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Genetics of self-compatibility in a self-incompatible wild diploid potato species *Solanum chacoense.* 2. Localization of an S locus inhibitor (Sli) gene on the potato genome using DNA markers *

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Summary

A self-compatible (SC) hybrid plant F_1-1 was obtained from a cross between a SC variant of a wild diploid potato species, *Solanum chacoense*, and a self-incompatible (SI) cultivated diploid species, *S. phureja*. The clone F_1-1 has previously been proposed to have a dominant S locus inhibitor gene (Sli) in a heterozygous condition. It was crossed as a male parent with a selected clone from a *S. stenotomum-S. phureja* population, resulting in a segregating population consisting of 116 hybrid plants. Self-compatibility was assessed by selfing each of the hybrids. Sixty-six of them were SC, while 35 were SI, showing a significant distortion from an expected Mendelian ratio of 1:1. A genetic linkage map was constructed using DNA markers to localize the Sli gene. A total of 28 RAPD and 127 RFLP markers identified 109 mapping positions on 12 linkage groups. The Sli gene was mapped at a distal end of chromosome 12. Since the S locus has been localized on chromosome 1 on the potato RFLP map, it is confirmed that the Sli gene is independent of the S locus.

Introduction

In general, diploid potatoes and their related wild tuber-bearing *Solanum* species are self-incompatible (SI); thus, controlled by the interaction of a pollen S gene with stylar S gene(s) (gametophytic self-incompatibility). However, self-compatible (SC) variants have often been described among genotypes of SI species (Cipar et al., 1964).

A SC variant was found in a wild diploid potato species *Solanum chacoense* Bitt., which was continuously selfed over seven generations (Hanneman, 1985). To investigate a genetic mechanism of self-compatibility in this variant, crossing experiments were conducted (Hosaka & Hanneman, 1998). The SC variant of *S. chacoense* was crossed with a cultivated diploid species, *S. phureja*, and the resulting F_1 hybrids were characterized through the use of a diallel mating scheme. Selfed or sib-mated F_2 families were also characterized for self-compatibility. Based on crossing behavior, it was proposed that a single dominant gene (named 'Sli') inhibited S gene expression in the pollen. Selfing of plants having Sli gene in a heterozygous condition resulted in SC and SI progeny, most likely because the pollen with and without Sli gene were both fertilized with eggs. Based on this observation, it was concluded that the Sli gene was expressed in a sporophytic fashion (Hosaka & Hanneman, 1998).

S allele-specific polymorphisms have been characterized for the mapping purpose in potato by Southern-hybridization with the cDNA clone coding for one allele of the S proteins (Gebhardt et al., 1991), or by isoelectric focusing of the stylar glycoproteins (Jacobs et al., 1995). Consequently, the potato S

* Reference to a specific brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of similar nature not mentioned.

Table 1. Primer sequences used in RAPD marker study

No.	Sequence (5′ to 3′)	Note[1]
28	TGGTCACTGG	
42	GATGACCGCC	OPC-05
43	GAACGGACTC	OPC-06
59	GGACCCAACC	OPD-02
77	ACCCGGTCAC	OPD-20
89	TTATCGCCCC	OPE-12
97	AACGGTGACC	OPE-20
99	TGCCGAGCTG	OPA-02
108	GGGTAACGCC	OPA-09
116	GACCGCTTGT	OPA-17
118	CAAACGTCGG	OPA-19
123	GGACTGGAGT	OPB-04
137	CCACAGCAGT	OPB-18
141	GAGGATCCCT	OPF-02
152	GGCTGCAGAA	OPF-13
155	GGAGTACTGG	OPF-16
165	GTGCCTAACC	OPG-06
168	CTGACGTCAC	OPG-09
172	CTCTCCGCCA	OPG-13
182	AGACGTCCAC	OPH-03
193	ACCAGGTTGG	OPH-14
198	CTGACCAGCC	OPH-19
290	GTCCACTGTG	OPM-11

[1] Primer identity given by Operon Technologies, Inc. (CA, USA).

locus has been mapped proximal to the reference marker TG24 on chromosome 1, which is similar to its chromosomal position in tomato (Tanksley & Loaiza-Figueroa, 1985). More specifically, S-RNase genes were mapped on chromosome 1 in *S. chacoense* (Rivard et al., 1996).

In this paper, self-compatibility caused by the Sli gene was investigated in a hybrid population. From the population, a genetic linkage map was constructed using RFLP and RAPD markers to localize Sli on the potato genome. Mapping results were used to test whether the Sli gene is linked to or independent of the S locus.

Materials and methods

A highly inbred line of *S. chacoense* (clonal identity of chc 525-3, SC) was crossed as a female with a cultivated diploid species, *S. phureja* (clonal identity of 1.22, SI). One clone of the F_1 progeny (clonal identity of F_1-1, SC) was crossed as a male with a selected clone (clonal identity of 93H100-1, SI) from a *S. stenotomum-S. phureja* bulk population. The hybrid family 94H89, consisting of 116 plants, was used as the mapping population. The proposed Sli genotypes were Sli^- for the chc 525-3 and F_1-1, and - - for 1.22 and 93H100-1 (Hosaka & Hanneman, 1998, and this study).

Plants were grown in pots in an insect-free screenhouse. Each plant of the mapping population was characterized for its self-compatibility. Anthers and petals were removed from flower buds one day prior to opening, and immediately, freshly collected pollen from the same plant was applied to the stigmas. Good pollen shedding was observed in all plants, indicating sufficient male fertility, although pollen stainability was not examined. Berry set was used as a mean of self-compatibility. Self-pollinations were made several times on different days, if berry set was not observed. Bulked pollen, collected from many other plants in the family, was applied to those which did not set berries to check female fertility. The female-fertile plants with no berry set after 10 self-pollinations were regarded as SI plants.

DNA isolation and detection procedures for RAPD markers were described previously (Hosaka & Hanneman, 1994). *Taq* DNA polymerase was purchased from TOYOBO Co., Ltd. (Osaka, Japan). Thermal cycling was performed using a BioOven (BioTherm Corporation, Arlington, VA, USA). Amplified DNA products were separated by electrophoresis on a 1.6% agarose gel. RAPD markers were designated as, for example, 108-570, which means a 570-bp amplified fragment obtained using decamer primer 108 (Table 1).

Total DNA for RFLP detection was extracted from young fresh leaves by a modification of the method of Doyle & Doyle (1987). DNA was precipitated with isopropanol and washed in 75% ethanol. The DNA pellet was suspended in 2 ml of TE (10 mM Tris, 1 mM EDTA) buffer with 5 μl of RNase (10 mg/ml). To remove polysaccharides (Michaels et al., 1994), 100 μl of 5 M NaCl were added to the DNA sample and then, 735 μl of 100% ethanol were mixed. After overnight incubation at 4 °C, polysaccharides were pelleted by centrifugation. DNA was ethanol-precipitated and resuspended in TE buffer. Approximately 4 μg of total DNA were digested with *Eco*RI, *Hin*dIII, *Pst*I or *Pvu*II restriction endonucleases (abbreviated as E, H, P and V, respectively), and Southern-blotted to a nylon membrane (Hybond

N^+, Amersham International plc, England) by alkaline transfer (Reed & Mann, 1985).

Probe DNA was amplified with 10.5 units of Expand ^{TM} High Fidelity (Boehringer Mannheim) in a volume of 50 μl (supplier's PCR buffer with 15 mM $MgCl_2$, 0.1 mM dNTPs, and 0.2 μM each of M13 primers 5'-GTAAAACGACGGCCAGT-3' and 5'-CAGGAAACAGCTATGAC-3'). Thermal cycling was performed using the BioOven (one cycle of 2 min at 93 °C, followed by 40 cycles of 1 sec at 96 °C, 30 sec at 55 °C and 1.5 min at 73 °C, then, terminated with one cycle of 1 min at 73 °C). PCR products were purified using SUPREC^{TM}-02 (Takara Shuzo Co., Ltd., Kyoto, Japan). Probe DNA concentration was measured by DyNA Quant^{TM} 200 (Hoefer Pharmacia Biotech Inc.) and adjusted to 10 ng/μl. Labeling of probe DNA and detection was performed using ECL direct nucleic acid labeling and detection systems (Amersham). Hybridization was carried out in rolling tubes.

The probes prefixed with 'TG' or 'CT' were tomato probes obtained from Dr. S.D. Tanksley, Cornell University, NY, USA, whose localization has been described for the tomato or potato genome (Tanksley et al., 1992). The probes prefixed with 'P' were random genomic clones from *S. phureja* 1.22 (Hosaka & Spooner, 1992).

Because of the highly heterozygous nature of the parental clones, various polymorphic DNA bands were observed in the present mapping population. In this study, only presence vs. absence of *S. chacoense*-specific bands with an expected Mendelian ratio of 1:1 was used. These markers are designated as, for example, P75E7500, which means a 7500-bp band detected on *Eco*RI digests probed with P75. Observed segregation ratios were analyzed for deviation using the x^2-test for goodness-of-fit. The skewed markers are shown by suffixing + or ++ (over-represented), and - or - - (under-represented), at significance levels of 5% or 1%, respectively. Using the program 'MAPMAKER' (Lander et al., 1987), the mapping population was treated as an F_2 backcross population and a genetic linkage map was constructed. Map distances were calculated using the Kosambi function (Kosambi, 1944).

Results

Self-compatibility was tested for a mapping population of 116 progenies from a cross 93H100-1 × F_1-1. A total of 1274 flowers were self-pollinated (Table 2). Sixty-six plants produced 348 berries, which were regarded to be SC. An average of 61% of pollinations set berries among the SC plants. However, extremely low berry setting frequencies were found in 94H89-51 (6%), 94H89-56 (4%) and 94H89-113 (10%), with each producing one berry containing 32, 129 and 15 seeds, respectively. Each berry contained an average of 114 seeds (Table 2). Extremely low seed set was found in 94H89-10 (16 seeds per berry) and 94H89-113 (15 seeds as mentioned above). The low berry setting or low number of seeds might be due to pseudo-compatibility. Seven plants (94H89-1, -21, -27, -84, -90, -91, and -99) failed to set berries with self-pollinations and bulk-pollen pollinations, and were regarded to be female-sterile. Thirty-five plants failed to set berries with over ten self-pollinations, but set berries with bulk-pollen pollinations; thus, they were convincingly SI. The remaining 8 plants were not determined for self-compatibility because of the insufficient number of pollinations made. Therefore, the segregation ratio was 66 SC vs. 35 SI, which is significantly skewed (x^2 = 9.51, 0.01 > p > 0.001) from an expected ratio of 1:1 for the presence of Sli.

A genetic map was constructed for the mapping population using DNA markers. The polymorphic bands transmitted from chc 525-3 via F_1-1 were scored for presence vs. absence. Forty-three TG probes, 11 CT probes and 71 P probes generated 127 RFLP markers (P136 and P435 generated two markers mapped on separate linkage groups). Twenty-three primers (Table 1) generated 28 *S. chacoense*-specific markers. A total of 155 molecular markers and the segregation of SC vs. SI were analyzed for linkage using the MAPMAKER program. Each of 45 markers cosegregated with one of the others. Thus, 110 loci were mapped, covering a span of 621 cM on 12 linkage groups (Figure 1). A LOD score of 5.0 was used, except for long map distances on groups 1 and 4, for which LOD scores of 4.0 and 3.3 were accepted, respectively. All the TG and CT probes used were mapped in similar alignment to that of the previously reported potato or tomato map (Tanksley et al., 1992). Thus, the linkage group numbers correspond to the chromosome numbers previously identified by Bonierbale et al. (1988).

The segregation of self-compatibility, associated with Sli, was mapped on one of the distal ends of linkage group 12, 10.0 cM away from the two cosegregated markers, CT156E5120 and 89-1320. If the above-mentioned four doubtful SC plants with ex-

Table 2. Crossing behavior (berries/pollinations) in the family 94H89 (93H100-1 × F_1-1)

Plant	Self[1]	Female[2]	Sli[3]
1	0/11	0/10	?
2	3/3 (277)		+
3	5/7 (118)		+
4	0/8	2/4	?
5	11/12 (101)		+
6	4/4 (115)		+
7	3/3 (197)		+
8	3/3 (110)		+
9	2/7 (89)		+
10	4/7 (16)		+
11	0/17	2/4	–
12	0/17	6/6	–
13	9/10 (92)		+
14	0/18	6/10	–
15	8/8 (108)		+
16	0/18	1/8	–
17	0/11	0/5	?
18	0/15	2/9	–
19	0/4	0/3	?
20	0/14	5/8	–
21	0/17	0/11	?
22	2/9 (99)		+
23	9/12 (151)		+
24	0/13	4/7	–
25	4/5 (124)		+
26	3/6 (53)		+
27	0/15	0/15	?
28	0/22	3/5	–
29	0/17	1/14	–
30	0/18	6/8	–
31	0/15	1/6	–
32	4/4 (82)		+
33	1/6 (95)		+
34	2/4 (127)		+
35	6/7 (103)		+
36	5/6 (119)		+
37	5/6 (275)		+
38	0/11	2/2	–
39	0/11	3/9	–
40	1/7 (92)		+
41	5/5 (96)		+
42	0/13	1/2	–
43	5/6 (68)		+
44	5/5 (136)		+
45	5/6 (168)		+
46	5/6 (146)		+
47	7/8 (163)		+
48	3/8 (189)		+
32	4/4 (82)		+
49	0/20	3/3	–

Table 2. Continued

Plant	Self[1]	Female[2]	Sli[3]
51	1/16 (32)		+
52	0/15	2/8	–
53	4/12 (134)		+
54	0/18	3/10	–
55	7/8 (84)		+
56	1/23 (129)		+
57	0/13	5/14	–
58	5/6 (103)		+
59	5/6 (43)		+
60	7/7 (71)		+
61	12/12 (88)		+
62	3/4 (65)		+
63	2/27 (39)		+
65	0/15	4/4	–
66	1/4 (126)		+
67	7/8 (125)		+
68	4/4 (74)		+
69	0/17	5/11	–
71	0/15	3/3	–
72	0/3		?
73	7/18 (90)		+
74	0/19	4/6	–
75	5/8 (80)		+
76	0/22	2/11	–
77	0/13	1/4	–
78	0/17	2/3	–
79	0/12	2/13	–
80	0/3	0/2	?
81	6/9 (103)		+
82	0/11	1/4	–
83	0/5		?
84	0/23	0/14	?
85	7/7 (156)		+
86	0/13	10/18	–
87	2/10 (43)		+
88	7/12 (134)		+
89	0/12	2/8	–
90	0/7	0/16	?
91	0/14	0/11	?
92	2/2 (183)		+
93	0/11	1/1	–
94	0/14	3/4	–
95	6/7 (184)		+
96	6/7 (176)		+
97	3/3 (81)		+
98	10/10 (157)		+
99	0/16	0/14	?
100	4/16 (194)		+
101	0/15	4/5	–
104	0/21	1/19	–
105	7/9 (68)		+

Table 2. Continued

Plant	Self[1]	Female[2]	Sli[3]
106	15/16 (78)		+
108	10/10 (134)		+
109	3/9 (41)		+
110	4/10 (44)		+
112	6/15 (32)		+
113	1/10 (15)		+
114	0/17	5/6	–
116	11/11 (220)		+
117	10/11 (74)		+
118	0/23	10/11	–
119	0/9	0/9	?
120	12/12 (118)		+
121	9/9 (61)		+
123	0/8	2/7	?
124	4/4 (127)		+
125	3/16 (62)		+

[1] No. of seeds per berry given in parenthesis.
[2] Female fertility was evaluated by pollinating with bulk pollen collected from many plants.
[3] + = presence, – = absence, ? = unable to determine.

tremely low berry or seed set were treated as SI, the same map distance was obtained (10.0 cM). And if those were removed from the data, tighter linkage was found (8.3 cM).

The probe TG24, a reference marker of the S locus, represented polymorphic bands between the grandparents, 1.22 and chc 525-3, in all four restriction digests. However, these bands were possessed by both parental clones F_1-1 and 93H100-1, making them impossible to score.

Segregation ratios were analyzed for deviation from an expected 1:1 ratio using x^2 test for goodness-of-fit (Figure 1). The segregation ratios of all markers of groups 2 and 11 were greatly distorted, with much less frequencies of the marker bands. For example, the most extreme distortion was found for TG497H2780 of group 11, where the segregation ratio was 8 presence : 108 absence. The Sli gene and the neighboring loci of chromosome 12 were also distorted towards higher frequencies of the marker bands; x^2 values were 4.17 for CT79E5990 and TG68H7370, 3.45 for TG283H2370, 8.83 for TG28P2850 and TG468E11300, 9.97 for TG296E3530, and 7.76 for CT156E5120. Significant localized distortion ($p < 0.01$) was observed in distal regions of linkage groups 1 and 10, where the marker bands were under-represented, and for groups 5 and 7, where those were over-represented.

Discussion

The SI × SC cross (90H100-1 × F_1-1) resulted in segregating progeny for self-compatibility. This confirmed the proposed hypothesis that a single dominant gene (Sli) is involved in dysfunction of the gametophytic self-incompatibility system (Hosaka & Hanneman, 1998), although the segregation ratio of the gene was considerably skewed from 1:1 segregation. By linkage analysis using DNA markers, Sli was mapped to the end of linkage group 12. It has been known that the self-incompatibility locus (S locus) of diploid potatoes exists on chromosome 1 (Gebhardt et al., 1991; Jacobs et al., 1995; Rivard et al., 1996). Thus, it is evident that Sli and the S locus are independent of each other and located on separate chromosomes.

The F_1-1 parent was expected to produce pollen with and without the Sli gene at an equal frequency, for the parent was heterozygous for Sli. The two types of pollen had the same possibility to be fertilized with eggs of 93H100-1, because sporophytic action of Sli in the F_1-1 parent dysfunctioned S gene interaction between F_1-1 and 93H100-1 (Hosaka & Hanneman, 1998). However, SC plants were significantly over-represented over their SI sibs in the mapping population, indicating a higher frequency of transmission of Sli to the progeny. A gametic advantage of the pollen having Sli might have been involved to some extent in pollen competition during the fertilization process. However, neighboring markers of chromosome 12 were also skewed, and the x^2 value was maximized at TG296E3530. Thus, the segregation distortion of Sli might be due to the other factors of the neighboring loci. The relative ease in identifying SC plants compared to SI plants might also have contributed to the over-representation of SC plants. SC plants were identified by as few as two self-pollinations (Table 2), while SI plants were confirmed only after clearing two criteria: failure of at least 10 self-pollinations made on separate days, and berries set after bulk-pollen pollinations to determine female fertility. Indeed, eight plants remained unconfirmed, due to less than 10 self-pollinations being made and no berry set (Table 2). Thus, a higher number of SC plants in the mapping population might not necessarily be associated with a gametophytic effect of Sli.

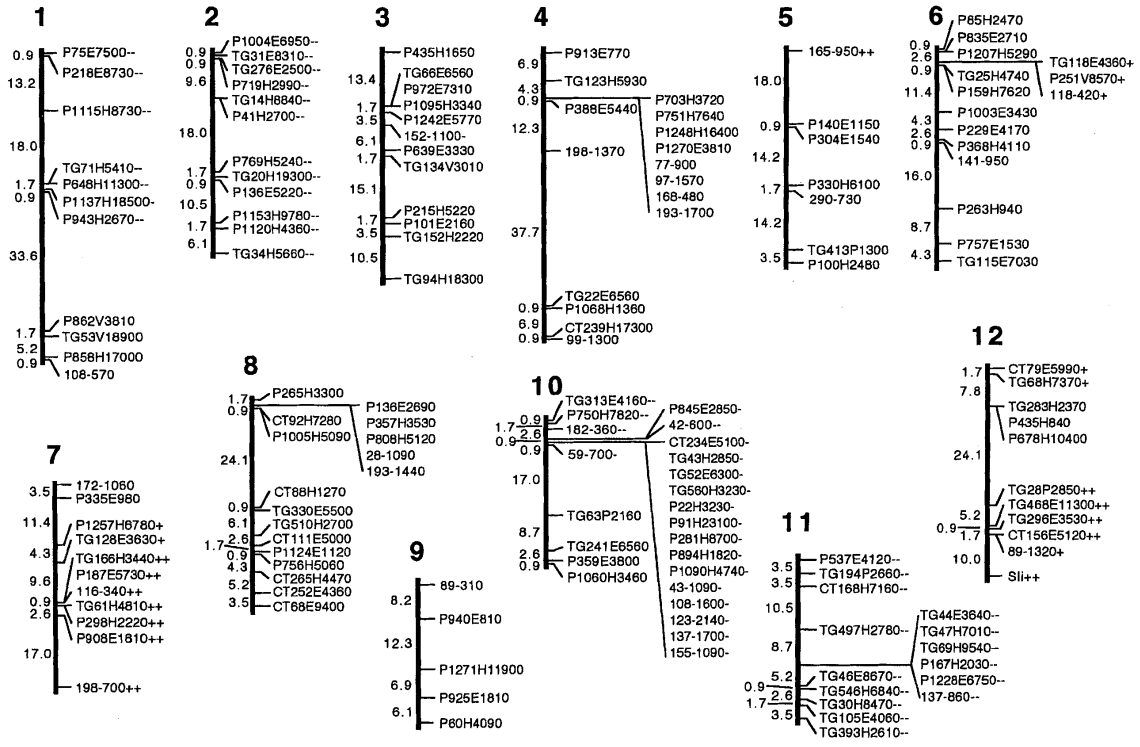

Figure 1. A genetic linkage map of chc 525-3, a self-compatible clone of *S. chacoense*, constructed using RFLP and RAPD markers. Markers with distorted segregation are indicated by suffixing + or ++ (the marker band was over-represented), and - or - - (under-represented), at significance levels of 5% or 1%, respectively. Map distances are indicated in centimorgans (cM).

In this study, skewed segregation of a whole chromosome was found for chromosomes 2 and 11, while localized skewedness was found for chromosomes 1, 5, 7, 10 and 12. Clusters of loci with distorted segregation ratios have been reported in various regions on potato maps (Bonierbale et al., 1988; Gebhardt et al., 1991; Jacobs et al., 1995; Rivard et al., 1996). The most prominent distortion of recombination was reported for chromosome 1, which was caused by the self-incompatibility locus (Gebhardt et al., 1991; Jacobs et al., 1995; Rivard et al., 1996). The accurate position of S locus on chromosome 1 was not identified because a reference marker of the S locus, TG24 could not be mapped. However, the S locus could be located somewhere between P75E7500 and TG71H5410 with two reasons; (1) the location of S locus is approximately 13 cM distal from TG71, inferred from the reported map distances between S locus and the flanking loci TG24 and TG71 (Gebhardt et al., 1991; Tanksley et al., 1992), and (2) S gene cDNA probe of *Petunia inflata* (gifted from Dr. T. -H. Kao, Pennsylvania State University) was mapped between P75 and P1115 on the RFLP map of 93H100-1 (unpublished data). Thus, the region including the S locus in chromosome 1 was significantly distorted. However, this should not be caused by the S locus, since the S gene had been rendered dysfunctional by Sli. The most extreme distortion in the present map was found for chromosomes 2 and 11 with unknown reasons.

The gametophytic self-incompatibility system prevents fertilization of pollen which has the same S as the pistillate parent. S-linked stylar proteins, or S gene products on the female side, have been identified in several Solanaceous species from the genera *Nicotiana*, *Petunia*, *Solanum*, and *Lycopersicon* (Kao & Huang, 1994). These proteins are highly basic glycoproteins with ribonuclease activity in common. However, S gene products in the pollen have not yet been identified (Thompson & Kirch, 1992). It has now been realized that the Sli gene interacts in some way with the gametophytic self-incompatibility system in pollen and results in SC plants. The Sli gene may inhibit S gene expression in the pollen, break down

the pollen-stigma recognition system, or protect pollen against stigma S-RNase. Although the real function of Sli gene remains unknown, it may play an important role for better understanding of the gametophytic self-incompatibility system.

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