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Comparison of *S*-alleles and S-glycoproteins between two wild populations of *Brassica campestris* in Turkey and Japan

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Summary. The number of identical *S*-alleles between two wild populations of *B. campestris*, one in Turkey, the other in Japan, that have been independent of one another for a long time was investigated. Diallel pollination tests between 38 *S*-allele homozygotes, i.e., 16 *S*-allele homozygotes from Turkey and 22 from Japan, revealed that these were 29 different *S*-alleles only 4 common ones. These *S*-alleles were differentiated by the iso-electric focusing (IEF) analysis of *S*-locus glycoproteins (SLGs) stained with an antiserum against SLG^8. All identical *S*-alleles had the major SLG band at the same pI value without exception, even though they were collected from different populations. However, the number of minor bands of SLGs varied between the two populations; the *S*-alleles in Balcesme had generally fewer minor bands than those in Oguni. The 29 independent *S*-alleles were numbered from S^{21} to S^{49} according to the pI value of the major SLG band. The major bands whose pI values were 7.5–8.5 were most common. Blot-hybridization patterns of genomic DNA hybridized with SLG^8 cDNA were not always the same among the strains of identical *S*-alleles obtained from different populations. Because about 20% of the *S*-alleles were shared between the two populations, it can be inferred that more than hundreds of *S*-alleles have been accumulated by mutation in *B. campestris* throughout the world.

Key words: *S*-allele – Glycoprotein – SLG – Self-incompatibility – *Brassica campestris*

Introduction

The self-incompatibility system of *Brassica* species is genetically explained by assuming sporophytic multiple *S*-alleles at a single locus (Bateman 1955). Many different *S*-alleles have been identified in cultivated *Brassica oleracea* (Ockendon 1974, 1982), and a few of them have been characterized by their S-glycoproteins and DNA (Nasrallah et al. 1987; Chen and Nasrallah 1990; Umbach et al. 1990; Trick and Flavell 1989; Gaude et al. 1991). However, few studies have been carried out on the wild *S*-alleles, i.e., those that have not been subjected to selection by man, except for the analysis of a very few alleles in Japan (Takayama et al. 1987; Isogai et al. 1987). In previous reports, we have isolated a number of *S*-alleles from two wild populations, from Oguni, Japan and Balcesme, Turkey, respectively (Nou et al. 1991, 1993), and these were characterized by their S-glycoproteins.

Since several proteins and genes that relate to *S*-alleles have been analyzed under different names up to date, we decided to use the terminology of Lalonde et al. (1989), Stein et al. (1991), and Hinata et al. (1993). The S-glycoproteins and *S*-locus glycoproteins are indicated by SLG (gothics) at the protein level and *SLG*(italics) at the DNA level, respectively.

In the study presented here we examined the identity of *S*-alleles and SLGs between these two populations of *B. campestris* to consider the diversification of *S*-alleles. Thirty different *S*-alleles were characterized by their SLGs at the protein level.

Materials and methods

Plants

S-allele homozygotes in *B. campestris* were isolated from two wild populations in Oguni, Japan and Balcesme, Turkey (Nou et al. 1991, 1993). Fifteen *S*-homozygous strains from the Oguni population (Oguni-new) and 16 from Balcesme were used. In addition, 7 *S*-allele homozygotes (S^5, S^7, S^8, S^9, S^{10}, S^{12}, S^{13}) isolated 15 years ago from a population in Oguni were also used in this study (Oguni-old). The locale where these 7 *S*-alleles were collected was about 1 km away from the new location, across a small river. The area for collection was changed because the old area had been converted to a paddy field. The S^8-, S^9-, and S^{12}-allele homozygotes had been partly characterized in earlier investigations (Takayama et al. 1986a, b, 1987; Isogai et al. 1987). The original populations of these homozygotes were denoted by JO, S, and TB,

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Table 1. Results of diallel pollination between 38 *S*-homozygotes isolated from the Balcesme, Turkey area (TB) and Oguni, Japan area (JO indicates newly isolated homozygotes and JOS, known ones). Twenty-nine *S*-alleles were distinguished and numbered from S^{21} to S^{49} as shown in the far-right colume

Male	JO							TB											
	7	7	9	11	12	23	38	20	22	24	26	26	42	45	45	82	82	83	87
Female	5	6	14	12	2	3	7	2	7	8	6	10	5	3	9	3	9	5	9
JO 7· 5	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7· 6	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9·14	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11·12	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12· 2	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23· 3	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
38· 7	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
TB 20· 2	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
22· 7	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
24· 8	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
26· 6	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
26·10	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
42· 5	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	±	+
45· 3	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
45· 9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
82· 3	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	−	+	+	+
82· 9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
83· 5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	±
87· 9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
JO S· 8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S·10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2·10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S· 7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9· 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S· 5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11· 6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S· 9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1· 6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2· 5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TB 53·10	+	+	+	+	+	+	+	±	+	+	+	+	+	+	+	±	+	+	+
JO 51· 6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
33· 5	+	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+
TB 46·12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
JO S·13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TB 25·13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
JO 1· 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TB 27· 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
JO S·12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+, More than 20 pollen tubes penetrating; ±, 5–20 pollen tubes penetrating; −, less than 5 or no pollen tubes penetrating

which correspond to Oguni-new, Oguni-old, and Balcesme, respectively.

The experimental methods for diallel pollination, the production of the polyclonal antibody, the electrophoresis of stigma proteins, and immunoblotting have been detailed in a previous report (Nou et al. 1991).

Estimation of the number of SLG minor bands

After differentiation of the stigma proteins by isoelectric focusing (IEF), the proteins were transferred to a nylon membrane, and the SLGs were stained with an antiserum against SLG[8]. As an alternative treatment the proteins on the gel plate were stained with Coomassie brilliant blue (CBB). The membrane or gel plate was scanned with the maximum absorbing wavelength (IEF immunoblotting: 550 nm; CBB staining: 600 nm) by a Dual-wavelength flying-spot Scanner (Shimadzu Co., Japan). In immunoblotting, when the peak value of a minor band was higher than a relative value (75%, 50% and 25% were used as critical values) to that of the major SLG band, the minor band was counted as one band. The number of minor bands was counted for each *S*-allele homozygote. In CBB staining, the number of minor bands of SLGs was estimated by comparing the band pattern of plate and the scanned data, because the background absorbance was variable according to the pI point.

Table 1 (continued)

JO S 8	S 10	2 10	S 7	9 1	S 5	11 6	S 9	1 6	2 5	TB 53 10	JO 51 6	33 5	TB 46 12	JO S 13	TB 25 13	JO 1 1	TB 27 1	JO S 12	*S*-alleles
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{29}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{41}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{36}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{45}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{44}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{39}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{25}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{33}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{38}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{32}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{47}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{30}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{31}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{48}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{37}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{49}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{21}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{40}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{23}
−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{43}
+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{27}
+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{26}
+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	S^{34}
+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	S^{28}
+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	S^{46}
+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+		+	+	
+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	S^{22}
+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	S^{35}
+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	S^{42}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	S^{24}
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	

+, More than 20 pollen tubes penetrating; ±, 5–20 pollen tubes penetrating; −, less than 5 or no pollen tubes penetrating

Genomic DNA isolation and DNA gel-blot analysis

Genomic DNA was extracted from 2 g of fresh leaf tissue by the cetyltrimethylammonium bromide (CTAB) method (Rogers and Bendich 1985) and digested with *Hin*dIII and *Eco*RI. Following electrophoresis on a 0.8% (w/v) agarose gel, the DNA was transferred to a Gene-Screen Plus membrane (Du Pont, USA). Non-radioactive labeling of DNA with the DIG-ELISA DNA labeling and detection kit (Boehringer Mannheim, FRG), hybridization, and detection were performed using cDNA for SLG^8 (Isogai et al. 1991) as a probe according to the manufacturer's instructions. The membrane was prehybridized in a solution containing 5× SSC, 0.1% sodium *N*-lauroyl sarcosinate, 0.02% SDS and 0.5% blocking reagent (I-Light, Tropix, USA) at 65° C overnight. Subsequently, it was hybridized with a solution containing the probe under the same conditions, and washed twice for 5 min with 0.5× SSC plus 0.1% SDS at 65° C. The hybridized S^8-cDNA probe was then allowed to react with antiserum against digoxigenin-bound alkaline phosphatase. The bound antibodies were detected chemiluminescently with AMPPD (Tropix, USA) as a substrate on X-ray film (Kodak, USA).

Results

S-allele identity

Tests of identity among 15 *S*-alleles from the Oguni-new population (JO) and 7 *S*-alleles isolated from the Oguni-old population (S^5–S^{13}) revealed that 4 *S*-alleles were

common between the two spots and 18 *S*-alleles were independent (Table 1). The 4 common *S*-alleles were S^{24} (JO1-1 and S-12), S^{26} (JO2-10 and S-7), S^{28} (JO11-6 and S-9), and S^{34} (JO9-1 and S-5).

Similar tests of identity were carried out on a total of 38 *S*-alleles, which consisted of 22 *S*-alleles from the Oguni population (Oguni-new plus Oguni-old) and 16 from that of Balcesme. Four *S*-alleles were common between the two populations, and 29 independent *S*-alleles were determined (Table 1). The pollination test was not completed for the remaining 1 *S*-allele (denoted as S^{99}), but this *S*-allele was considered to be independent of the others because of its distinct band pattern of S-glycoproteins. The common *S*-alleles between the Japanese and Turkish populations were S^{22} (JO51-6 and TB53-10), S^{24} (JO1-1, S-12 and TB27-1), S^{35} (JO33-5 and TB46-12), and S^{42} (S-13 and TB25-13).

Table 2 shows the frequency distribution of 30 independent *S*-alleles shown by the 76 *S*-allele homozygotes isolated to date. Of these 30 independent *S*-alleles, 10 *S*-alleles were found once, 9 *S*-alleles twice, 4 *S*-alleles three times, 3 *S*-alleles, four times, 2 *S*-alleles five times; S^{32} and S^{35} were the most frequently observed (seven times). According to our data, S^{32} was codominant to S^{24}, S^{30} and S^{38}, dominant to S^{35}; and S^{35} was codominant to S^{23}, dominant to S^{38}, and recessive to S^{32}.

Table 2. Frequency of the isolated 30 *S*-alleles (S^{21}–S^{49}) found in respective populations, i.e., Balcesme, Turkey (TB) and Oguni, Japan (JO), as shown by plant number

S-allele	*S*-homozygotes
S^{29}	JO:7-5
S^{25}	JO:38-7
S^{43}	JO:S-8
S^{27}	JO:S-10
S^{33}	TB:20-2
S^{47}	TB:26-6
S^{31}	TB:42-5
S^{48}	TB:45-3
S^{40}	TB:83-5
S^{23}	TB:87-9
S^{41}	JO:7-6, 38-6
S^{36}	JO:9-14, 42-7
S^{34}	JO:9-1, S-5
S^{42}	JO:S-13, TB:25-13
S^{22}	JO:51-6, TB:53-10
S^{30}	TB:26-10, 32-14
S^{37}	TB:45-9, 79-1
S^{49}	TB:42-13, 82-3
S^{21}	TB:50-9, 82-9
S^{44}	JO:12-2, 36-8, 39-9
S^{39}	JO:23-3, 51-5, 54-6
S^{28}	JO:11-6, 12-1, S-9
S^{38}	JO:22-7, 24-6, 51-10
S^{24}	JO:1-1, 57-13, S-12, TB:27-1
S^{46}	JO:1-6, 2-5, 19-2, 37-7
S^{99}[a]	JO:17-7, 18-13, 44-6, 58-5
S^{45}	JO:11-12, 19-5, 23-11, 25-9, 49-8
S^{26}	JO:2-1, 25-10, 44-5, S-7
S^{32}	TB:7-7, 16-13, 24-8, 27-11, 32-11, 50-11, 55-3
S^{35}	JO:33-5, TB:6-12, 7-12, 46-12, 51-7, 52-7, 87-2

[a] S^{99} is another *S*-allele whose pollination test has not completed but which is assumed to be different from the others on the basis of its SLG band pattern

It is not conclusive whether the frequent *S*-alleles are recessive or not, because our survey of dominant-recessive relations among other *S*-alleles has not yet been completed.

Relationships between S-alleles and SLG

The SLGs of these 38 *S*-homozygotes were differentiated by IEF analysis following staining with the antiserum against SLG^8 (Fig. 1). The pI values of the major SLGs were seen to be similar between identical *S*-alleles, even if the latter were taken from different populations.

SLGs were compared among 29 independent *S*-alleles by arranging them according to the pI value of their major bands (Fig. 2). The pI values of 9 *S*-alleles, S^{21}–S^{29}, were less than 7.0, and 2 *S*-alleles (S^{48}, S^{49}) had very high pI values; the remaining 18 *S*-alleles had pI values that fell between 7.0 and 8.5. In some of these 18 *S*-alleles, the pI values of the major bands were very close to one another and could be grouped together: the pI values for S^{30}, S^{31}, S^{32}, and S^{33} were about pI 7.5; those of S^{36}, S^{37}, S^{38}, S^{39}, and S^{40} were about pI 8.0; and those for S^{42}, S^{43}, S^{44}, and S^{45} were about pI 8.4. Repeated pollination tests confirmed the independence of these *S*-alleles.

The number of minor bands of SLGs that stained with SLG^8-antiserum was compared among *S*-alleles homozygotes by scanning densitometric analysis (Table 3). When minor bands with a peak value of more than 75% of that of the respective major band was counted, *S*-alleles with one to four minor bands were observed to be frequent in the Balcesme population, while those with four to seven minor bands were frequent in the Oguni population. When the critical value was 50%, *S*-alleles in the Balcesme population had one to five minor bands, while those in the Oguni population had a number of bands that varied up to ten. A similar tendency was observed when a critical value of 25% was adopted. In CBB staining, the mode of the number of stainable bands was 2 in the Balcesme population but 3 in the Oguni population, though the difference was not significant.

The different *S*-alleles were examined by gel-blot analysis of genomic DNA from S^{42}, S^{43}, S^{44}, and S^{45}, whose SLGs had pI values close to 8.4 (Fig. 3a). An SLG^8 cDNA was used as probe. When the genomic DNA was digested with *Hin*dIII, the patterns of the gel-blot clearly differentiated these 4 *S*-allele homozygotes, indicating their difference on the genomic constitution relating SLG (Fig. 3b). In Fig. 3b, however, the two bands marked by asterisks were common to all *S*-alleles, those marked by an open circle were common to S^{42}, S^{44}, and S^{45}, and those marked by a closed circle were common to S^{42}, S^{43}, and S^{44}. These *S*-alleles had at least in part, similar components of genomic DNA. Similar gel-blot analysis after *Eco*RI digestion also distinguished 15 different S-homozygotes.

Figure 4 shows another gel-blot analysis after *Eco*RI digestion which differentiated 3 strains homozygous for the same S^{24} allele but that originated from different populations. Clear differences in the banding pattern were observed between 1 strain of Balcesme, TB27-1,

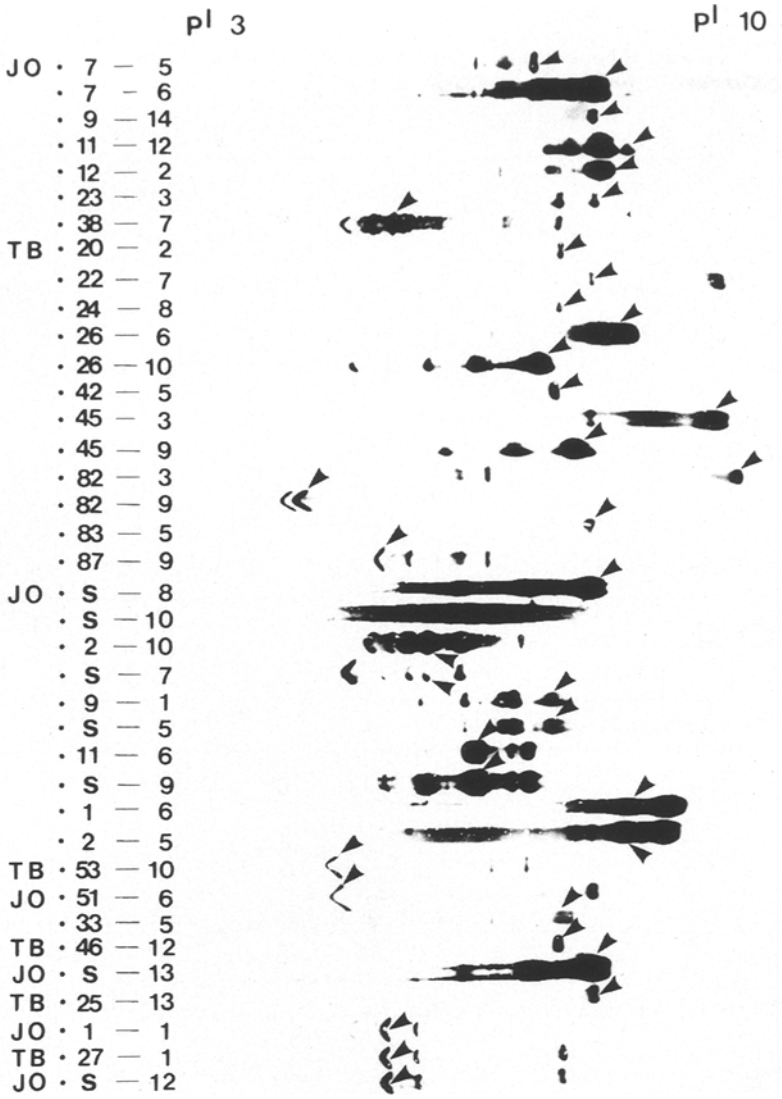

Fig. 1. IEF-immunoblot profiles stained with SLG[8] antiserum for 38 *S*-homozygotes isolated from Oguni, Japan and Balcesme, Turkey populations, indicating that the major SLG bands (*arrowheads*) in *S*-homozygotes with identical *S*-alleles (see Table 1) have the same pI value

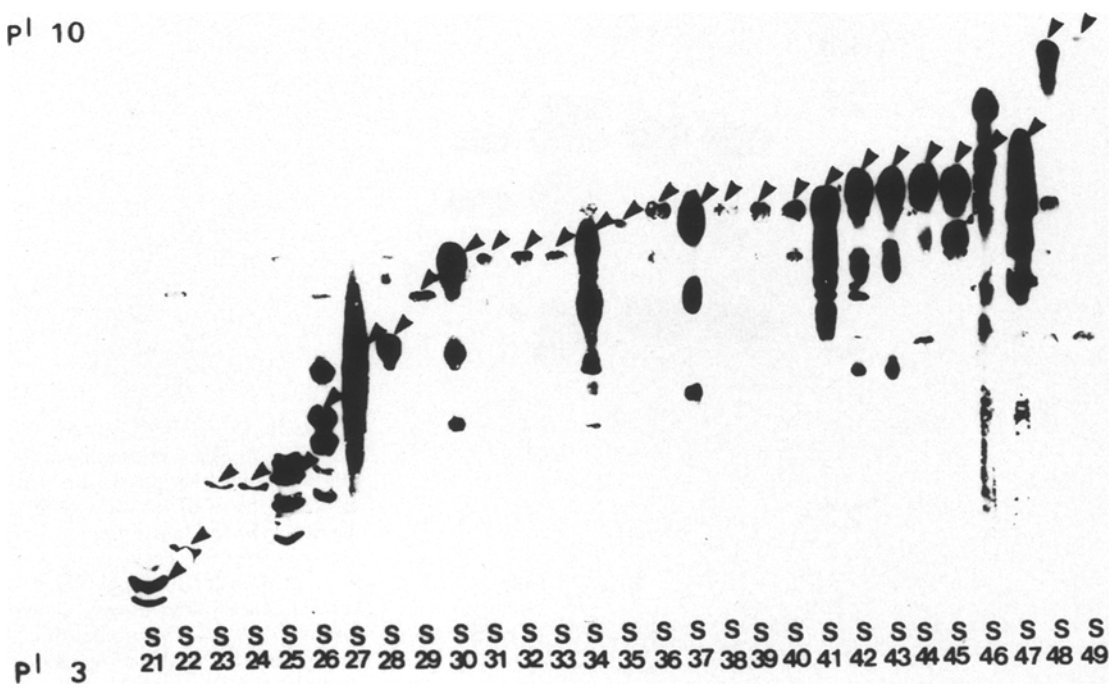

Fig. 2. IEF-immunoblot profiles of SLGs for 29 different *S*-alleles isolated in *B. campestris* to date arranged according to the pI value of the major SLG band. *Arrowheads* indicate the major SLG band

Table 3. Comparison of the number of SLG minor bands between S-homozygotes of the Balcesme and Oguni areas when counted on IEF immunoblotting membranes (a) and CBB-stained ones (b)

a

Number of minor bands[a]	Number of *S*-allele homozygotes					
	Critical value 25%		Critical value 50%		Critical value 75%	
	Balcesme	Oguni	Balcesme	Oguni	Balcesme	Oguni
1	2	0	4	2	8	3
2	3	2	4	3	6	12
3	1	1	6	3	2	2
4	7	6	2	4	1	2
5	1	2	1	3	0	0
6	1	3	0	2	0	1
7	2	3	0	0	0	1
8	0	0	0	2	0	0
9	0	1	0	1	0	0
>10	0	3	0	1	0	0
Total	17	21	17	21	17	21

b

Number of stainable bands	Number of *S*-allele homozygotes	
	Balcesme	Oguni
1	0	1
2	18	12
3	12	23
4	5	4
Total	35	40

[a] A minor bands was counted as such when its absorbing value was greater than a critical value, 75%, 50% and 25%, of that of the respective major band

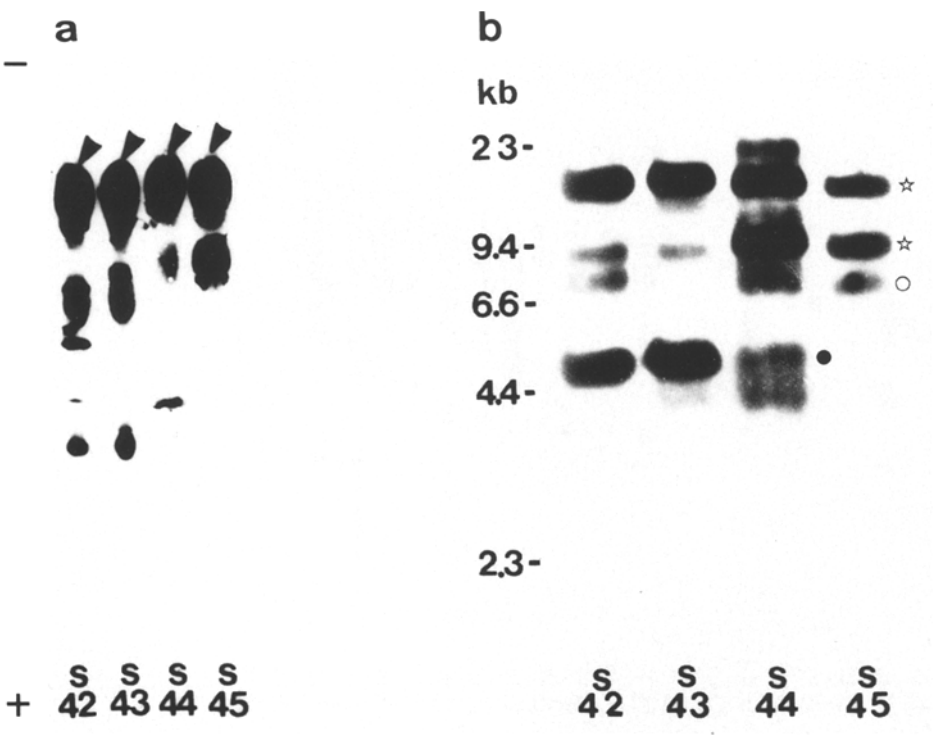

Fig. 3a, b. Protein IEF-gel-blot stained with SLG[8] antiserum (**a**) and DNA gel-blot hybridized with the cDNA clone of SLG[8] after *Hin*dIII digestion (**b**) for four different *S*-allele homozygotes that have very similar pI values for the SLG major band. Clear differences were shown by the DNA gel-blot profile, though some common bands (☆, ○, ●) were also observed

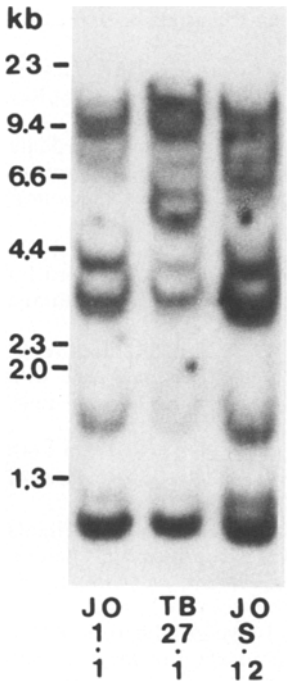

Fig. 4. DNA gel-blot analysis for three S^{24} homozygotes of different origins (JO1-1, Oguni-new; TB27-1, Balcesme; and JOS-12, Oguni-old), indicating polymorphism of the genomic DNA that hybridizes with the SLG^{8}-cDNA probe. The DNA was digested with *Eco*RI

and 2 of Oguni, JO1-1 and JOS-12. The band patterns of the latter 2 of Oguni were rather similar to one another, except for one band at about 5 kb.

Discussion

Number of S-alleles

In our comparison of the two locations of the Oguni population, 4 *S*-alleles were identical between the 15 *S*-alleles from Oguni-new and 7 *S*-alleles from Oguni-old. In the comparison between 22 *S*-alleles of Japan and 16 *S*-alleles of Turkey, 4 *S*-alleles were identical. If we assume that a population contains N number of *S*-alleles, with p ($0<p<1$) being the rate of *S*-alleles shared by another population and having the same value in every population, the total number (NT) of *S*-alleles of the whole populations could be calculated as: $NT=S\{1-(1-p)^{n+1}\}/p$, where n is the number of populations. If n is infinity, $NT=S/p$. NT was calculated for several values of S and p as shown in Table 4. In the Oguni populations, if the number of *S*-alleles in each sampling spot (N) is assumed to be 20, and the p values are 0.20 and 0.75, NT would be 100 and 27, respectively. We may be able to assume that the total number of *S*-alleles in this area are 27–100.

Table 4. Total number of *S*-alleles (NT) calculated by a formula $NT=N/p$, where N is the number of *S*-alleles in each population and p is the rate of common *S*-alleles between two populations

N	NT				
	p=0.20	0.25	0.33	0.50	0.75
20	100	80	60	40	27
30	150	120	90	60	40
40	200	160	120	80	53
50	250	200	150	100	67
60	300	240	180	120	80

As for the comparison between the Japanese and Turkish populations, if the number of *S*-alleles (N) is 30, and the p values are 0.20 and 0.33, then the total *S*-alleles of *B. campestris* in the whole world (NT) would be 150 and 90, respectively. If N is 50, and p is 0.25, NT would be 200. Although this calculation is based on very little data combined with hypothetical assumptions, we may be able to assume at this moment that variation in the *S*-locus consists of more than 100 *S*-alleles.

It is probable that *S*-alleles do not have any differential fitness. They are most likely transferred to the progeny through heterozygous plants. Mutated *S*-alleles may have been accumulated in each population, and some of them may have been eliminted only by random drift. In this way, different types of mutated *S*-alleles have accumulated in each population, and the total number of the mutated *S*-alleles would have to be more than hundreds. One problem is how to explain such variation in *S*-alleles at a single locus in terms of DNA and protein architecture.

S-alleles S^{32} and S^{35} were the ones most frequently observed and most of these were found in the Balcesme population; the next most frequent *S*-alleles, such as S^{24}, S^{46}, S^{99}, S^{45}, and S^{26}, were found in the Oguni population. Ockendon (1974) suggested that recessive *S*-alleles were liable to be accumulated in a population. Studies on the dominant-recessive relations among all *S*-alleles are in progress.

Characterization of S-alleles by SLG

We were able to characterize *S*-alleles by the pI values of the major SLGs, even though the *S*-genes were collected from different places. This strongly suggests that the SLGs may play an important role in recognition events.

SDS-PAGE analysis on the SLGs of these *S*-alleles within each group revealed a slight difference in band stainability, probably indicating a difference in the number of polysaccharide residues (Nou et al. 1991, 1993). However, it was difficult to ascribe the differences in *S*-alleles to the SDS-PAGE banding pattern.

Most of the *S*-alleles contained more than one minor band of SLGs in the IEF-immunoblot analysis. These bands were inherited in correlation with *S*-alleles as well as with the major SLG band, and non-specific immunostaining artifacts could be excluded (Nou et al. 1991, 1993). As discussed in these earlier reports two possibilities can be considered. First, S-glycoproteins are modified during or after translation, second, these plural bands are functionally different products of several sequences of genomic DNA that have homology to SLGs (Nou et al. 1993).

The presence of minor bands may recall a study on NS-glycoproteins, which are highly homologous to SLGs in terms of amino acid sequences but whose locus

is different from that of *SLGs* (Watanabe et al. 1992). It was pointed out that the NS-glycoproteins showed ladder-like figures in IEF-immunoblot analysis with an antiserum against NS^1-glycoprotein. Although why they produce so many proteins has not yet been analyzed, a very similar mechanism may be assumed to work upon the protein configuration of SLGs and NS-glycoproteins.

S-alleles in the two populations differ in the number of their minor bands. One possibility is that the number of minor bands is determined by the difference in genetic background. Elucidation of the mechanism and role of the minor bands may provide us with a clue to understanding the recognition reactions of self-incompatibility.

Southern analyses of genomic DNA probed with a cDNA clone of *SLG* revealed that there are multiple bands of hybridization when restriction enzymes of *Eco*RI and *Hin*dIII are used. In SLG^8 there is no *Eco*RI restriction site within its coding region (Dwyer et al. 1991). It could be assumed that in their nuclear genome, there are several sequences homologous to the *SLG* structural genes and that there is variation in the DNA sequences of the region flanking the *SLG* gene. Accordingly, the *S*-homozygotes having identical *S*-alleles do not always have the same pattern after Southern blotting.

So far, the numbering of *S*-alleles has been chronologically recorded depending both upon the order of isolation and the researchers involved. In the present study, we numbered *S*-alleles systematically according to the pI value of the major SLG band, since *S*-alleles were characterized by these values. Characterization of these *S* genes at the DNA level is in progress.

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References

Bateman AJ (1955) Self-incompatibility systems in angiosperms. III. Cruciferae. Heredity 9:52–68

Chen CH, Nasrallah JB (1990) A new class of *S* sequences defined by a pollen recessive self-incompatibility allele of *Brassica olevacea*. Mol Gen Genet 222:241–248

Dwyer KG, Balnet MA, Nasrallah JB, Nasrallah ME (1991) DNA sequences of self-incompatibility genes from *Brassica campestris* and *B. oleracea*: polymorphism predating speciation. Plant Mol Biol 16:481–486

Gaude T, Denoroy L, Dumas C (1991) Use of a fast protein electrophoretic purification procedure for N-terminal sequence analysis to identify *S*-locus-related proteins in stigmas of *Brassica oleracea*. Electrophoresis 12:646–653

Hinata K, Watanabe M, Toriyama K, Isogai A (1993) A review of recent studies on homomorphic self-incompatibility. Int Rev Cytol 143:257–296

Isogai A, Takayama S, Tsukamoto C, Ueda Y, Shiozawa H, Hinata K, Okazaki K, Suzuki A (1987) *S*-locus-specific glycoproteins associated with self-incompatibility in *Brassica campestris*. Plant Cell Pyhsiol 28:1279–1291

Isogai A, Yamagawa S, Watanabe M, Hinata K, Suzuki A (1991) S- and NS-glycoproteins of *Brassica campestris*. In: Third Int Congr Plant Mol Biol molecular biology of plant growth and development. Abstr no. 526

Lalonde BA, Nasrallah ME, Dwyer KG, Chen CH, Barlow B, Nasrallah JB (1989) A highly conserved *Brassica* gene with homology to the *S*-locus-specific glycoprotein structural gene. Plant Cell 1:249–258

Nasrallah JB, Kao TH, Goldberg ML, Nasrallah ME (1987) Amino acid sequence of glycoproteins encoded by three alleles of the *S*-locus of *Brassica oleracea*. Nature 326:617–619

Nou IS, Watanabe M, Isogai A, Shiozawa H, Suzuki A, Hinata K (1991) Variation of *S*-alleles and S-glycoproteins in a naturalized population of self-incompatible *Brassica campestris* L. Jpn J Genet 66:227–239

Nou IS, Watanabe M, Isuzugawa K, Isogai A, Hinata K (1993) Isolation of *S*-alleles from a wild population of *Brassica campestris* L. at Balcesme, Turkey and their characterization by S-glycoproteins. Sex Plant Reprod 6:71–78

Ockendon DJ (1974) Distribution of self-incompatibility alleles and breeding structure of open-pollinated cultivars of Brussels sprouts. Heredity 33:159–171

Ockendon DJ (1982) An *S*-allele survey of cabbage (*Brassica oleracea* var 'capitata'). Euphytica 31:325–331

Rogers SO, Bendich AJ (1985) Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol Biol 5:69

Stein JC, Howlett B, Boyes DC, Nasrallah ME, Nasrallah JB (1991) Molecular cloning of a putative receptor protein kinase gene encoded at the self-incompatibility locus of *Brassica oleracea*. Proc Natl Acad Sci USA 88:8816–8820

Takayama S, Isogai A, Tsukamoto C, Ueda Y, Hinata K, Okazaki K, Suzuki A (1986a) Isolation and some characterization of *S*-locus-specific glycoproteins associated with self-incompatibility in *Brassica campestris*. Agric Biol Chem 50:1365–1367

Takayama S, Isogai A, Tsukamoto C, Ueda Y, Hinata K, Okazaki K, Koseki K, Suzuki A (1986b) Structure of carbohydrate chains of S-glycoproteins in *Brassica campestris* associated with self-incompatibility. Agric Biol Chem 50:1673–1676

Takayama S, Isogai A, Tsukamoto C, Ueda Y, Hinata K, Okazaki K, Suzuki A (1987) Sequences of S-glycoproteins, products of the *Brassica campestris* self-incompatibility locus. Nature 326:102–105

Trick M, Flavell RB (1989) A homologous *S* genotype of *Brassica oleracea* expresses two *S*-like genes. Mol Gen Genet 218:112–117

Umbach AL, Lalonde BA, Kandasamy MK, Nasrallah JB, Nasrallah ME (1990) Immunodetection of protein glycoforms encoded by two independent genes of the self-incompatibility multigene family of *Brassica*. Plant Physiol 93:739–747

Watanabe M, Nou IS, Takayama S, Yamakawa S, Isogai A, Suzuki A, Takeuchi T, Hinata K (1992) Variations in and inheritance of NS-glycoprotein in self-incompatible *Brassica campestris* L. Plant Cell Physiol 33:343–351