COMPARATIVE STUDIES ON S-GLYCOPROTEINS PURIFIED FROM
DIFFERENT S-GENOTYPES IN SELF-INCOMPATIBLE BRASSICA
SPECIES II. IMMUNOLOGICAL SPECIFICITIES

KOKICHI HINATA, TAKESHI NISHIO AND JUN KIMURA

ABSTRACT

Antisera were prepared by immunization of apparently purified S-glycoproteins; one from an S allele of Brassica campestris and two from S alleles of Brassica oleracea. Each antiserum was reactive not only with the homologous S-glycoprotein but also with the heterologous ones, i.e. with the S-glycoproteins of the other S alleles of the same locus. In double diffusion tests, a spur against the heterologous S-glycoproteins suggested heterogeneity of the glycoproteins. The heterogeneity appears to involve a component of the molecule in which the genotypic specificity of an S-glycoprotein resides, probably, for the recognition site. Some molecular components are common to all tested S-glycoproteins and in this respect are like the public antigens of the MHC locus of mammals. The common molecular components were recognized between the S-allele-specific glycoproteins within Brassica oleracea and also between them and those of Brassica campestris. No S-specific substances were detected in buffer-soluble homogenates of style, ovary or anther. However, these homogenates contained substances that had structures similar to the corresponding common parts of the S-glycoproteins.

IN Cruciferae, the genetic system of self-incompatibility is controlled by the sporophytic action of multiple S alleles of a single locus (BateMAN 1955). Existence of S-specific substances in stigmas of self-incompatible Brassicas has been identified by immunological reactions against antisera made from crude stigma homogenates (NASRALLAH and WALLACE 1967, SEDGLEY 1974, KUCERA and POLAK 1975, SAREEN and KAKAR 1977). As shown in our earlier experiments, S-glycoproteins defined by their isoelectric points (pI) were purified by chemical procedures (NISHIO and HINATA 1979, 1982). The present experiments were undertaken, therefore, to learn (1) whether the S-glycoproteins thus defined have immunological specificity and (2) whether an S-glycoprotein cross-reacts with antisera made against different S-glycoproteins.

Symbols °S1 and °S4 were again used, respectively, to distinguish between the S alleles of Brassica oleracea and Brassica campestris (NISHIO and HINATA 1982).

MATERIALS AND METHODS

Plant materials were the homozygotes °S18, °S22, °S7, °S4 and °S12 of Brassica oleracea and °S7, °S9 and °S9 of Brassica campestris. The Brassica oleracea genetic strains were provided by N. P. A.

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van Marrewijk, Institute for Horticultural Plant Breeding, The Netherlands. Genotypes \( ^6S^{39} \) and \( ^6S^{22} \) are dominant alleles, \( ^6S' \) and \( ^6S^2 \) are recessive alleles and \( ^6S^{13} \) is intermediate (van Marrewijk, personal communication). The homozygous \( S \) alleles of \( B. \) campestris were isolated from a naturalized population from Oguni, Japan. Our electrofocusing analysis of crude stigma homogenates assigned a single S-glycoprotein band with a different PI to every \( S \) allele except \( ^6S'^{11} \), for which we found three S-glycoprotein bands. Stigmas were collected from these plants as previously described (Nishio and Hinata 1979, 1982).

Preparation of antigen and antisera: Antigens used for antisera production were from \( ^6S', ^6S^{39} \) and \( ^6S^{22} \) homozygotes. For \( ^6S'^{7} \) 12,000 stigmas and for \( ^6S^{39} \) 6,000 were homogenized in a phosphate-saline solution (0.01 M phosphate buffer pH 7.1 plus 8.5 gm/l of NaCl) with a mortar and pestle and centrifuged at 10,000 \( \times \) g for 20 min. Each homogenate was divided among 16 tubes (5 mm in diameter) for acrylamide gel electrofocusing. After determining the position of an S-glycoprotein band from one stained tube, the gel part containing the S-glycoprotein was collected from the other 15 tubes. The collected sections were homogenized in phosphate-saline solution with a glass homogenizer and divided for two injections. The homogenate was used as the antigen. For \( ^6S'^{22} \), the S-glycoprotein that was purified in the former experiment (Nishio and Hinata 1982) was used. That purified S-glycoprotein was divided into two parts. One part, containing 0.4 mg protein, was dissolved into 1 ml phosphate-saline and used as the antigen.

Before injection, antigens were mixed with Freund's complete adjuvant (1:1) and emulsified by ultrasonic waves. Antisera were prepared in rabbits by two intramuscular injections into the legs and back at an interval of 3 weeks. Two weeks after the second injection the serum was harvested and the titre of antibodies was determined against the crude stigma homogenates.

Immunoelectrophoresis: This was carried out in 1% agar medium containing 0.05 M barbital buffer pH 8.4 according to Clausen (1969).

Immunodiffusion after electrofocusing: Acrylamide gel electrofocusing was carried out according to Nishio and Hinata (1978) with 20 stigmas applied to each 3 mm column. After a parallel run of two gel columns for each S-glycoprotein, one was stained with Coomassie brilliant blue G250 to determine the band position of the S-glycoprotein and the other column was used in the immunodiffusion. The diffusion medium was the phosphate-saline solution containing 1.0% agar and 0.1% sodium azide. This was melted and poured onto a 25 x 75 mm microscope slide to a depth of 3 mm. After the medium solidified, one trough 3 mm wide was made along the center line for the antiserum, and twenty holes 3 mm in diameter were arranged in rows 3 mm from both sides of the trough. After electrofocusing the acrylamide gel column was cut into sequential pieces 3 mm thick and the pieces were put in the holes in order. Gaps between the medium and the gel piece were filled with melted agar at 45°. After filling the trough with antiserum, the plates were kept at 3° for two or three days.

Double diffusion: The composition of the medium was the same as that used in the immunodiffusion. The antigen wells were positioned at a distance of 5 mm from the central serum well. Test antigens were crude extracts prepared from 20 stigmas which were homogenized in 0.1 ml phosphate-saline solution and centrifuged at 10,000 g for 20 min.

Undiluted antiserum containing 0.1% sodium azide were used for the immunodiffusion experiments, unless otherwise indicated.

Absorption: Stigma homogenates were made from 200 stigmas in 1 ml phosphate-saline with the same stigma to saline ratio as for the test antigens. Antiserum to be absorbed was mixed with the homogenates at 1:1 or 1:2 (v/v) and kept at 37° for 30 min. Then, the mixture was centrifuged at 10,000 g for 20 min and the supernatant was used as an absorbed antiserum.

RESULTS

Titer and immunoelectrophoresis: The antisera had titers of 1/4 for Anti\(^+S'\), 1/4 for Anti\(^+S^{39}\) and 1/8 for Anti\(^+S^{22}\) according to a ring test. In immunoelectrophoresis, Anti\(^+S'\) gave an apparent single precipitin arc to the \( ^6S' \) stigma homoge-
nate (Figure 1A). Similarly, Anti\textsuperscript{oS\textsuperscript{39}} made one arc to the \textsuperscript{oS\textsuperscript{39}} stigma homogenate. Against \textsuperscript{oS\textsuperscript{22}}, Anti\textsuperscript{oS\textsuperscript{22}} gave two precipitin arcs that coalesced with each other (Figure 1B). The \textsuperscript{oS\textsuperscript{22}} stigma homogenate reacted with Anti\textsuperscript{oS\textsuperscript{39}} and gave two coalesced arcs with Anti\textsuperscript{oS\textsuperscript{3}}.

**Immunodiffusion after electrofocusing:** Immunological tests were made against sequential pieces of acrylamide gel columns containing the numerous electrofused proteins from stigma homogenates. Figure 2 A–C gives the result for the \textsuperscript{oS\textsuperscript{22}} stigma homogenate. A precipitin line formed against Anti\textsuperscript{oS\textsuperscript{22}} at the fifth gel piece which corresponded to the \textsuperscript{oS\textsuperscript{22}} glycoprotein band position (Figure 2 A,B). The same was observed for \textsuperscript{oS\textsuperscript{22}} against Anti\textsuperscript{oS\textsuperscript{39}} but the band was weaker (Figure 2C). For the \textsuperscript{cS\textsuperscript{30}} stigma homogenate the tenth gel piece, where electro-

**Figure 1.—Immunoelectrophoresis of stigma homogenates reacted with the antisera of respective S-glycoproteins.**

A: \textsuperscript{cS\textsuperscript{7}} stigma homogenate.
B: \textsuperscript{oS\textsuperscript{22}} stigma homogenate.

**Figure 2.—Immunodiffusion after electrofocused stigma homogenates of \textsuperscript{oS\textsuperscript{22}} (A–C) and \textsuperscript{cS\textsuperscript{7}} (D–F).**

A-C: Band profile (A) of \textsuperscript{oS\textsuperscript{22}} stigma homogenate after electrofocusing (arrow: \textsuperscript{oS\textsuperscript{22}} glycoprotein band). The gel pieces of the electrofocused column were reacted with Anti\textsuperscript{oS\textsuperscript{22}} (B) and Anti\textsuperscript{oS\textsuperscript{39}} (C). The 5th piece that involved the \textsuperscript{oS\textsuperscript{22}} glycoprotein formed a precipitin arc in every case.

D-F: Band profile (D) of \textsuperscript{cS\textsuperscript{7}} stigma homogenate after electrofocusing (arrow: \textsuperscript{cS\textsuperscript{7}} glycoprotein band). The gel pieces of the electrofocused column were reacted with Anti\textsuperscript{cS\textsuperscript{7}} (E) and Anti\textsuperscript{oS\textsuperscript{39}} (F). The 18th piece that involved the \textsuperscript{cS\textsuperscript{7}} glycoprotein formed a precipitin arc in every case.
focusing showed the presence of the \( S^{29} \) glycoprotein, reacted with the Anti\( S^{39} \) as with Anti\( S^{22} \). Thus, a precipitin line from the electrofocused stigma homogenate is formed only at the gel position of the S-glycoprotein. Also, the antisera of the two tested \( B. oleracea \) \( S \) alleles are reactive not only with their homologous S-glycoproteins but also with heterologous \( S \) alleles of \( B. oleracea \). Since three S-glycoproteins of different pI were found in electrofocused gels of \( S^{13} \) stigmas, this stigma homogenate was reacted with Anti\( S^{22} \). Precipitin lines formed against the 14th to 17th gel pieces, those involving the three S-glycoprotein bands.

To identify possible interspecific relations between the \( S \) alleles of \( B. oleracea \) vs. \( B. campestris \), the \( S^{7} \) stigma homogenate was reacted with Anti\( S^{7} \), Anti\( S^{22} \)

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**Figure 3.**—Representative demonstrations of double diffusion tests concerning Anti\( S^{7} \) (A-C) and Anti\( S^{22} \) (D-F). Test antigens were the stigma homogenates of \( S^{7}(1) \), \( S^{8}(2) \), \( S^{9}(3) \), \( S^{22}(4) \), \( S^{39}(5) \) and \( S^{7}(6) \). The experimental scheme is shown below and an explanation of the precipitin lines are in the text.

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Test antigens</th>
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<tbody>
<tr>
<td>A: Anti( S^{7} )</td>
<td>( B. campestris )</td>
</tr>
<tr>
<td>B: Absorbed Anti( S^{7} ) with ( S^{8} ) in 1:1</td>
<td>( S^{7}, S^{8} ) &amp; ( S^{9} )</td>
</tr>
<tr>
<td>C: Anti( S^{7} )</td>
<td>( B. campestris ) ( S^{7} ), ( B. oleracea ) ( S^{22} ) &amp; ( S^{39} )</td>
</tr>
<tr>
<td>D: Diluted Anti( S^{22} ) in 1:2*</td>
<td>( B. oleracea ) ( S^{22}, S^{39} ) &amp; ( S^{7} )</td>
</tr>
<tr>
<td>E: Absorbed Anti( S^{22} ) with ( S^{7} ) in 1:1</td>
<td>Same as D</td>
</tr>
<tr>
<td>F: Absorbed Anti( S^{22} ) with ( S^{7} ) in 1:2</td>
<td>Same as D</td>
</tr>
</tbody>
</table>

* This dilution was made to compare Figure 3 F.
S-GLYCOPROTEINS IN Brassica

and Anti\textsuperscript{\textregistered}S\textsuperscript{22} (Figure 2D–F). In every case a precipitin line was formed to the 18th gel piece, which corresponded to the \textsuperscript{\textregistered}S\textsuperscript{7} glycoprotein band. Thus, antisera are reactive with homologous and also heterologous S-glycoproteins of these two species.

**Double diffusion:** Anti\textsuperscript{\textregistered}S\textsuperscript{7} was reacted against the stigma homogenates of \textsuperscript{\textregistered}S\textsuperscript{7}, \textsuperscript{\textregistered}S\textsuperscript{8} and \textsuperscript{\textregistered}S\textsuperscript{6}. A strong precipitin line was observed in each homologous combination and it fused with a weak precipitin line in heterologous combinations (Figure 3A). Further, this line formed a spur against heterologous antigens. When the Anti\textsuperscript{\textregistered}S\textsuperscript{7} was absorbed with the stigma homogenate of \textsuperscript{\textregistered}S\textsuperscript{8}, a precipitin line was formed in the homologous combination but not in the heterologous combinations under both absorbing ratios of 1:1 and 1:2 (Figure 3B). To test the interspecific relations, this same Anti\textsuperscript{\textregistered}S\textsuperscript{7} was reacted against stigma homogenates of \textsuperscript{\textregistered}S\textsuperscript{22}, \textsuperscript{\textregistered}S\textsuperscript{39} and \textsuperscript{\textregistered}S\textsuperscript{7}. A fused precipitin line appeared against all three S-glycoproteins with a spur against the heterologous combinations (Figure 3C). Besides this fused line, two or more precipitin lines formed against \textsuperscript{\textregistered}S\textsuperscript{39}. One of them fused with the line of \textsuperscript{\textregistered}S\textsuperscript{7} but the others did not. In all the heterologous combinations so far investigated, the Anti\textsuperscript{\textregistered}S\textsuperscript{7} made the fused precipitin line and a spur. The intensity of the fused line differed among the different S-genotypes.

Anti\textsuperscript{\textregistered}S\textsuperscript{22} made a thick precipitin line in the homologous combination under the present experimental conditions (Figure 3D) that seemed to involve two lines. The strongest line fused with a line to \textsuperscript{\textregistered}S\textsuperscript{39} stigma homogenate and formed a spur against it. No corresponding precipitin line was observed against \textsuperscript{\textregistered}S\textsuperscript{7} stigma homogenate. A weak line fused to the inside of these lines against every combination. When the inciting \textsuperscript{\textregistered}S\textsuperscript{22} antigen (purified) was used, this inside line was not formed. Therefore, we considered that certain molecules having a structure partly similar to \textsuperscript{\textregistered}S\textsuperscript{22} glycoprotein are present in the crude stigma homogenates and thus the inner fused line is formed. When this Anti\textsuperscript{\textregistered}S\textsuperscript{22} was absorbed with \textsuperscript{\textregistered}S\textsuperscript{7} stigma homogenate in a 1:1 ratio, the inner fused line disappeared, but the outer fused line against \textsuperscript{\textregistered}S\textsuperscript{39} and \textsuperscript{\textregistered}S\textsuperscript{22} was still observed (Figure 3E). Figure 3E shows clearly that the precipitin line against \textsuperscript{\textregistered}S\textsuperscript{22} contain two lines. When the absorbing ratio was increased to 1:2, a single precipitin line was found only in the homologous \textsuperscript{\textregistered}S\textsuperscript{22} (Figure 3F). In the interspecific comparison, a precipitin line was also formed to the stigma homogenates of B. campestris, and a spur was observed when the homologous stigma homogenate was provided in parallel.

Anti\textsuperscript{\textregistered}S\textsuperscript{39} gave a precipitin line to the homologous and heterologous combinations, and the lines fused with each other. No spur was formed, however.

The double diffusion results for crude stigma homogenate are summarized in Table 1. The unabsorbed antisera made precipitin line(s) against every S allele genotype. With absorption in a 1:1 ratio for Anti\textsuperscript{\textregistered}S\textsuperscript{22} and Anti\textsuperscript{\textregistered}S\textsuperscript{7} genotype differences were found. Both antisera made precipitin lines with \textsuperscript{\textregistered}S\textsuperscript{22}, \textsuperscript{\textregistered}S\textsuperscript{39}, \textsuperscript{\textregistered}S\textsuperscript{18} and \textsuperscript{\textregistered}S\textsuperscript{7} but not with \textsuperscript{\textregistered}S\textsuperscript{5}, \textsuperscript{\textregistered}S\textsuperscript{2}, \textsuperscript{\textregistered}S\textsuperscript{8} and \textsuperscript{\textregistered}S\textsuperscript{9}. When the absorbing ratio was 1:2, these antisera did not cross-react with the heterologous S-glycoproteins except between \textsuperscript{\textregistered}S\textsuperscript{39} stigma homogenate and Anti\textsuperscript{\textregistered}S\textsuperscript{7} absorbed with \textsuperscript{\textregistered}S\textsuperscript{8} or \textsuperscript{\textregistered}S\textsuperscript{9} stigma homogenate. Absorbed Anti\textsuperscript{\textregistered}S\textsuperscript{39} never made any precipitin lines against heterologous S-glycoproteins.
TABLE 1

Precipitin line formation between absorbed antisera and test antigens in double diffusion

<table>
<thead>
<tr>
<th>Antigen: Stigma homogenate</th>
<th>Anti $S_1$</th>
<th>$A^S_2$</th>
<th>$A^S_3$</th>
<th>Anti $S_1$</th>
<th>$A^S_2$</th>
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<th>Anti $S_1$</th>
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<th>$A^S_3$</th>
<th>Anti $S_1$</th>
<th>$A^S_2$</th>
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<tbody>
<tr>
<td>$S_{22}$</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>$S_{29}$</td>
<td>+</td>
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<td>+</td>
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<td>$S_i$</td>
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<tr>
<td>$S_{13}$</td>
<td>+</td>
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<td>$S_r$</td>
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Precipitin line was formed +, and not formed -. 
* This presentation means Anti$S_{22}$ was absorbed with $S_{29}$ stigma homogenate in the ratio of 1:1 or 1:2 (v/v).

When the three antisera were tested against the homogenates of ovary, style and anther, a weak and fused precipitin line appeared against every one of them. The weak precipitin line for these tissues disappeared in the reactions with absorbed antisera.

DISCUSSION

Anti$S_i$ made a precipitin arc in immunoelectrophoresis of homologous stigma homogenates and also against the $S_i$ glycoprotein band of the electrofocused stigma homogenate. This suggests homogeneity of $S_i$ glycoprotein. Anti$S_{22}$ also gave a thick precipitin line at its $S_i$-glycoprotein position on gels with electrofocused homologous stigma homogenate. Immunoelectrophoresis of $S_{22}$ stigma homogenate gave two coalesced lines. These two lines may correspond to the immunized two glycoproteins that have the same PI but different molecular weight (NISHIO and HINATA 1982). Since these two lines were also observed in the immunoelectrophoresis of $S_{22}$ stigma homogenate against Anti$S_{29}$ as well as against Anti$S_i$, the two glycoproteins seemed to have similar molecular structure. We have considered the possible heterogeneity of $S_{22}$ glycoprotein. (In connection with the heterogeneity of $S$ alleles, the $S_{13}$ allele may be taken as another example. We observed three S-glycoproteins with different PI's, and all three S-glycoproteins were cross reactive with Anti$S_{22}$.) Two alternative explanations are being considered. One is that the presence of more than one S-glycoprotein is caused by the cleavage of polypeptide chains during the chemical processing. The other is that the $S$ locus is sometimes duplicated and produces more than one S-glycoprotein. In support of the $S$-locus duplication explanation, D. H. WALLACE (personal communication) has pollination and seed set data showing that an inbred strain that would never breed true for self-incompatibility was always simultaneously active for the two alleles $S_{22}$ and $S_i$. Relative activities of these duplicated alleles continued to vary among the sibling plots of suc-
cessive generations of inbreeding. For the S alleles studied here, there is no
evidence favoring such locus duplication vs. molecule cleavage.

Absorbed Anti'S' and Anti'S'' showed specific reactions to the homologous
stigma homogenates in the double diffusion tests. The existence of immunological
specificity for apparently purified S-glycoproteins is supported by evidence that
they differ in amino acid composition (Nishio and Hinata 1982). It can be in-
ferred that the apparently purified S-glycoproteins defined by their pI correspond
to the immunologically detected S-specific substances found in crude stigma ex-
tracts by many researchers (Nasrallah and Wallace 1967, Nasrallah, Bar-
ber and Wallace 1970, Sedgley 1974, Kucera and Polak 1975, Sareen and
Kakar 1977).

For Anti'S''0, a single precipitin line was observed in immunoelectrophoresis
and also in immunodiffusion of electrofocused stigma homogenate. This appar-
etly purified S-glycoprotein did not, however, show any genotypic specificity
in double diffusion tests. In spite of 'S'' being a dominant S allele, no antibodies
seemed to be produced against the specific site of this S-glycoprotein. This is
probably because 50% fewer stigmas were used in preparing the antigen.

Antisera made from a purified S-glycoprotein precipitated not only with its
homologous S-glycoprotein but also with the other S-glycoproteins. This result
suggests that an S-glycoprotein has several determinant structures that can give
immunological reactions. Of these determinant molecular structures, one is spe-
cific to each S-glycoprotein and another is common to all S-glycoproteins. Every
S-glycoprotein is reactive with concanavalin A, which suggests the possibility of
the presence of common structures between them, at least in carbohydrate chains
(Nishio and Hinata 1978). Nasrallah (1979) reported the lack of cross-
reactions between S-specific proteins of stigma using antisera that was made from
crude stigma homogenate and absorbed with crude stigma homogenate of other
S-allele genotypes. It may be that when the common structures of S' and S''
glycoproteins are also common to S' glycoprotein, the Anti-S' absorbed with S'
stigma homogenate cannot make precipitin lines against the S' glycoprotein.
The double diffusion results in Table 1 may show several cases of this. The data
indicate that the common structure is also heterogeneous among different S-
glycoproteins and that the common structure includes a part common to all
S-glycoproteins plus another part shared with a few S-glycoproteins.

In mammalian histocompatibility, the major histocompatibility complex
(MHC) is believed to be concerned with recognition reactions. The H-2 locus
(MHC) in mouse is a gene complex and its antigens have private and public
parts in their structures (Klein 1971, 1974). For self-incompatibility of higher
plants, the S-glycoproteins are considered to be the most probable substances
used in the recognition reactions between stigma and pollen. The multiple S
alleles of an S locus are well known, and the S locus is considered to be a gene
complex having distinct specificity and activity parts (Lewis 1965). The specific
and common structures of the apparently purified S-glycoproteins identified in
this study are comparable to the private and public parts of the MHC antigens.
We suppose the loci concerned with the major recognition reactions of plants and
animals may have similarities of structure.
The common structure of the S-glycoproteins in *B. oleracea* was also common, at least in a part, to those in *B. campestris*. The two species have different chromosome numbers but are related closely enough that they can be crossed with each other by using embryo culture; artificial amphidiploids have frequently been produced (Tsunoda, Hinata and Gomez-Campo 1980). Differentiation of these species apparently has not advanced so far as to change the common structure of their S-glycoproteins.

The double diffusion experiment indicates that the style, ovary and anther contain some substances with a structure similar to a part of the S-glycoprotein molecules. This structure is considered to be a part of the common structure of the S-glycoproteins because the precipitin line disappeared when absorbed antiserum was used. No immunological S-specificity was detected in the tested buffer homogenate of these tissues. Although further studies, particularly with pollen, are needed to determine whether S-glycoproteins are really absent or are present but in an insoluble state, the present result suggests that S-specific substances in pollen may have different molecular structures from S-glycoproteins in stigma.

The cross-reactions between different S-glycoproteins provide helpful information for practical hybrid breeding, where quick determination of individual S-genotype is necessary. We have developed a rapid method for analyzing S-glycoproteins using cellulose-acetate electrofocusing (Nishio and Hinata 1980, Hinata and Nishio 1981). One problem with this procedure is in identifying the S-glycoprotein band among the many glycoproteins in the stigma. The different S-glycoproteins might be identified by their cross-reactions with an available antiserum against a purified S-glycoprotein. In the first inbred generation of a heterozygous plant, two homozygous genotypes might then be separated by the difference between the reactive S-glycoproteins after electrofocusing.

We express sincere thanks to M. Katsuno and S. Hoshi, Lab. of Animal Hygiene, Tohoku University for their valuable advice and for providing facilities for making antibodies. Thanks are also due to Y. Fujio, Lab. of Breeding of Aquatic Animals and Plants, for reading manuscripts; and to S. Tsunoda, Lab. of Plant Breeding, for suggestions and encouragement. Particular thanks are due to D. H. Wallace, Dept. of Plant Breeding and Biometry, Cornell University, U.S. for providing his data and for revising of manuscripts.

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**LITERATURE CITED**


S-GLYCOPROTEINS IN BRASSICA


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