Characterization of *S* Haplotype in a New Self-Compatible *Brassica rapa* cultivar Dahuangyoucai

Xingguo ZHANG[1,2], *Chaozhi MA*[2], *Dongmei YIN*[1], *Wei ZHU*[1], *Changbin GAO*[2], *Jianfeng ZHANG*[2] *and Tingdong FU*[2]

[1]*College of Agronomy, Henan Agricultural University, Zhengzhou, P.R. China;* [2]*National Key Laboratory of Crop Genetic Improvement, National Centre of Rapeseed Improvement in Wuhan, Huazhong Agricultural University, Wuhan, P.R. China*

Abstract

Zhang X., Ma C., Yin D., Zhu W., Gao C., Zhang J., Fu T. (2013): **Characterization of *S* haplotype in a new self-compatible** *Brassica rapa* **cultivar Dahuangyoucai**. Czech J. Genet. Plant Breed., **49**: 157–163.

The most important *Brassica* species, *B. rapa*, is naturally self-incompatible. Self-compatible mutants would be useful for dissecting the molecular mechanism of self-incompatibility (SI), a process that promotes outcrossing by recognizing and refusing self-pollens. The *S* haplotype in a new self-compatible *B. rapa* cultivar, Dahuangyoucai, was characterized for the first time in this study. Sequence analysis of the *S*-locus genes, *SLG* (*S*-locus glycoprotein), *SRK* (*S*-locus receptor kinase) and *SCR* (*S*-locus cysteine-rich protein) revealed that Dahuangyoucai contained *S* haplotype highly similar to *S-f2*, a non-functional class I *S* haplotype identified in another self-compatible *B. rapa* cultivar, Yellow Sarson. Mutations of *MLPK* (*M*-locus protein kinase) and non-transcription of the male determinant, *SCR*, were observed in this cultivar, which is similar to the situation reported in Yellow Sarson. With respect to the female determinant, *SRK*, no transcript was detected in Yellow Sarson but two fragments were detected in Dahuangyoucai. One fragment was highly similar to *SRK-f2*, but the other fragment was different from the signal factors previously identified in the SI reaction. The results suggest that Dahuangyoucai and Yellow Sarson have the same origin and a similar mechanism of self-compatibility, but diverge after mutations in *SRK*, *SCR* and *MLPK*. Further studying the self-compatibility of Dahuangyoucai might identify novel factors involved in the SI signalling cascade and provide new insights into the mechanisms of SI in Brassicaceae.

Keywords: *Brassica rapa* L.; gene expression; *M*-locus protein kinase (*MLPK*); *S*-locus genes; self-incompatibility

Self-incompatibility (SI), which promotes outbreeding and maintains genetic diversity by recognizing self from non-self pollen, is a well-known and common reproductive system found in flowering plants. SI in the genus *Brassica* is sporophytically controlled by a single polymorphic locus, the *S*-locus (Bateman 1955). There are three genes at the *S*-locus: *SLG* (*S*-locus glycoprotein) (Nasrallah *et al.* 1988), *SRK* (*S*-locus receptor kinase) (Stein *et al.* 1991) and *SCR*/*SP11* (*S*-locus cysteine-rich protein/*S*-locus protein 11) (Schopfer *et al.* 1999; Suzuki *et al.* 1999). *SRK* and *SCR* are proved to encode the female and the male determinant of the SI recognition specificity, respectively. *SLG* is the first gene isolated to be associated with the SI phenotype, and shares a high sequence similarity to the extracellular domain (*S* domain) of *SRK*, but its function in SI reaction is still unknown (Takasaki *et al.* 2000; Silva *et al.* 2001). These three genes are segregated as a unit, the *S* haplotype (Nasrallah & Nasrallah 1993). According to the nucleotide sequence of *SLG* alleles, *S* haplotypes are classi-

fied into two classes, class I and class II, and class I *S* haplotypes are generally dominant over class II *S* haplotypes in the pollen and confer a strong SI phenotype (Nasrallah *et al*. 1991).

Upon self-pollination, the pollen-borne ligand, SCR, binds specifically to the stigma surface with the extracellular domain of its cognate, SRK, in an *S*-haplotype specific manner, which activates the kinase domain of SRK and triggers the signalling cascade that results in the SI reaction. Some downstream signal factors, including three positive mediators, MLPK (M-locus protein kinase) (Murase *et al*. 2004), ARC1 (Arm-repeat containing 1) (Stone *et al*. 2003) and rdr6 (RNA-dependent RNA polymerase) (Tantikanjana *et al*. 2009), and two negative regulators, Exo70A1 (exocyst complex subunit) (Samuel *et al*. 2009) and THL (Thioredoxin-h-like) (Cabrillac *et al*. 2001), have been characterized comprehensively. The knocked-down expression or increased expression of these factors leads to concomitant changes in the self-pollen rejection responses. However, the complete regulatory network of the signal transduction pathway downstream of SRK remains poorly understood.

Self-compatible mutants of *Brassica rapa* or *B. oleracea* would be useful for dissecting the mechanism of SI in these species. Inability of the *S*-locus genes, *SRK* and/or *SCR* (Fujimoto *et al*. 2006; Okamoto *et al*. 2007), and the disruption of downstream factors in the signalling cascade, like *MLPK* (Murase *et al.* 2004) and *ARC1* (Stone *et al*. 2003), might give rise to self-compatible *B. rapa* and/or *B. oleracea* lines. Studies on the self-compatible *B. rapa* var. Yellow Sarson have revealed that it has a non-functional class I *S* haplotype, *S-f2*, that results from a 4892 bp retrotransposon insertion into the first intron of *SRK* and a 89-bp deletion in the promoter of *SCR* (Fujimoto *et al*. 2006). A recessive epistatic modifying gene, *MLPK*, controls the self-compatible phenotype in Yellow Sarson (Hinata *et al*. 1983) and the inactivity of MLPK resulting from a point mutation at amino acid 194 is also responsible for self-compatibility (Murase *et al*. 2004). Several other self-compatible *B. rapa* lines have been reported (Isokawa *et al*. 2010), but the full molecular and genetic mechanisms in these lines remain to be determined (Takayama & Isogai 2005). Screening a new self-compatible *B. rapa* line is possible in China, as it is one of the major origins of *B. rapa*, and this is important before a comprehensive SI model can be proposed in the Brassicaceae.

Tang *et al*. (2006) used four different pollination methods to investigate the silique setting rate and seeds per silique of one *B. rapa* cultivar, Dahuangyoucai, and primary results revealed that it was normally self-compatible. In this study, the *S* haplotype of Dahuangyoucai as a new genetic resource was characterized for the first time. The *S*-locus genes and *MLPK* were analysed to identify the putative molecular mechanism underlying its self-compatible phenotype. The potential of self-compatible mutants for dissecting the SI mechanism in the SI signalling cascade in the Brassicaceae are discussed.

MATERIAL AND METHODS

Plant material. The self-compatible *B. rapa* cultivar Dahuangyoucai was obtained from Qinghai Academy of Agriculture and Forestry Sciences (Xining, China). Plants were grown in the field under normal field conditions at Huazhong Agricultural University (Wuhan, China). A self-incompatible *B. rapa* tester line with *S* haplotype *S47* was used as a control for the SI phenotype.

Phenotype investigation. Pollen-tube behaviour after self-pollination was observed using a fluorescent microscope (Nikon Eclipse 80i, Nikon Corp., Tokyo, Japan). Flower buds were emasculated just one day before anthesis and pollinated with pollen from the same plant. 24 h after pollination pollinated buds were fixed in formaldehyde-acetic acid-alcohol fixation (FAA composition: 50% ethanol, 10% formalin, 5% acetic acid) for 24 h under room conditions. Then, stigmas of these flower buds were hydrolyzed in 1 N NaOH for 12 h, and pollen tubes were stained with aniline blue solution (0.1% aniline blue in 2% K_3PO_4), as described by Isokawa *et al*. (2010). Three flower buds were used each time and the experiment was replicated on three different days. During flowering, the major inflorescence was bagged for self-pollination and the SCI (self-compatibility index = the number of seeds/the number of flowers) was calculated according to Zhang *et al.* (2008).

Polymerase chain reaction (PCR) amplification analysis of *S* haplotypes. Genomic DNA from each plant was extracted from young leaves according to the cetyl trimethyl ammonium bromide (CTAB) method (Doyle & Doyle 1990). Primer pairs specific of class I or class II *S* haplotypes (Nishio *et al*. 1996, 1997) were used for primary identification of *S* haplotype class. New primer pairs were designed for further characterization

and gene expression analysis using the Oligo 3 primer software (http://redb.croplab.org/modules/redbtools/primer3.php). PCR were performed in final volumes of 20 µl. Thermal conditions were as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles at 94°C for 30 s, 55°C for 45 s, and 72°C for 1 min. PCR products were electrophoretically fractionated in 1% agarose TAE gels and stained with ethidium bromide. Expected fragments (the size of fragments is shown in Table 1) were cloned using the pMD18-T vector (TaKaRa, Shanghai Genemy BioTech Co., Ltd., China) and sequenced. For each fragment, at least three clones were sequenced.

Reverse transcriptase (RT)-PCR of gene expression. Total RNA was extracted from buds one day before anthesis using Tripure reagent (Bioteke, http://www.bioteke.com/chn/). RNA was reverse-transcribed into first strand cDNA using M-MLV reverse transcriptase and an oligo (dT) primer (Fermentas, Thermo Fisher Scientific Co., Beijing, China) according to the manufacturer's instructions. After the RT-PCR reaction, cDNA was subjected to PCR with specific primers (Table 1), while the *actin* gene was amplified as a positive standard control (Actin-F: CGGTCCAGATTCGTCATACTCAGCC; Actin-R: AAATGTGATGTGGATATCAGGAAGG). PCR was performed using 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 55°C, and extension for 30 s at 72°C. Amplified fragments were cloned and sequenced.

RESULTS

Confirmation of self-compatible phenotype

Unlike the self-incompatible tester line (Figure 1a), Dahuangyoucai showed numerous pollen tubes on stigma papilla cells upon self-pollination (Figure 1b). The mean SCI of Dahuangyoucai was 6.27, which

Table 1. Primer name, primer sequence, amplified DNA and cDNA fragments, homologous gene and sequence identity referred to in this study

Primer pair[a]	Sequence (5'–3')[b]	DNA fragment (bp)	cDNA fragment (bp)	Homologous gene	Identity (%)
PS3 + PS21	ATGAAAGGGGTACAGAACAT CTCAAGTCCCACTGCTGCGG	–			
PK1 + PK4	CTGCGATCATGTTCTGCCTCTGG CAATCCCAAAATCCGAGATCT	–			
PS5 + PS15	ATGAAAGGCGTAAGAAAAACCTA CCGTGTTTTATTTTAAGAGAAAGAGCT	1336		*BraSLG-f2a*	99
SRK-1 + SRK-2	TGTTTCTTGCTCGTCTTCGTT TTCCCGTCACAGTAAGCGTAA	908		*BraSRK-f2*	100
SRK-N1 + SRK-N2	TAACCAAACCATATCCAAGTGT GGCTCTGTCGCTTACAAACTCT	772		*BraSRK-f2*	100
SRK-N3 + SRK-N4	TGTTTGAGATGGGAGTGGGTGG GAAACCAATCAAACATTGAATT	727		*BraSRK-f2*	100
SCR-P3 + SCR-P4	GAATTTTTGATTATCTAACT TCTTTGCACCAATCGTAGCT	451		*BraSCR-f2*	100
MLPK-1 + MLPK-2	GACAAACAGAGGCGAAGCAGA GCTATCTGACTTCGGTTTGGC	623		*MLPK-f2*	100
SCR-1 + SCR-2	AATCTGCAATTTATGCTTTAT TAGCATGGCCAAGGACCGGTT	554	–	*BraSCR-f2*	100
SRK-R3 + SRK-R6	AAAGGCTATCAAAGACATCACT GGGCATCAATGACTGAGCAGGTGTA	1178	903 491	*BraSRK-f2* *AthS3*	100 97
MLPK-2S + MLPK-2A	AGCTCAGAGGCAAATGATACAC AGCAGACGGACGTGGATACG	N	1125 1170	*MLPK-f2* *ARK1B*	99 88

[a]forward primer + reverse primer; [b]upper sequence is the forward primer; – no amplification; N – no sequencing

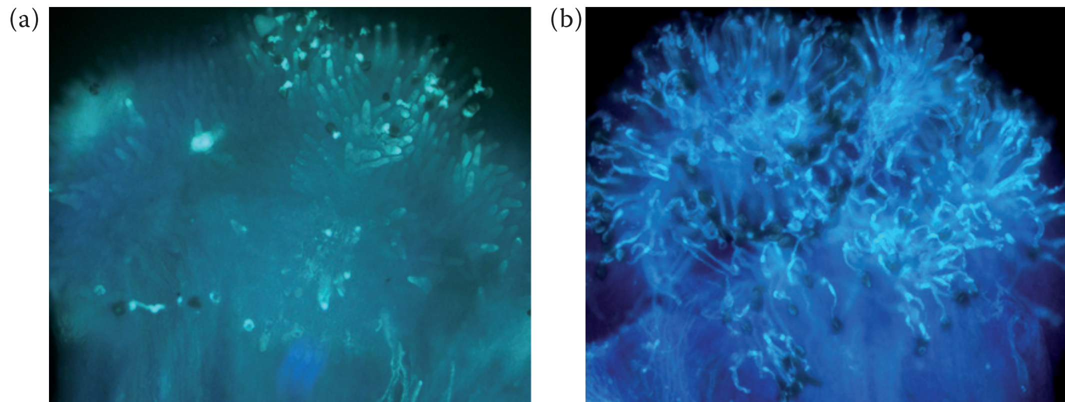

Figure 1. Representative visualizations of self-incompatible and self-compatible phenotypes in self-pollination tests; pollinations were performed one day before anthesis; (a): self-incompatible *S47S47*, no obvious pollen tube observed after self-pollination; (b): self-compatible Dahuangyoucai, numerous pollen tubes generated after self-pollination

was close to its average seed per open-pollinated flower (7.95). The mean SCI of the SI control tester line was 0.82. Pollen tube observations and SCI data confirmed that Dahuangyoucai was self-compatible.

Characterization of *S* haplotype in Dahuangyoucai

Class I *S*-haplotype specific primer pair, PS5 and PS15, and class II *S* haplotype specific primer pair, PS3 and PS21, were used to screen the *S* haplotype of the Dahuangyoucai cultivar (Table 1). An expected fragment of 1336 bp was isolated from the Dahuangyoucai cv. using PS5 and PS15, and it showed 99% sequence identity to *BraSLG-f2a*. No amplification was observed when PS3 and PS21 were used. So the *S* haplotype of the Dahuangyoucai cv. was deduced to be of class I type and to have a high similarity with *S-f2*; however, the class I *SRK* kinase-domain specific primer pair PK1 and PK4 generated no amplification in the Dahuangyoucai cv.

Three primer pairs were designed based on the *S* domain of *BraSRK-f2* (SRK-1 and SRK-2), and on the kinase domain of *BraSRK-f2* (SRK-R3 and SRK-R6) and *BraSCR-f2* (SCR-1 and SCR-2) (Figure 2). SRK-1 and SRK-2 amplified a fragment of 908 bp, which was 100% and 99% identical to *BraSRK-f2* and *BraSRK54*, respectively. SRK-R3 and SRK-R6 produced a fragment of 1178 bp, which showed 99% identity to *BraSRK-f2* and differed just in three point mutations (two transitions and one transversion) (Figure 3). A fragment of 554 bp amplified by SCR-1 and SCR-2 was identical to *BraSCR-f2*, and highly similar to *BraSCR54* (99% sequence identity).

Three primer pairs were designed to correspond with the partial insertion sequence of *BraSRK-f2* (SRK-N1 and SRK-N2; SRK-N3 and SRK-N4) and the whole deletion sequence of *BraSCR-f2* (SCR-P3 and SCR-P4). SRK-N1 and SRK-N2 amplified a fragment of 772 bp, while SRK-N3 and SRK-N4

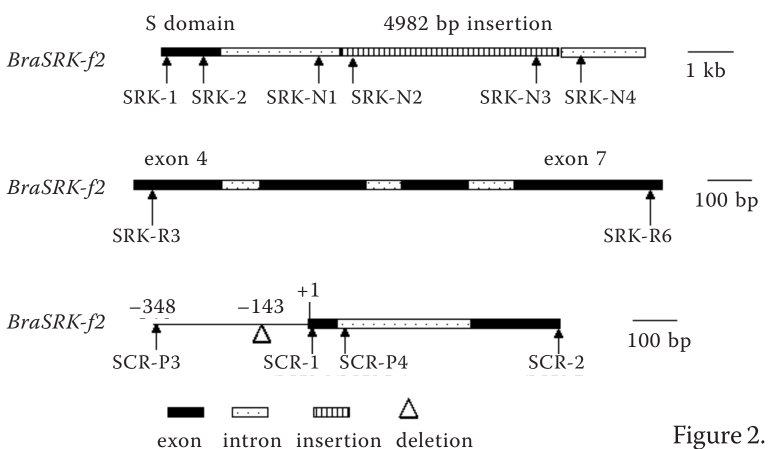

Figure 2. Position of the primers designed in this study

```
SRK-Dahuangyoucai  721 bp   CAATCTTCCAAGCTATGTAAGTTTAAGAACCAATAATATTCTATCTACTCTCGAGATTGC
BraSRK-f2        14605 bp   CGATCTTCCAAGCTATGTAAGTTTAAGAACCAATAATATTCTATCTACTCTCGAGATTGC
                            * **********************************************************

SRK-Dahuangyoucai 1081 bp   AGCAAGTTATTATGCAAATAATCCTTCCTCAAGTAAGCAATTCGATGACGATGAATCCTG
BraSRK-f2        14245 bp   AGCAAGTTATTCTGCAAGTAATCCTTCCTCAAGTAAGCAATTCGATGACGATGAATCCTG
                            *********** ***** ******************************************
```

Figure 3. Three point mutations in the coding sequence of *SRK* in the Dahuangyoucai cv. compared with *BraSRK-f2*; boxes highlight the point mutations

amplified a fragment of the expected length, 727 bp. Each of these fragments was 100% identical to corresponding sections of the insertion sequence in *BraSRK-f2*. SCR-P3 and SCR-P4 gave rise to a fragment of 451 bp as expected, and this showed 100% identity to the promoter of *BraSCR-f2*. These results suggested that the *S* haplotype of Dahuangyoucai was highly similar, if not identical, to *S-f2*.

Expression of SRK and SCR in Dahuangyoucai

RT-PCR of flower buds of the Dahuangyoucai cv., SRK-R3 + SRK-R6 primer pair gave two expression signals, including one expected fragment (903 bp) and one small fragment (491 bp), while the SCR-1 and SCR-2 primer pair failed to amplify a transcript (Figure 4). Comparison of 1178 bp genomic DNA and 903 cDNA fragments amplified by SRK-R3+SRK-R6 primer pair indicated that three introns were separated as 88, 82 and 105 bp, which corresponded exactly with introns 4, 5 and 6 of *BraSRK-f2*, respectively. The sequence of 491 bp fragment was similar to that of 903 bp fragment only in the primer sequences. EST databank searches revealed that this fragment was highly similar (97%) to a cDNA sequence involved in pollen development in *B. napus* and the 40S ribosomal protein S3 in *Arabidopsis thaliana*.

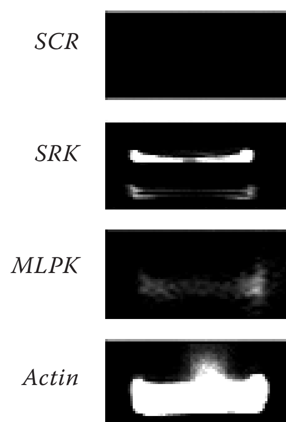

Figure 4. RT-PCR analysis of *SCR*, *SRK* and *MLPK* in the Dahuangyoucai; cv. Actin was used as positive control

Mutation and expression of MLPK in Dahuangyoucai

One primer pair (MLPK-1 and MLPK-2) was designed according to the cDNA sequences of *MLPK-f2*, and PCR resulted in a fragment of 623 bp in length from the Dahuangyoucai cv. When compared with the cDNA sequence of *MLPK-f2*, this fragment was separated into two regions (1-112 bp and 192-623 bp) that shared 100% identity to *MLPK-f2*; and the remaining middle 79 bp was deduced to be the intron sequence.

RT-PCR analysis using the primer pair MLPK-2S and MLPK-2A showed that *MLPK* was transcribed normally and with just one signal. However, two fragments of 1125 bp and 1170 bp were specifically sequenced. The longer fragment contained two insertions, 12 bp located at the 1030 bp position and 33 bp at the 1080 bp position, and the sequence shared 88% identity with the protein kinase *APK1B* from *A. thaliana*. The other fragment of 1125 bp was 99% identical to *MLPK-f2*, and a point mutation of C to G at residue 194 of *MLPK-f2* was also found. This result was similar to the situation of *S-f2*, where two MLPK isoforms were shown to interact with SRK (Kakita *et al.* 2007).

DISCUSSION

B. rapa cultivar Dahuangyoucai was selected from a landrace in 1958 by Qinghai Academy of Agriculture and Forestry Sciences (Xining, China) (Institute of China Oil Crops Research 1988). It has many unique traits such as erect plant type, high oil content, large and round seeds and yellow-coloured seeds, and several of these traits

have been transferred into *B. napus* (personal communication with professor D. Du). The self-compatibility of Dahuangyoucai was recognized for some time (Tang *et al*. 2006), but its *S* haplotype was not reported previously in the literature. This study is the first report on characterization of the *S* haplotype and dissecting the molecular mechanism of self-compatibility in the Dahuangyoucai cv. Stigma exsertion, a morphological feature of flowers that is important during the transition of mating systems (Goodwillie *et al*. 2010) and that may promote cross-pollination in plants (Barrett 2002), was observed in Dahuangyoucai.

The expression of *S*-locus genes is essential for the SI phenotype. Results from PCR using class I and class II *S*-haplotype specific primer pairs based on *SLG*, the first *S*-locus gene identified (Nasrallah *et al.* 1988), indicated that *SLG* in Dahuangyoucai was highly similar to *BraSLG-f2a*. Fragments amplified by primer pairs designed according to sequences from *BraSRK-f2* and *BraSCR-f2* were also completely or highly identical to the corresponding regions of *S-f2*. RT-PCR of flower buds from Dahuangyoucai detected no transcript of *SCR* but two fragments of *SRK*, which might be due to the fact that the primers were not fully specific. In view of the observation of stigma exsertion, this fragment was compared to *rdr6*, which is a mutant gene that simultaneously enhances SI and causes stigma exsertion but does not increase the transcript levels of *SRK* in the SI reaction (Tantikanjana *et al*. 2009); however, no sequence similarity was observed. The differential expression of *S*-locus genes indicated that the *S* haplotype in Dahuangyoucai might share the same ancestral *S* haplotype as *S-f2*, but it may have diverged during its evolution and domestication, in light that Yellow Sarson line is an old cultivar (Institute of China Oil Crops Research 1988). The allelic relationship of *S*-locus genes is being investigated by classical genetic methods in our research group now, which will be helpful to uncover more differences between Dahuangyoucai and Yellow Sarson.

The investigation of new self-compatible lines of different origins will not only aid the understanding of SI evolution in the Brassicaceae, but also will be useful tools to enhance our understanding of receptor kinase signalling in plants. China is one of the major origins of *B. rapa*, and thus contains a large number of cultivars showing diverse genetic variation. The nucleotide sequences and RT-PCR analysis of *S*-locus genes and *MLPK* suggest that Dahuangyoucai and Yellow Sarson share the same origin and similar self-compatibility mechanisms, but these cultivars have diverged after mutations in *SRK*, *SCR* and *MLPK*. The SI signalling cascade in the Brassicaceae was proposed to be a complex network, which might involve the co-ordinate evolution of SI and pistil development (Tantikanjana *et al*. 2009), endocytosis and endosomal regulation (Ivanov *et al*. 2009), and defence against pathogens (Rea *et al*. 2010). Improved understanding of Dahuangyoucai will further elucidate the molecular mechanism and evolution of SI in the Brassicaceae.

Acknowledgements. We thank Dr. D. Du (Qinghai Academy of Agriculture and Forestry Sciences, Xining, China) for his advice and discussions on the manuscript. We also thank him for kindly providing seeds used in this study. The authors are grateful to several anonymous reviewers for their helpful suggestions on revising the manuscript. This work was supported by grants from the National High Technology Research and Development Program (2011AA10A104) and the National Natural Science Funds (30971802).

References

Barrett S.C.H. (2002): The evolution of plant sexual diversity. Nature Reviews Genetics, **3**: 274–284.

Bateman A.J. (1955): Self-incompatibility systems in angiosperms. III. Cruciferae. Heredity, **9**: 53–58.

Cabrillac D., Cock J.M., Dumas C., Gaude T. (2001): The *S*-locus receptor kinase is inhibited by thioredoxins and activated by pollen coat proteins. Nature, **410**: 220–223.

Doyle J.J., Doyle J.L. (1990): Isolation of plant DNA from fresh tissue. Focus, **12**: 13–15.

Fujimoto R., Sugimura T., Nishio T. (2006): Gene conversion from *SLG* to *SRK* resulting in self-compatibility in *Brassica rapa*. FEBS Letters, **580**: 425–430.

Goodwillie C., Sargent R.D., Eckert C.G., Elle E., Geber M.A., Johnston M.O., Kalisz S., Moeller D.A., Ree R.H., VallejoMarin M., Winn A.A. (2010): Correlated evolution of mating system and loral display traits in flowering plants and its implications for the distribution of mating system variation. New Phytologist, **185**: 311–321.

Hinata K., Okazaki K., Nishio K. (1983): Gene analysis of self-compatibility in *Brassica campestris* var. yellow sarson (a case of recessive epistatic modifier). In: Proc. 6th Int. Rapeseed Congr., Paris, 354–359.

Institute of China Oil Crops Research (1988): List of China Oilseed Varieties. 1st Ed., China Agricultural Press, Beijing. (in Chinese).

Isokawa S., Osaka M., Shirasawa A., Kikuta R., Komatsu S., Horisaki A., Niikura S., Takada Y., Shiba H., Isogai A., Takayama S., Suzuki G., Suwabe K., Watanabe M. (2010): Novel self-compatible of *Brassica rapa* L. isolated from the Japanese bulk-populations. Genes & Genetic Systems, **85**: 87–96.

Ivanov R., Gaude T. (2009): Endocytosis and endosomal regulation of the *S*-receptor kinase during the self-incompatibility response in *Brassica oleracea*. Plant Cell, **21**: 2107–2117.

Kakita M., Murase K., Iwano M., Matsumoto T., Watanabe M., Shiba H., Isogai A., Takayama S. (2007): Two distinct forms of *M*-locus protein kinase localize to the plasma membrane and interact directly with *S*-locus receptor kinase to transduce self-incompatibility signalling in *Brassica rapa*. Plant Cell, **19**: 3961–3973.

Murase K., Shiba H., Iwano M., Che F.S., Watanabe M., Isogai A., Takayama S. (2004): A membrane-anchored protein kinase involved in *Brassica* self-incompatibility signaling. Science, **303**: 1516–1519.

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

Nasrallah J.B., Yu S.M., Nasrallah M.E. (1988): Self-incompatibility genes of *Brassica oleracea*: expression, isolation, and structure. Proceedings of the National Academy of Sciences of the USA, **85**: 5551–5555.

Nasrallah J.B., Nishio T., Nasrallah M.E. (1991): The self-incompatibility genes of *Brassica*: expression and use in genetic ablation of floral tissues. Annual Review of Plant Physiology and Plant Molecular Biology, **42**: 393–422.

Nishio T., Kusaba M., Watanabe M., Hinata K. (1996): Registration of *S* alleles in *Brassica campestris* L. by the restriction fragment sizes of *SLG*s. Theoretical and Applied Genetics, **92**: 388–394.

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 oleracea* L. Theoretical and Applied Genetics, **95**: 335–342.

Okamoto S., Odashima M., Fujimoto R., Sato Y., Kitashiba H., Nishio T. (2007): Self-compatibility in *Brassica napus* is caused by independent mutations in *S*-locus genes. Plant Journal, **50**: 391–400.

Rea A.C., Liu P., Nasrallah J.B. (2010) A transgenic self-incompatible *Arabidopsis thaliana* model for evolutionary and mechanistic studies of crucifer self-incompatibility. Journal of Experimental Botany, **61**: 1897–1906.

Samuel M.A., Chong Y.T., Haasen K.E., Aldea-Brydges M.G., Stone S.L., Goring D.R. (2009): Cellular pathways regulating responses to compatible and self-incompatible pollen in *Brassica* and *Arabidopsis* stigmas intersect at Exo70A1, a putative component of the exocyst complex. Plant Cell, **21**: 2655–2671.

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

Silva N.F., Stone S.L., Christie L.N., Sulaman W., Nazarian K.A.P. (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. Molecular Genetics and Genomics, **265**: 552–559.

Stein J.C., Howlett B., Boyes D.C., Nasrallah M.E., Nasrallah J.B. (1991): Molecular cloning of a putative rector protein kinase encoded at the self-incompatibility locus of *Brassia oleracea*. Proceedings of the National Academy of Sciences of the USA, **88**: 8816–8820.

Stone S.L., Anderson E.M., Mullen R.T., Goring D.R. (2003): ARC1 is an E3 ubiquitin ligase and promotes the ubiquitination of proteins during the rejection of self-incompatible *Brassica* pollen. Plant Cell, **15**: 885–898.

Suzuki G., Kai N., Hirose T., Fukai 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.

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. (2005): Self-incompatibility in plants. Annual Review of Plant Biology, **56**: 467–489.

Tang G.Y., Xu Q.L., Wang R.S., Li j., Du D.Z. (2006): Primary study on self-compatibility in Dahuang Rape of *Brassica chinensis*. Science and Technology of Qinghai Agriculture, **1**: 8–9 (in Chinese)

Tantikanjana T., Rizvi N., Nasrallah M.E., Nasrallah J.B. (2009): A dual role for the *S*-locus receptor kinase in self-incompatibility and pistil development revealed by an *Arabidopsis* rdr6 mutation. Plant Cell, **21**: 2642–2654.

Zhang X.G., Ma C.Z., Fu T.D., Li Y.Y., Wang T.H., Chen Q.F., Tu J.X., Shen J.X. (2008): Development of SCAR markers linked to self-incompatibility in *Brassica napus* L. Molecular Breeding, **21**: 305–315.

Received for publication August 8, 2012
Accepted after corrections September 5, 2013

Corresponding author:

Dr. Chaozhi Ma, Huazhong Agricultural University, National Center of Rapeseed Improvement in Wuhan, National Key Laboratory of Crop Genetic Improvement, Wuhan 430070, China
e-mail: yuanbeauty@mail.hzau.edu.cn