JARQ 41 (1), 1 – 5 (2007) http://www.jircas.affrc.go.jp

REVIEW

Use of Self-Compatibility and Modifier Genes for Breeding and Genetic Analysis in Common Buckwheat (*Fagopyrum esculentum*)

Katsuhiro MATSUI[1]*, Takeshi NISHIO[2] and Takahisa TETSUKA[1]

[1] Research Team for Biomass and Industrial Crop, National Agricultural Research Center for Kyushu Okinawa Region, (Koshi, Kumamoto 861–1192, Japan)
[2] Laboratory of Plant Breeding and Genetics, Tohoku University (Sendai, Miyagi 981–8555, Japan)

Abstract

Common buckwheat plants have heteromorphic self-incompatibility. Using two self-fertilizing lines, we revealed that there are two distinct systems of self-compatibility, one using a self-compatible allele, S^h, the other using modifier genes located outside the *S* locus and suppressing the functions of the *S*-locus genes. S^h appears to have been produced by recombination in the *S* supergene ($gi^S I^P PA/gi^S I^P PA$), in which each gene is functional. The modifier genes control the intensity of self-incompatibility and have three distinctive features: (1) Pollen shows cross-compatibility with styles of all flower types, although the compatibility is influenced by the genetic background of those plants. (2) Flower morphology of F_1 plants is controlled by the genotype of the *S* locus. (3) F_1 plants show high self-compatibility, although the level of self-compatibility is influenced by genetic background. The use of these genes for buckwheat breeding is discussed.

Discipline: Plant breeding
Additional key words: heteromorphic incompatibility, pollen tube growth, *S* locus, *S* supergene

Introduction

In many flowering plants, self-incompatibility (SI) is an important system to prevent inbreeding and to promote outbreeding. In most species studied, the SI system is controlled by a single locus, *S*. SI is primarily a reaction between haploid pollen grains or pollen tubes and diploid stigmas or styles. SI is classified into two distinct types by whether the SI response is related to floral morphology, such as style length, anther height and pollen size (heteromorphic SI), or not (homomorphic SI).

There are two distinct types of homomorphic SI: gametophytic (GSI) and sporophytic control (SSI) of SI response. In the GSI system, the pollen SI phenotype is determined by its own genotype. In the SSI system, the pollen SI phenotype is determined by the genotype of its diploid parent. Heteromorphic incompatibility also is due to SSI.

Recent studies of SI have clarified the mechanisms of homomorphic SSI and GSI at the molecular level[4,6,16]. The molecular basis of heteromorphic incompatibility has yet not been clarified.

There are also two types of heteromorphic incompatibility: distylous and tristylous. Most species with heteromorphic flowers have distylous SI. Common buckwheat is a distylous self-incompatible species with two types of floral architecture: thrum, having short styles and high anthers; and pin, having long styles and low anthers[1]. 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[5]. Recently, a self-compatible allele, S^h, which is derived from *F. homotropicum*, has been reported[13,18]. The flower morphology of a plant with S^h allele is long-homostyle.

Here we review the genetic aspects of heteromorphic incompatibility in common buckwheat and discuss the use of the self-compatible allele and genes suppressing the SI functions in buckwheat breeding and genetic analysis.

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*Corresponding author: e-mail matsuik@affrc.go.jp
Received 3 March 2006; accepted 13 June 2006.

S supergene hypothesis and breakdown of self-incompatibility with homomorphic variants in buckwheat

The incompatibility response is based on the interaction between pollen and style or stigma. This response in the heteromorphic incompatibility system has a close relationship with flower morphology. Pin flowers have a long style, low anthers, and small pollen grains (Fig. 1A). Thrum flowers have a short style, high anthers, and large pollen grains (Fig. 1B). Dowrick (1956)[3] postulated that the genes controlling flower morphology and self-incompatibility in *Primula*, which has a distylous self-incompatibility system, are distinct but tightly linked with each other (*S* supergene), indicating that the *S* supergene segregates as a simple Mendelian factor. Sharma and Boyes (1961)[15] considered that the *S* locus of common buckwheat is similar to the *S* supergene proposed in *Primula*. 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 (Fig. 2). 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 5 genes are unknown.

We found that a self-compatible line that was produced by an interspecific cross between common buckwheat and *F. homotropicum* shows the pollen–style interaction in accordance with the *S* supergene hypothesis[9]. The flower morphology is long homostyle (Fig. 1C) and the pollen size is similar to that of thrum. The pollen tubes of the self-compatible plants were compatible with the styles of the pin plants but incompatible with the styles of thrum plants. On the other hand, the pollen tubes of pin flowers were incompatible with the styles of the long homostyle plants, but the pollen tubes of thrum flowers were compatible with the styles of the long homostyle plants (Fig. 3). These reactions can be explained by assuming that the genotype of the S^h allele is gi^SI^PPA/gi^SI^PPA. The orders of *g* and i^S and of i^P, *p* and *a* were not revealed. The dominance relationship of S^h with *S* and *s* ($S > S^h > s$) can be explained by the dominance relationship of each gene in the *S* supergene (Fig. 4).

Fig. 1. Flower morphology in common buckwheat
A: Pin, B: Thrum, C: Long homostyle, D: Short homostyle.

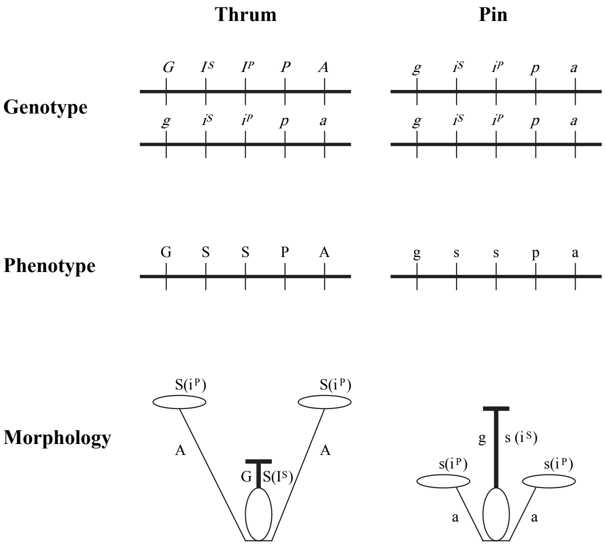

Fig. 2. The *S* supergene in buckwheat
G: Style length, I^s: Stylar incompatibility, I^p: Pollen incompatibility, *P*: Pollen size, *A*: Anther height.

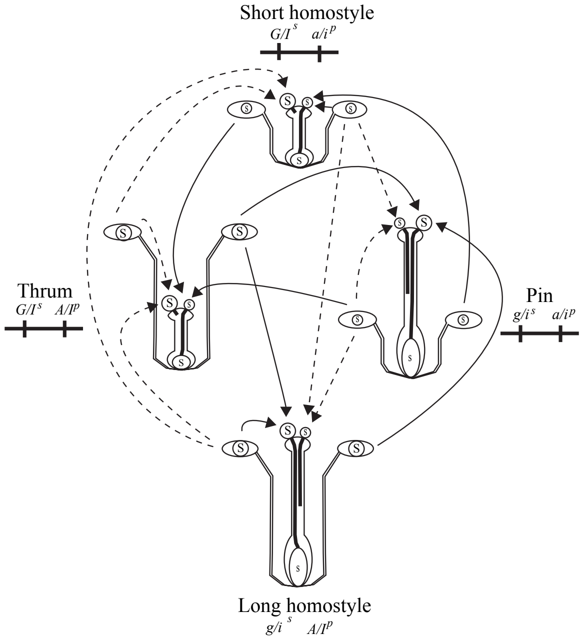

Fig. 3. Expected compatibility interactions among pin, thrum, long homostyle, and short homostyle plants
Crosses shown by arrows with unbroken lines are compatible crosses and arrows with broken lines indicate incompatible crosses.

	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
The S^{sh} alleles are tentatively designated here if the short homostyle has occurred by the recombination in the *S* supergene. The genotype of *S/s* is the 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 the action of self-incompatibility.

Effects of modifier genes on heterostyly in buckwheat

Self-fertile common buckwheat lines have been obtained by spontaneous or artificial mutation[7,14,15,17]. Marshall (1970)[8] developed a self-fertile buckwheat line derived from a mutant of common buckwheat, and named it "Pennline 10". The flower morphology of "Pennline 10" is the short homostyle (Fig. 1D). We suspected if the short homostyle character of "Pennline 10" has occurred by recombination in the *S* supergene, the genotype in the *S* supergene should be GI^Si^Ppa/GI^Si^Ppa (Figs. 3 & 4) and the manner of compatibility should react according to the expected reaction of Fig. 3. However, the pollen–pistil reaction between "Pennline 10" and pin or thrum plants did not fit the expected reaction. Furthermore, F_2 segregation of flower morphology did not fit a 3:1 single-factorial ratio (Table 1). These results suggested that the self-compatibility and short homostyle of "Pennline 10" are controlled by genes outside the *S* supergene[10]. The *S* locus of "Pennline 10" was found to have *ss* genotype like pin, and the self-fertility of "Pennline 10" was inferred not to be derived from recombination in the *S* supergene.

The distinctive features of the modifier genes are as follows: (1) Pollen of "Pennline 10" shows cross-compatibility with styles of all the flower types, although the compatibility is influenced by the genetic background of the recipient plants. (2) Flower morphology of F_1 plants is controlled by the genotype of the *S* locus. (3) F_1 plants show high self-compatibility, the level of which is influenced by the genetic background. Genetic background in this case includes differences in ecotype, such as summer and autumn types.

Major genes or polygenes outside the *S* locus responsible for breakdown of self-incompatibility have been reported in many plants[2]. Furthermore, many reports have demonstrated that polygenes control the intensity of self-incompatibility[2], and the self-compatibility of "Pennline 10" is likely due to the expression of such genes.

Use of self-compatible allele and modifier genes for breeding and genetic analysis

The yield of buckwheat is low, and is influenced largely by environmental conditions. Self-incompatibility is thought to be one of the reasons, because seed production needs crossing mediated by insects such as bees. Many breeders and researchers have tried to produce self-fertilizing plants. Here we describe two ways to get self-fertilized seeds in buckwheat: use of the self-compatible S^h allele and use of modifier genes.

To produce self-fertilizing cultivars, use of the self-compatible S^h allele is better than the use of modifier genes. Control of self-compatibility by a single gene enables easy selection of self-fertilizing plants having other desirable agricultural traits, such as high yields, lodging resistance, and disease resistance. An additional benefit is the ease of selection of self-compatible plants by flower morphology. However, care is needed when seeds are obtained by self-fertilization without any isolation and bagging in the selection of self-fertilized seeds, because self-compatible plants can be crossed with other plants including self-incompatible plants.

A self-compatible line with the S^h allele and a self-fertilizing line with modifier genes could be powerful tools for genetic analysis. Mutant plants such as dwarf

Table 1. Flower morphology of F_2 plants

Line	Flower morphology				
	F_1	F_2			
		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 02AL10 to 02AL13 were produced by the cross between thrum plants and "Pennline 10", and lines 02AL14 to 02AL17 were produced by the cross between pin plants and "Pennline 10".

plants are sometimes recognized in common buckwheat (self-incompatibility) fields. However, in most cases, the mutant plants are produced by homozygosity of recessive genes and do not appear in both pin and thrum forms simultaneously. Thus, it would take a long time to fix the mutant trait. Crossing for genetic analysis of the mutant character is usually performed between a self-incompatible plant (female parent) and a self-compatible plant (pollen parent) because of the high possibility seeds on the self-incompatible plant are produced by crossing with the self-compatible plant, and without obtaining many self-fertilized seeds. When the flower morphology of a mutant plant is pin (*ss*), a self-compatible line with the S^h allele (long homostyle) would be better than a self-fertilizing line with modifier genes, such as "Pennline 10" because it gives a fully compatible combination. On the other hand, when the flower morphology of the mutant plant is thrum, a self-fertilizing line with modifier genes such as "Pennline 10" would be better than a self-compatible line with the S^h allele (long homostyle), because the pollen of the "Pennline 10" is cross-compatible with the styles of all flower types and produces self-fertilized seeds.

We have performed genetic analysis of some mutant traits such as dwarf and deficiency of anthocyanin characters (in preparation) with self-compatible lines. We have also produced self-compatible lines (long homostyle) with the non-brittle pedicel trait based on the results of genetic analysis[11,12]. The self-compatible lines with the non-brittle pedicel trait are more useful for genetic analysis and surpass other lines as parental lines for breeding. The production of new buckwheat cultivars with good agronomical traits would be accelerated by using the self-compatible lines.

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