GENE ANALYSIS OF SELF-COMPATIBILITY IN BRASSICA CAMPESTRIS VAR. YELLOW SARSON (A CASE OF RECESSIVE EPISTATIC MODIFIER)

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Brassica campestris L. is known to be self-incompatible in general, while it involves a self-compatible cultivar, yellow sarson. Yellow sarson whose name is due to its yellow seed colour is considered to have lost the self-incompatibility in the course of cultivation by ancient farmers in India (1). Analysis of the genetic factors controlling the self-compatibility may contribute to the knowledges about; 1. how self-compatible plants have lost the self-incompatibility, 2. how the self-incompatibility is regulated by genes, and 3. how to raise self-compatible strains if necessary in a breeding program.

In such experiments, presence or absence of S alleles is often questioned, when the self-incompatibility in the progeny plants is masked by the other genetic factors. Therefore, the presence of S alleles was monitored by S-glycoproteins in each plant in the present experiment. Close correlation of the S-glycoproteins and S alleles and the chemical nature of the S-glycoproteins have been reported (2,3).

Materials and methods

The material strain of yellow sarson (strain no. C634; abbreviate as C6) was of Indian origin and provided through USDA. Two self-incompatible strains homozygous for S^8 and S^{12} were isolated from a naturalized population at Oguni, Yamagata, Japan and their S-glycoproteins were analyzed (abbreviate as S8 and S12, respectively).

The C6 was hybridized with S8 and S12 and their F_1, F_2 and backcrossed progenies were tested for their incompatibility by selfing and test-pollinating with the parental strains. Incompatibility of the pollination combinations was determined by observing the pollinated stigmas if occurs the disturbance of pollen-tube intrusion into stigma papillae (2). S-glycoprotein analysis was carried out for every progeny plants by cellulose-acetate electrofocusing (4). To S8 and S12, respective glycoprotein bands were observed, while no specific bands were detected to C6 (5).

Results

In the hybrid between C6 and S8, the F_1 was incompatible in selfing and the reciprocal pollinations with the S8, while it was compatible in the reciprocal pollinations with the C6. The F_2 segregated into three types on the incompatibility and S-glycoproteins. Type I plants were compatible in all the test-pollinations and selfing and did not possessed the S8-glycoproteins. Type II plants had the S8-glycoprotein and their pollen was incompatible with the stigma of S8 but every other test-pollination was compatible. Type III plants having the S8-glycoprotein showed the same incompatibility relations as the F_1. Two backcrossed progenies with C6 was as same as the II type of the F_2. Of 7 backcrossed progenies with S8, 5 were the III type but the other 2 plants were incompatible with C6 pollen, being described as III' type (Table 1).

The segregation of the progenies on the incompatibility relations was explained by the following assumptions:

1. Yellow sarson has lost the S allele activity or keeps its activity very low.
2. There are M vs m alleles whose locus is independent of S alleles.
3. The m gene suppresses the action of S alleles on the pistil side but not on the pollen side.

The expected segregation of genotypes in the progenies under these assumptions is presented in Table 2. The table also shows the expectation of the incompatibility relations in the diallel pollinations between the F_2 plants. These expectations explained well the results of the Table 1 and those of the diallel pollination experiments shown in Table 3, except the appearance of the III' type plants. Good agreement was found on the presence or absence of the S8-glycoprotein.

The present scheme was also applied to the family C6 x S12 as shown in Table 4. Characteristic points of this family were as follows; 1, the expression of incompatibility fluctuated in the styles of the III type plants in the F_2 and diallel pollinations between the F_2 plants, and 2, the III' type plants were found in the F_2 and the backcrossed progenies with S12.

The segregation ratios of the three types in the F_2 agreed well between the expectations and the experimental results (Table 5).

It was often observed that the pollen-tube intrusion occurred in the callose forming stigma papillae in the diallel pollinations.

Discussion

Several cases of self-compatibility has been reported in Brassica species so far (6, 7, 8, 9). The present case of the recessive epistatic gene raises our interests because it is active only in the stigma. Presence of the M gene in the stigma of the self-incompatible plants suggests that at least two factors should be satisfied for the normal expression of incompatibility. One is controlled by the S genes and the other by the M gene.

On the other hand, self-incompatibility reaction involves two phases; one is the recognition reaction and the following phase is the disturbance of the pollen-tube intrusion into papilla cell wall. The recognition reaction is controlled by the S genes. On the action of the M gene, two possibilities may be considered. The M gene participates in the chemical and/or structural background for the occurrence of the recognition reaction, or it is a necessary factor for the disturbance of the pollen-tube intrusion. Because the pollen-tube intrusion in the diallel pollinations was observed with the callose formation in the papillae, we are tending to take the latter possibility. That is, the M gene concerns with the disturbance of the pollen-tube intrusion, and the callose formation is an indication of the occurrence of the recognition reaction by the S genes.

The S-glycoproteins in stigma have been postulated to be the most possible substances that participate in the recognition reaction in self-incompatible Crucifers (3). The S-glycoproteins are heterogeneous among S alleles and this heterogeneity involves S-genotype specific structures and other structures that are common to several S-genotypes. In inter-subspecies and inter-species pollinations, it can be expected that the common structures between the parental S alleles undergo a reaction with a certain degree of performance and that C6 keeps low activity in the common structures of the S alleles, though its glycoprotein has not been detected. Characteristics of the III' type that appeared in the F_2 and backcrossed progenies were the reciprocal differences of the incompatibility in the pollinations between the plants and C6. When stigmas had the M gene and the C6 plants kept a weak activity of S alleles, the reciprocal differences could be observed, since the m gene did not mask the activity of S alleles on the pollen side. Such assumption could be applied to the unilateral incompatibility pointed out by Lewis and Crowe (10) and Sampson (11).

It is postulated that a recessive epistatic gene m is the major factor causing the self-compatibility in yellow sarson. When the mutation occurred in a self-incompatible population, the m gene might be kept in the allogamous population for a fairly long period, because the m gene is recessive and not active on the pollen side.

The accumulation of m gene in the population depended upon the amount and degree of the deleterious genes that had lowered the fittness of selfed progenies. The deleterious effect could be, sometimes, reduced by an intensive farming or some other circumstances, and the population became partially autogamous. In such conditions, genetic combinations emerged frequently between self-compatibility and the yellow seed colour. Thus, self-compatible plants with yellow seeds have been finally established by farmers' selection.

Besides, we would like to point out that the m gene found in the present study is useful for the hybrid breeding by cytoplasmic male sterility in B. campestris. Breeding a good maintainer is necessary for the success of this breeding system. However, the S gene of the maintainer is inevitably transferred to the male sterile line along with the male sterility genes, and the maintenance becomes very difficult due to the identity of the S gene (12). This difficulty can be cleared away by the incorporation of the m gene.

References

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Table 1 Incompatibility behaviour of the hybrid progenies of C6 x S^8 -

Progenies	No. of plants	Test cross x S^8	S^8x	Self	x C6	C6 x	S^8band	Types
F_1	5	-	-	-	+	+	+	III
F_2	4	+	+	+	+	+	-	I
	3	+	-	+	+	+	+	II
	6	-	-	-	+	+	+	III
F_1 x C6	2	+	-	+	+	+	+	II
F_1 x S^8	5	-	-	-	+	+	+	III
	2	-	-	-	-	+	+	III'

x S^8 (x C6): Pistils of progeny plants were test crossed with the S^8 pollen (with the yellow sarson pollen).

S^8 x (C6 x): Pollen of progeny plants was examined on the S^8 stigma (on the yellow sarson stigma).

+ : Many pollen tubes penetrate the stigma papilla.

- : A few or no pollen tubes penetrate the stigma papilla.

Table2 Expected incompatibility behaviour of the hybrid progenies from the cross between self-incompatible (B. campestris) and self-compatible (yellow sarson) strains under the recessive epistatic gene(m)assumption

Test cross:

Progenies	Phenotypes	Test cross x S^i	S^ix	self	x C6	C6 x	S^iband
F_1	S^iM	-	-	-	+	+	+
F_2	I:S^cm,S^cM	+	+	+	+	+	-
	II:S^im	+	-	+	+	+	+
	III:S^iM	-	-	-	+	+	+

F_2 diallel cross:

Female	Phenotypes	Male I S^c-	II S^im	III S^iM	S^iband
I	S^cm, S^cM	+	+	+	-
II	S^im	+	+	+	+
III	S^iM	+	-	-	+

Respective allelic genes S^ivs.S^c and M vs.m are independent each other.
m suppresses the expression of S^i at the pistil side but not at the pollen side.

Table 3 Diallel cross of the C6 x S^8 F_2 family

Female	Male 6	7	4	5	10	13	15	11	3	2	S^8band	Types
6	+++	++	+++	+++	+++	++	+++	+++	+ +	+	-	I
7	++	++	++	++	++	+	+	+	+	+	-	I
4	+++	++	+++	+++	+++	++	+++	+++	+	+	-	I
5	+++	++	+++	+++	+++	++	+++	+++	+	+	+	II
10	+++	++	+++	+-+	+++	++	+++	+++	+ +	+	+	II
13	++	++	++	++	++	++	++	++	+	+	+	II
15	+++	++	-++	--+	--+	--	--+	--+	- -	-	+	III
11	+++	+-	+++	---	---	--	---	---	- -	-	+	III
3	+++	++	-++	--+	---	--	---	---	- -	-	+	III
2	+	+	-	-	-	-	-	-	-	-	+	III

Score + and - represents compatible and incompatible results in view of pollen tube penetration.

Results of the 1st, 2nd and 3rd crossing experiments are presented from left to right in respective cross combinations (blanck: without trial).

Table 4 Incompatibility behaviour of the hybrid progenies of C6 x S^{12}

Progenies	No. of plants	Test cross x S^{12}	S^{12}x	Self	x C6	C6 x	S^{12}band	Types
F_1	2	-	-	-	+	+	+	III
F_2	2	+	+	+	+	+	-	I
	3	+	-	+	+	+	+	II
	8	+,-*	-	+,-*	+	+	+	III
	1	+,-*	-	+,-*	-	+	+	III'
F_1 x C6	4	+	+	+	+	+	-	I
	2	+	-	+	+	+	+	II
F_1 x S^{12}	3	-	-	-	+	+	+	III
	2	-	-	-	-	+	+	III'

+ : Many pollen tubes penetrate the stigma papilla, compatible.

- : A few or no pollen tubes penetrate the stigma papilla, incompatible.

+,-* : Results are variable: It tended to be incompatible at the early flowering stage while compatible at the late stage.

Table 5 χ^2 test for F_2 segregation

F_2 families	Test cross types I	II	III	Others	Total	χ^2	P
C6 x S^8	4(3.25)	3(2.44)	6(7.31)	-	13	0.54	.80-.70
C6 x S^{12}	2(3.25)	3(2.44)	8(7.31)	1	13	0.67	.80-.70
Total	6(6.50)	6(4.88)	14(14.6)	1	26	0.32	.90-.80
Expect. ratio	4	: 3	: 9				