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SELF-INCOMPATIBILITY ANTIGENS AND S GENE EXPRESSION IN *BRASSICA*

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Summary

Immunodiffusion tests performed on stigma extracts belonging to 21 incompatibility genotypes of *Brassica oleracea* var. acephala and to five genotypes of *B. oleracea* var. capitata showed no cross-reactions among the self-incompatibility (S) antigens found in this tissue. The lack of immunological relatedness between stigma antigens and the inability to detect S antigens in the pollen is exploited in formulating a testable hypothesis for analysing the structure and/or function of the self-incompatibility locus in plants.

1. Introduction

In higher plants, self-incompatibility is attributed to an exchange of molecular and cellular " recognition " signals between the male gametophyte and cells of the pistil (Lewis, 1954; Heslop-Harrison, 1975; Clark and Knox, 1978). Genetic analyses of several species of Cruciferae indicated that multiple alleles at the S locus control incompatibility (Bateman, 1955; Thompson and Taylor, 1965). The S alleles in the genus *Brassica* have been shown to produce, in stigma cells, proteinaceous molecules or antigens that are detectable by antibodies obtained following the immunisation of rabbits with stigma homogenates (Nasrallah and Wallace, 1967; Sedgley, 1974; Kučera and Polàk, 1975). Although our earlier immunogenetic studies pointed to a one allele-one antigen relationship in *Brassica*, it remained to be seen whether cross-reactions between different S antigens would be encountered. This report indicates an apparent lack of cross-reaction among members of a large sample of S antigens.

2. Materials and methods

(i) B. oleracea var. acephala *genotypes*

Stigmas from the following 21 kale genotypes were collected at the Plant Breeding Institute, Cambridge, England, courtesy of Dr K. F. Thompson (the *S* allele numbers are those of Thompson). Following harvest, stigmas were allowed to dry overnight at room temperature and stored in airtight vials.

S_1S_1	S_4S_4	S_7S_7	$S_{11}S_{11}$	$S_{15}S_{15}$	$S_{18}S_{18}$	$S_{23}S_{23}$
S_2S_2	S_5S_5	S_8S_8	$S_{13}S_{13}$	$S_{16}S_{16}$	$S_{20}S_{20}$	$S_{25}S_{25}$
S_3S_3	S_6S_6	$S_{10}S_{10}$	$S_{14}S_{14}$	$S_{17}S_{17}$	$S_{21}S_{21}$	$S_{26}S_{26}$

Homozygotes for any given allele will be denoted henceforth by the single allele.

(ii) B. oleracea var. capitata *genotypes*

Five cabbage genotypes were used with *S* allele designations assigned independently of the kale material of Thompson. Three genotypes, S_1S_1, S_2S_2, and S_3S_3, were described by Nasrallah and Wallace (1967) and will be referred to as CS_1, CS_2, and CS_3 in this paper. Two additional genotypes, CS_5 (S_5S_5) and CS_8 (S_8S_8), were kindly supplied by Dr D. H. Wallace of Cornell University.

(iii) *Antisera against stigma homogenates*

For a description of the two antisera, AHS_1 and AHS_2, produced against CS_1 and CS_2 stigma homogenates, see Nasrallah and Wallace (1967) and the protocol given below.

(iv) *Antisera against stigma extracts* (AES_2)

Stigma extracts were prepared by soaking dried cabbage CS_2 stigmas in buffered physiological saline (phosphate buffer *p*H 7·0) for 2 hours at 5°C followed by freezing, thawing and collection of supernatant. New Zealand White rabbits were immunised by administering subcutaneously and intramuscularly a 1 : 1 mixture of stigma extracts and Freund's Adjuvant (Difco). During each of the 2nd, 3rd, and 4th weeks, three booster intravenous injections were given per rabbit and the antisera harvested at the end of the 5th week. Extracts from 3000 stigmas were used per rabbit.

(v) *Test antigens*

Approximately 15 dried stigmas were pulverised in a spot plate and the resulting powder was suspended in 30 μl of buffered saline and then loaded into wells in Ouchterlony double diffusion plates. Testing of kale genotypes was performed at the Scottish Horticultural Research Institute, Invergowrie, Scotland.

(vi) *Absorption of antisera*

The absorption procedure with AHS_1 and AHS_2 was described by Nasrallah and Wallace (1967). With AES_2, the absorption was modified by adding 300 dried stigmas of CS_1, CS_2, CS_3, CS_5 or CS_8 genotypes to 1 ml of antiserum. The resulting mixture was left for 12 hours at 5°C and then centrifuged at 2000 *g* for 15 min. before using the supernatant in double diffusion tests.

3. Results

(i) *Stigma tests*

When stigma homogenates from each of the 21 kale genotypes were reacted against heterologously absorbed AHS_2 in double diffusion tests, only S_{21} formed a specific precipitation band typical of CS_2 (fig. 1). Homogenates of CS_2 and kale S_{21} loaded in adjacent wells gave a reaction of identity indicating that the same antigen was present in the two genotypes. Pollination tests had already shown S_{21} of kale to be identical to CS_2 (Thompson, 1968). Similar tests with heterologously absorbed AHS_1

showed that S_2 of kale produced a precipitation band typical of CS_1. Once again, results of pollination tests had shown that the kale S_2 allele was identical to CS_1 (Thompson, 1968). A reaction of identity was evident between CS_1 and kale S_2. However, another kale genotype, namely S_7, also reacted in a manner similar to kale S_2 (fig. 2); this fact suggested a possible cross-reaction between two different antigens. When Thompson was informed of the results, he kindly checked the tested S_7 genotype by appropriate pollinations and concluded: " The clones, from which stigmas were obtained for allele S_7, were heterozygous for alleles S_7 and S_2, confirming the serology results " (Thompson, personal communication). The precipitation band opposite S_7 (fig. 2) is in all probability due to the presence of the S_2 antigen in the tested line, and does not therefore represent a cross-reaction.

A second antiserum against CS_2 stigma extracts (AES_2) gave results consistent with those of AHS_2. Absorption of AES_2 with each of CS_1, CS_3, CS_5, and CS_8 stigma homogenates gave a precipitation band opposite the CS_2 genotypes in double diffusion tests. Absorption of AES_2 with homozygous or heterozygous genotypes carrying the CS_2 allele abolishes all precipitation bands indicating complete absorption of the antiserum.

(ii) *Pollen tests*

Pollen, mature anthers and young developing anthers including both pre-meiotic and post-meiotic stages were homogenised in buffered saline and reacted at different concentrations against AHS_1, AHS_2, and AES_2. Immunodiffusion tests similar to those used with stigma homogenates failed to reveal detectable levels of CS_1 and CS_2 stigma antigens in CS_1 and CS_2 pollen or anther tissue. Furthermore, whole pollen, pollen homogenates and anther homogenates were used as absorbing antigens with AHS_1, AHS_2 and AES_2. The absorbed antisera formed the characteristic CS_1 and CS_2 precipitation bands with the respective stigma homogenates, with no detectable weakening, and therefore no apparent absorption of the *S* specific antibody molecules.

4. Discussion

The self-incompatibility system is useful for exploring the control of the highly specific recognition mechanism between cells of pollen and pistil. The pollen component of the *S* allele system has yielded least to inquiry and little is known about its control (Heslop-Harrison *et al.*, 1974; Ferrari and Wallace, 1976). On the other hand, the stigma reaction was shown to be mediated by proteinaceous molecules that are readily detectable by immunochemical methods (Nasrallah and Wallace, 1967; Nasrallah, Barber and Wallace, 1970). Such findings had led us to postulate that, in the stigma, the *S* alleles function by each producing a unique polypeptide, the primary structure of which determines its antigenic properties. However, in the light of the data presented here, it is difficult to reconcile this hypothesis with the demonstrated absence of cross-reactions among the large number of *S* antigens examined to date. Had the *S* antigens been polypeptide variants of one protein species, one would expect that immunological cross-reactions between such biochemically related molecules would have been uncovered.

In view of the above and of the recently reported correlation of *S* alleles

with specific PAS-positive molecules (Hinata and Nishio, 1978), and alternative mechanism of gene action is presented. It envisages the function of alleles at the *S* locus as that of linking specificity markers such as sugars or sugar derivatives to a proteinaceous core molecule coded for by another locus (or loci). Evidence for the involvement of more than one locus in incompatibility systems has been demonstrated in several angiosperm families (Lundqvist, 1975) and in a self-fertile mutant of *Brassica* (Nasrallah, 1974). The *S* controlled markers could conceivably take part in the interactions that lead to incompatibility and also act as haptens that help elicit the observed antibody response. Ultimately, the characterisation of the structure of the stigma antigens will settle this question.

As for the pollen determinants of specificity, our results indicate that in *Brassica*, the S stigma antigens are not found in this tissue as is the case in *Oenothera* (Lewis *et al.*, 1966) and *Petunia* (Linskens, 1960). It appears that in Cruciferae, the pollen recognition molecules are not identical to those of the stigma, and therefore that different genetic units are responsible for the pollen reaction. Regardless of the precise structural organisation of the *S* gene complex, the function of the pollen genetic elements must be closely co-ordinated with their stigma counterparts. As a working hypothesis, it is postulated that such co-ordination is achieved by the expression of a specific glycoprotein antigen in the stigma, and of a complementary but different receptor molecule in the pollen.

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