K. (2000): Genomic organization of *SLG/SRK* region of the *S*-locus in *Brassica* species. Annals. Bot. 85(Suppl.A): 155-162.

Watanabe, M., Hatakeyama, K., Takada, Z., Hinata, K. (2001): Molecular aspects of self-incompatibility in *Brassica* species. Plant Cell Physiol. 42: 560-565.

Watanabe, M., Takayama,S., Isogai, A., Hinata, K. (2003): Recent Progresses on self-incompatibility research in *Brassica* species. Breeding science 53: 199-208.

Xu, F.S., Wang, Y.H., Meng, J. (2001): Mapping boron efficiency gene(s) in *Brassica napus* using RFLP and AFLP markers. . *Plant Breed* **120**: 319-324.

Zhao, J., Meng, J. (2003): Genetic analysis of loci associated with parential resistance to *Sclerotinia sclerotiorum* in rapeseed (*Brassica napus* L.). *Theor Appl Genet* **106**: 759-764.

Zhi-wen, L., Ting-dong, F., Jin-xing, T., Bao-yuan, C. (2005): Inheritance of seed colour and identification of RAPD and AFLP markers linked to the seed colour gene in rapeseed (*Brassica napus* L.). *Theor Appl Genet* **110**: 303-310.

12. LIST OF ABBREVIATION

AFLP - Amplified Fragment Length Polymorphism

ACGM – Amplified consensus gene marker

CAPS – cleaved amplified polymorphic sequences

CHA – chemical hybridizing agens

CMS – cytoplasmic male sterility

DGMS – dominant genic male sterility

DUS - Distinctness, Uniformity and Stability

EST – expressed sequence tag

GMS – genic male sterility

GSI – gametophic self-incompatibility

MAS – marker-assisted selection

PCR – polymerase chain reaction

QTL – quantitative trait loci

RAPD - random amplified polymorphic DNA

RFLP - restriction fragment length polymorphism

RGA – resistance gene analog

RGMS – recessive genic male sterility

SI - self-incompatibility, self-incompatible

SSI – sporophitic self-incompatibility

SC - self-compatibility, self-compatible

SCAR - sequence characterized amplified region

SCR - *S*-locus cysteine-rich protein

SLG - *S*-locus glycoprotein

SP11 - *S*-locus protein 11

SRK - *S*-locus receptor kinase

SRK-KD – kinase domain

SRK – TD – transmembrane domain

SSR - simple sequence repeat

STS - sequence-tagged site

13. SHRNUTÍ

Struktura a funkce S-lokusu u řepky (*Brassica napus* L.)

Autoinkompatibilita (AI) je jedním z možných mechanismů, který lze použít pro hybridní šlechtění řepky, kde je důležité zabránit samosprášení. Geny které jsou důležité v autoinkompatibilní reakci leží na *S*-lokusu, který byl podrobněji zkoumán u příbuzných druhů jako je *Brassica oleracea* nebo *Brassica rapa*. Práce je rozčleněna na šest studií zabývajících se autoinkompatibilitou a molekulárními markery a čtyři studie z nich jsou původní.

Hlavními cíly bylo najít specifický molekulární marker pro recesivní typ autoinkompatibility, ktera se vyskytuje AI (autoinkompatibilních) linií řepky. Jako vhodný kandidátní gen byl zvolen gen *SCR*, který funguje jako pylový determinant u AI příbuzných druhů *Brassica rapa a Brassica oleracea*. Tento gen se v práci podařilo indentifikovat i u *Brassica napus* a byla nalezena i jeho alela, která se pomocí metody PCR specificky amplifikovala pouze u části štěpící populace dihaploidních rostlin řepky. Tento markerový gen segregoval přesně podle teoretického štěpného poměru 1:1 autoinkompatibilních rostlin ku autokompatibilním rostlinám. Nevýhodou tohoto markeru je, že ho lze použít pouze u linií odvozených z linie Tandem 6/85, protože tento marker je alelově specifický.

Protože již byl znám markerový gen *SLG* pro třídu I *S*-haplotypů, které jsou považovány za dominantní, byl tento markerový gen použit s ke srovnání s nalezeným markerovým genem *SCR*. U řepky, kde se přirozeně vyskytuje pouze recesivní typ autoinkompatibility to znamená, že tento gen se vyskytuje pouze v autokompatibilních rostlinách. Přestože bylo prokázáno, že gen *SLG* není považován v autoinkompatibilní reakci za rozhodující, oba markerové geny určovaly stejný genotyp.

Markery stanovený genotyp byl tedy porovnán s fenotypem a bylo kupodivu zjištěno, že fenotyp neodpovídá teoretickému štěpnému poměru dihaploidních rostlin odvozených z F_1 generace po křížení AI linie s AK donorem kvality, tedy 1:1, nýbž je 1:3, což by odpovídalo dvoum nezávislým lokusům řídícím autoinkompatibilitu. Je tedy možné usuzovat, že existuje nějaký další supresorový lokus, který ovlivňuje AI reakci. Pomocí obou molekulárních markerů tedy nelze stoprocentně selektovat AI rostliny, ale lze významně snížit procento AK rostlin, protože pomocí markerových genů ležících na S-lokusu je možné zjistit 90% takovýchto rostlin ve štěpící dihaploidní populaci.

S-lokus je je velmi polymorfní, což bylo využito ve sledování variability mezi AI liniemi a některými odrůdami na základě porovnání sekvencí genu *SLG* třídy II. Sledováno bylo různé zastoupení alel u AI linií a odrůd *Brassica napus* a sekvence byly také porovnány s různými sekvencemi genu *SLG* tříd I i II, které pocházely z *B. napus*, *B. oleracea*, *B. rapa* a *Raphanus sativus* z databáze National Center for Biotechnology Information dostupné online. U analyzovaných AI linií a odrůd řepky bylo nalezeno 3 až 7 odlišných alel, což je méně než by se dalo předpokládat u tak polymorfního lokusu. Z toho se dá usuzovat na sníženou základnu pro výběr šlechtitelského materiálu, která byla způsobena dlouhodobou selekcí. Po porovnání s ostatními sekvencemi z databáze bylo zjištěno, že velmi rozdílné jsou sekvence *SLG* genu mezi jednotlivými třídami než mezi rozdílnými druhy.