Genes Outside the *S* Supergene Suppress S Functions in Buckwheat (*Fagopyrum esculentum*)

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● *Background and Aims* Common buckwheat (*Fagopyrum esculentum*) is a dimorphic self-incompatible plant with either pin or thrum flowers. The *S* supergene is thought to govern self-incompatibility, flower morphology and pollen size in buckwheat. Two major types of self-fertile lines have been reported. One is a type with long-homostyle flowers, Kyukei SC2 (KSC2), and the other is a type with short-homostyle flowers, Pennline 10. To clarify whether the locus controlling flower morphology and self-fertility of Pennline 10 is the same as that of KSC2, pollen tube tests and genetic analysis have been performed.
● *Methods* Pollen tube growth was assessed in the styles and flower morphology of KSC2, Pennline 10, F_1 and F_2 plants that were produced by the crosses between plants with pin or thrum and Pennline 10.
● *Key Results* Pollen tubes of Pennline 10 reached ovules of all flower types. The flower morphology of F_1 plants produced by the cross between thrum and Pennline 10 were thrum or pin, and when pin plants were used as maternal plants, all the F_1 plants were pin. Both plants with pin or short-pin flowers, whose ratio of style length to anther height was smaller than that of pin, appeared in F_2 populations of thrum × Pennline 10 as well as in those of pin × Pennline 10.
● *Conclusion* The results suggest that Pennline 10 possesses the *s* allele as pin does, not an allele produced by the recombination in the *S* supergene, and that the short style length of Pennline 10 is controlled by multiple genes outside the *S* supergene.   © 2004 Annals of Botany Company

Key words: Self-incompatibility, heteromorphic flowers, modifier genes, pollen tube growth test, genetic analysis, *Fagopyrum esculentum*.

INTRODUCTION

Self-incompatibility (SI) is a genetic mechanism to prevent self-fertilization after pollination. Most species with heteromorphic flowers have di-allelic SI. Distylous incompatibility encompasses two types of floral architecture: thrum, having short styles and high anthers; and pin, having long styles and low anthers. This characteristic is controlled by a single gene complex that segregates as a simple Mendelian factor, with one dominant allele (*S*) found only in thrum plants and one recessive allele (*s*) present in the heterozygous state in thrum plants and in the homozygous state in pin plants (Garber and Quisenberry, 1927). Self-incompatibility is primarily a reaction between haploid pollen tubes and a diploid style, but thrum pollen, despite segregation of *S* and *s*, behaves as if it were all *S* type. This effect is because of the sporophytic determination of the pollen reaction and *S* being dominant over *s*.

Common buckwheat (*Fagopyrum esculentum*) has typical distylous sporophytic self-incompatibility. Sharma and Boyes (1961) considered the *S* locus of common buckwheat to be similar to the *S* supergene proposed to occur in *Primula* (Dowrick, 1956). They postulated that the *S* supergene of buckwheat consists of five genes: *G*, style length; I^S, stylar incompatibility; I^P, pollen incompatibility; *P*, pollen size; and *A*, anther height. Pin has small pollen grains, and

thrum has larger pollen grains. Pin-linked characters are recessive, and thrum-linked characters are dominant, and therefore the genotype of pin is gi^Si^Ppa/gi^Si^Ppa and that of thrum is GI^SI^PPA/gi^Si^Ppa, although the nature and correct order of these five genes are unknown.

Self-fertile common buckwheat lines have been obtained by spontaneous or artificial mutation (Schoch-Bodmer, 1934; Tatebe, 1953; Sharma and Boyes, 1961; Marshall, 1969). Marshall (1970) developed a self-fertile buckwheat line derived from a mutant of common buckwheat, and named it Pennline 10. In 1991, self-compatible wild buckwheat, *Fagopyrum homotropicum*, which is very similar to *F. esculentum* ssp. *ancestralis* except for long-homostylous flowers and self-compatibility, was discovered in Yunnan province, China (Ohnishi, 1998). Self-compatible common buckwheat lines have been produced by interspecific crosses between *F. esculentum* and *F. homotropicum* with embryo rescue (Campbell, 1995; Aii *et al.*, 1998; Woo *et al.*, 1999; Matsui *et al.*, 2003*b*). The flower morphology of the self-compatible lines is long homostyle and is controlled by a single gene (Campbell, 1995; Aii *et al.*, 1998; Woo *et al.*, 1999; Matsui *et al.*, 2003*a, b*). The allele controlling homomorphic flowers was designated as S^h, and the dominance relationship of S^h with *S* and *s* was found to be $S>S^h>s$ (Woo *et al.*, 1999). Matsui *et al.* (2003*b*) suggested that self-compatibility, flower morphology, and the dominance relationship are due to the genotype of gi^SI^PPA/gi^SI^PPA caused by the recombination in the *S* supergene.

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However, the self-fertilization of Pennline 10 has not been investigated in detail. In the present study, it is inferred that genes outside the *S* supergene control functions of the *S* locus in Pennline 10.

MATERIALS AND METHODS

Plant materials

Two self-fertile buckwheat lines were used—Pennline 10 (kindly provided by National Seed Storage Laboratory USDA-ARS) and Kyukei SC2 (KSC2), produced by a cross between *Fagopyrum esculentum* and *F. homotropicum* (Matsui *et al*., 2003*b*)—and also two self-incompatible cultivars, Botansoba and Shinano 1. Pennline 10 has short-homostylous flowers (reduced style) (Marshall, 1969), and KSC2 has long-homostylous flowers (Matsui *et al*., 2003*b*). F_1 plants were produced by hand pollination between either Botansoba or Shinano 1 and Pennline 10. F_1 plants produced by the cross between Shinano 1 and Pennline 10 with pin and thrum flowers were designated as F_1P and F_1T, respectively. F_2 populations were obtained by self-pollination of the F_1 plants.

Observation of pollen tube growth

To identify cross-compatibility and -incompatibility of Pennline 10, pollen tube growth was evaluated. Incompatibility reactions were evaluated based on pollen tube growth rather than seed sets because seed sets were easily influenced by enviromental conditions. Branches with buds and flowers were collected and stood in bottles with water in a dark room at 20 °C. The next morning, the flowers which were just starting to open were detached from the branch, emasculated, put on 0·8 % agar plates, and cross-pollinated. A check was not made for self-pollen grains on the stigmas with a lens because it had been confirmed that few self-pollen grains pollinated in this test. At 6 or 24 h later, the styles were collected and fixed with acetic acid : ethanol (3 : 7). After being rinsed with distilled water for 15 min, the styles were treated with 1 N sodium hydroxide for 120 min at 60 °C, briefly washed with distilled water, and then stained with 0·1 % aniline blue for 60 min at 60 °C. After a brief rinse with distilled water, the pollen germination and pollen tube growth were examined by fluorescence microscopy (Leica, Wetzlar, Germany).

RESULTS

Cross-compatibility of Pennline 10

When pollen grains of Pennline 10 were crossed with the pistils of pin, thrum and long-homostyle flowers, the pollen tubes reached into the ovules (Figs 1A and B and 2). Therefore, the pollen grains of Pennline 10 were compatible with all style types, suggesting that pollen grains of Pennline 10 have lost the *S* function. In the crosses on the pistils of Pennline 10 of pollen from other plants with different flower morphology, pollen tube growth was unstable (Fig. 2). In addition, pollen tube growth of the crosses between pin and
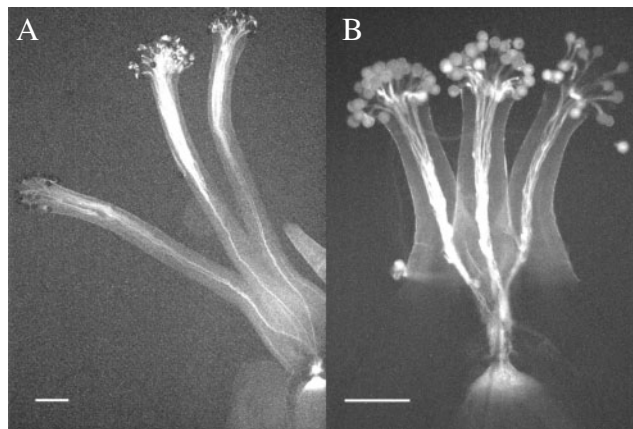

Fig. 1. Pollen tube growth of Pennline 10 on the styles of self-incompatible plants. Pollen tubes reach the base of the pin style (A) and the thrum style (B). Bars = 0·2 mm.

F_1P, between F_1P and F_1P, and between KSC2 and F_1P was unstable. However, pollen–style interactions in other cross combinations were distinct (Fig. 2).

Flower morphology of F_1 and F_2

To clarify whether the loss of S function of pollen is caused by the deletion of I^P in the *S* supergene or controlled by genes outside the *S* supergene, the flower morphology of F_1 and F_2 plants was evaluated. Twenty-five F_1 seeds, obtained by using Pennline 10 pollen, were grown in a glasshouse or a growth chamber. When thrum plants were used as maternal plants, the flower morphology of F_1 plants were thrum or pin, and when pin plants were used as maternal plants, all the F_1 plants were pin (Table 1). All the F_1 plants produced by the cross between Botansoba and Pennline 10 set selfed seeds, but plants produced by the cross between Shinano 1 and Pennline 10 set no or few F_2 seeds. These results suggest that the self-compatibility of Pennline 10 is influenced by the genetic background. Plants having pin flowers appeared in all eight F_2 populations (02AL10 to 02AL17) derived from the cross between Botansoba with either pin or thrum flowers and Pennline 10, including those derived from F_1 plants having thrum flowers, F_1T (Table 2). Two populations, 02AL10 and 02AL13, had no short-homostyle plant, and an intermediate flower phenotype, short-pin, whose ratio of style length to anther height is smaller than that of pin and larger than that of Pennline 10, was observed.

DISCUSSION

Distylous self-incompatibility such as that of buckwheat and *Primula* is mainly controlled by the *S* supergene (Dowrick, 1956; Sharma and Boyes, 1961; Matsui *et al*., 2003*b*). Self-compatible variants probably resulting from recombination in the *S* allele have been reported. Wedderburn and Richards (1992) reported that homostyly in some homostyle species had arisen secondarily by recombination
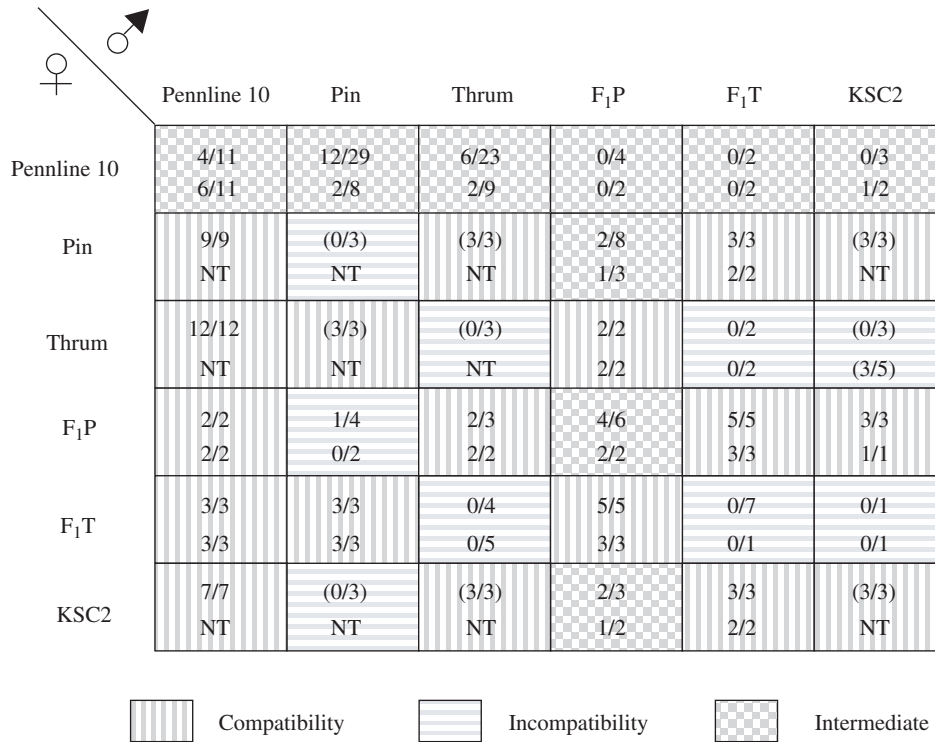
FIG. 2. Pollen tube growth in each cross combination. Data are presented as number of styles with pollen tubes reaching the ovule/number of pistils pollinated. Upper values are findings at 6 h after pollination, and lower values are those at 24 h after pollination. Values in parentheses are from Matsui *et al.* (2003*b*). NT, not tested.

TABLE 1. *Flower morphology of F₁ plants*

Cross combination		Flower morphology of F₁ plants	
Female	Male	Thrum	Pin
Botansoba (T)	Pennline 10 (SH)	3	2
Botansoba (P)	Pennline 10 (SH)	0	4
Shinano 1 (T)	Pennline 10 (SH)	6	3
Shinano 1 (P)	Pennline 10 (SH)	0	7

P, pin; T, thrum; SH, short homostyle.

TABLE 2. *Flower morphology of F₂ plants*

		Flower morphology			
		F₂			
Line	F₁	Thrum	Pin	Short pin	Short homostyle
02AL10	Thrum	6	2	3	0
02AL11	Thrum	19	7	9	2
02AL12	Pin	0	48	27	1
02AL13	Thrum	33	21	3	0
02AL14	Pin	0	24	7	3
02AL15	Pin	0	17	1	1
02AL16	Pin	0	18	14	7
02AL17	Pin	0	25	4	2

All lines were produced by the cross between Botansoba and Pennline 10. Lines from 02AL10 to 02AL13 were produced by the cross between thrum plants and Pennline 10, and from 02AL14 to 02AL17 were produced by the cross between pin plants and Pennline 10.

within the *S* complex linkage group in *Primula*. Matsui *et al.* (2003*b*) reported that a self-compatible allele, S^h, derived from *F. homotropicum* had arisen by recombination in the *S* supergene. If the short-homostyle trait of Pennline 10 had arisen by recombination in the *S* supergene, its genotype would be considered to be $GI^s i^p a / GI^s i^p a$ (Fig. 3). If this model is correct the pollen tubes of the short-homostylous plants should be compatible with the styles of thrum plants but incompatible with the styles of pin plants and the styles of the short-homostylous plants should be incompatible with thrum pollen but compatible with pin pollen. In addition, the pollen tubes of short-homostylous plants should be incompatible with the style of long-homostylous plants, and the reciprocal cross also should be incompatible, because the genotype of long homostyle is $gi^S I^P PA / gi^S I^P PA$ (Fig. 3). Furthermore, the flower morphology of F₁ plants produced

by the cross between thrum and Pennline 10 should be thrum or short homostyle (Fig. 4), and only short-homostylous plants should be produced by the cross between pin and Pennline 10 (Fig. 4).

However, in the present study, pollen of Pennline 10 was compatible with all the flower types. In addition, short-homostylous plants were not obtained, and pin plants appeared in the F₁ plants produced by the cross between thrum and short homostyle. Furthermore, short-homostyle plants were not found and only pin plants appeared among

the F$_1$ plants of the pin × short-homostyle cross. These results indicate that short homostyle of Pennline 10 was not generated through recombination in the *S* supergene.

If the self-compatible gene of Pennline 10 is due to deletions of *I^P* and *A*, F$_2$ plants derived from the self-pollination of the F$_1$ thrum plant that arose from the cross between thrum and Pennline 10 should segregate thrum and short homostyle in a 3 : 1 mono-factorial ratio. In addition, F$_2$ plants derived from the self-pollination of F$_1$ pin plants that

arose from the cross between either thrum or pin and Pennline 10 should segregate short homostyle and pin in a 3 : 1 mono-factorial ratio. However, the F$_2$ plants derived from the F$_1$ thrum plants included pin plants, and thrum plants were not observed among the F$_2$ plants derived from the F$_1$ pin plants. Furthermore, plants with short-pin flowers occurred in both F$_2$ populations. These results suggest that the *S* locus of Pennline 10 is *ss* genotype same as pin and that modifier genes affect self-compatibility and style length.

Polygenes or major genes outside the *S* locus responsible for breakdown of self-incompatibility have been reported to occur in many plants, e.g. alsike clover (Townsend, 1969), *Brassica* (Thompson and Taylor, 1966; Nasrallah and Wallace, 1968; Hinata *et al.*, 1983) and *Petunia* (Tsukamoto *et al.*, 2003). Mather (1950) reported a mutant that has a gene that shortens the length of pin stigmas in *P. sinensis*. Kurian and Richards (1997) reported that there are at least two loci with additive effect on the genes on the style length, stigma papilla length and style cell length. In the present study, flower morphology in the F$_1$ population was pin or thrum, indicating that the modifier genes did not have an effect because of their heterozygosity. However, pollen tube growth was unstable in the pin × F$_1$P, F$_1$P × F$_1$P, and KSC2 × F$_1$P crosses, suggesting that the genes for self-compatibility might show partial dominance or operate a late-acting system sensitive to environmental conditions. An intermediate flower phenotype, short-pin, recognized in the F$_2$ population was probably due to homozygosity of some of the modifier genes, and short homostyles are probably produced when all of the polygenes are in their homozygous forms in a plant.

The compatibility or incompatibility of Pennline 10 was not clarified when it was used as the style parent. The reason pollen tubes did not reach the ovule by self-pollination of Pennline 10 may be the influence of various environmental factors on the expression of polygenes. High seed fertility of Pennline 10 might be caused not by self-compatibility but by genes controlling intensity of
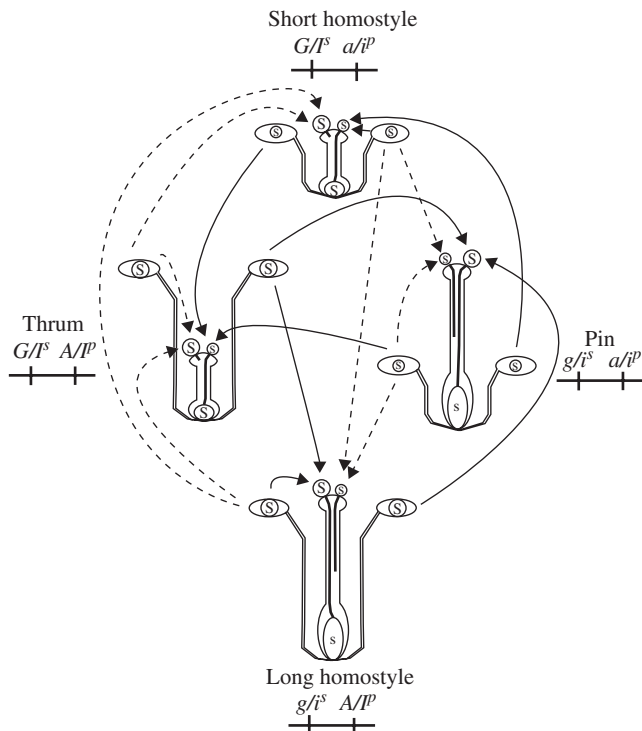

FIG. 3. Expected compatibility interactions among a pin, thrum, long-homostyle and short-homostyle plants. Crosses shown by arrows are compatible cross and arrows with broken lines are incompatible cross.

Figure 3 labels:
Short homostyle *G/I^s a/i^p*
Thrum *G/I^s A/I^p*
Pin *g/i^s a/i^p*
Long homostyle *g/i^s A/I^p*

	Genotype of the *S* locus[*]									
	s/s	*S/S*	*S^h/S^h*	*S^{sh}/S^{sh}*	*S/s*	*S^h/s*	*S^{sh}/s*	*S/S^h*	*S/S^{sh}*	*S^h/S^{sh}*
Genotype of style length (*g*) and anther height (*a*)	*ggaa*	*GGAA*	*ggAA*	*GGaa*	*GgAa*	*ggAa*	*Ggaa*	*GgAA*	*GGAa*	*GgAa*
Phenotype of style length (*g*) and anther height (*a*)	ga	GA	gA	Ga	GA	gA	Ga	GA	GA	GA
Flower morphology	Pin	Thrum	LH	SH	Thrum	LH	SH	Thrum	Thrum	Thrum
Dominance relationship of the *S* allele	——	——	——	——	*S>s*	*S^h>s*	*S^{sh}>s*	*S>S^h*	*S>S^{sh}*	——

FIG. 4. Expected dominance relationships among *s*, *S*, *S^h* and *S^{sh}* alleles based on the dominance relationships of the style length, *g*, and anther height, *a*, genes. *S^{sh}*, tentatively designated here as short homostyle, occurs by recombination in the *S* supergene. [*]Genotypes of *S/s* is normal type for thrum under a natural environment. Genotypes of *S/S* and *S^h/S^{sh}* plants are tentatively designated here because these plants should not be produced by self-incompatibility action.

self-incompatibility. Further study is needed to clarify the compatibility or incompatibility of the style of Pennline 10.

Many reports demonstrate that polygenes control the intensity of self-incompatibility (Nasrallah and Wallace, 1968; Crowe, 1971; Richards and Thurling, 1973), and the self-fertilization of Pennline 10 is likely to be due to such genes. Seed production of buckwheat is influenced by day length and temperature, suggesting that the expression of the involved genes is influenced by various environmental conditions. There is no report of QTL analysis of the intensity of self-incompatibility with molecular maps in buckwheat. QTL analysis would give further information on heteromorphic self-incompatibility.

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