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Production of inbred progenies of diploid potatoes using an S-locus inhibitor (*Sli*) gene, and their characterization

R.K. Bihman and K. Hosaka

Abstract: To develop inbred lines from self-incompatible, cultivated diploid potatoes, an S-locus inhibitor (*Sli*) gene derived from a self-compatible variant of a wild potato species, *Solanum chacoense*, was incorporated into various cultivated diploid potatoes. The progeny was selfed twice by the action of the *Sli* gene to obtain 74 S_2 inbred clones belonging to 8 families. More than 40% of them were either non-flowering or pollen sterile. Among the pollen fertile clones, self-compatible clones occurred with a much lower frequency (20.9%) than expected (83.3%). The result demonstrated that self-compatibility was introduced and expressed in the gene pool of cultivated diploid potatoes by an action of the *Sli* gene, although serious inbreeding depression associated with selfing occurred. The genotypes of S_2 inbreds were surveyed using 46 *S. chacoense* - specific RFLP (restriction fragment length polymorphism) markers covering the whole potato genome. More than half of the markers (67.4%) showed distorted segregation. Particularly, all markers on chromosome 12 were overrepresented in the S_2 inbreds. This confirms our earlier finding that the *Sli* gene locates on chromosome 12 and the alleles linked with this gene are preferentially transmitted because of its essential requirement for selfing.

Key words: RFLP markers, inbreds, diploid potato, distorted segregation.

Résumé : Afin de développer des lignées fixées à partir de variétés diploïdes autoincompatibles de la pomme de terre, un gène inhibiteur du locus S (*Sli*) provenant d'un variant autocompatible d'une espèce sauvage de pomme de terre, *Solanum chacoense*, a été incorporé chez divers cultivars diploïdes de pomme de terre. La progéniture a été autofécondée à deux reprises grâce à l'action du gène *Sli* ce qui a permis d'obtenir 74 clones S_2 appartenant à huit familles. Plus de 40 % de ces plantes ne fleurissaient pas ou étaient androstériles. Parmi les clones androfertiles, la proportion observée de clones autocompatibles (20,9 %) était plus faible que celle attendue (83,3 %). Ce résultat a montré que l'autocompatibilité avait été introduite et exprimée dans le génome de pommes de terre cultivées diploïdes par suite de la présence du gène *Sli* mais qu'une importante dépression consanguine était associée à l'autofécondation. Le génotype des inbreds S_2 a été examiné à l'aide de 46 marqueurs RFLP (polymorphisme de longueur des fragments de restriction) spécifiques du *S. chacoense* et distribués sur l'ensemble du génome. Plus de la moitié des marqueurs (67,4 %) ont montré une distorsion de la ségrégation. En particulier, tous les marqueurs du chromosome 12 étaient surabondants dans les inbreds S_2 . Ceci confirme des résultats antérieurs des auteurs, lesquels avaient trouvé que le gène *Sli* était situé sur le chromosome 12 et que des allèles liés à ce gène étaient transmis préférentiellement en raison de la nécessité de ce locus pour permettre l'autofécondation.

Mots clés : marqueurs RFLP, inbreds, pomme de terre diploïde, distorsion de la ségrégation.

[Traduit par la Rédaction]

Introduction

Cultivated potato (*Solanum tuberosum* L.) is a highly heterozygous tetraploid ($2n = 4x = 48$) with tetrasomic inheritance, thus segregation in this species is more complicated than in those with disomic inheritance (Howard 1970; Ross 1986; Hawkes 1990). Potato suffers from serious inbreeding de-

pression on selfing (Krantz 1924; De Jong and Rowe 1971). Tetraploidy and inbreeding depression are respon dihaploids can simplify genetical ansible, to a very large extent, for the difficulty of advancing potato breeding and genetics. Use of diploid species and selection studies in potato. However, lack of specific stocks and tester lines of diploid materials is still an impediment to the achievement of these goals.

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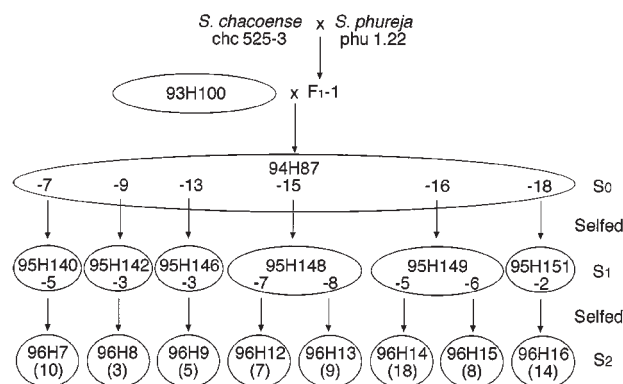
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Fig. 1. Origin of the inbreds used in this study. Each family is circled, inside which parental clones of next selfed generation are given by hyphenation after the family name. The number in parentheses in each S_2 family is the number of selected clones used for this study.

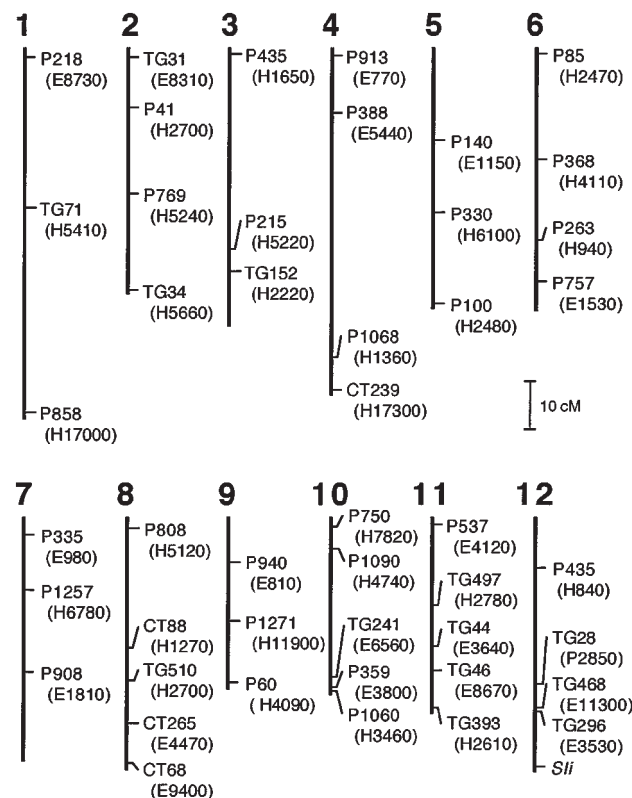


Cultivated potato has a large collection of related cultivated and wild tuber-bearing *Solanum* species. The majority of these species are diploid ($2n = 2x = 24$) (Hawkes 1990) and most of them are self-sterile due to the presence of the gametophytic self-incompatibility (Pushkarnath 1942; Pandey 1962; Cipar et al. 1964b), which leads to the extreme difficulty of developing inbred lines. However, self-compatible variants have often been described in self-incompatible diploid species (Pushkarnath 1942; Cipar et al. 1964b; De Jong and Rowe 1971; Olsder and Hermesen 1976; De Jong 1977; Cappadocia et al. 1986; Birhman 1986). Such variants may be used in the production of inbred progenies of diploid potatoes.

A number of self-compatible clones of *S. chacoense* Bitt., a wild diploid ($2n = 2x = 24$) tuber-bearing *Solanum* species, were identified by Hanneman (1985). A highly inbred clone of one such variant was crossed with a cultivated diploid species, *S. phureja*, and the resulting interspecific F_1 hybrids and their selfed and sib-mated F_2 progenies were investigated for self-compatibility (Hosaka and Hanneman 1998a). Self-compatibility was found to be conditioned by the presence of a dominant allele of an *S*-locus (self-incompatibility locus) inhibitor gene (*Sli*) that was nonallelic to the *S* locus. Its action was sporophytic in contrast to the gametophytic nature of the *S* locus (Hosaka and Hanneman 1998a). Since homozygotes of the *Sli* gene have not been identified, they may be lethal (Hosaka and Hanneman 1998a). Using RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) markers, the *Sli* gene was mapped at a distal end of chromosome 12 (Hosaka and Hanneman 1998b).

To develop inbreds of cultivated potatoes, one of the interspecific F_1 hybrids having the *Sli* gene was crossed with cultivated diploid potatoes of diverse origins. This paper reports on the production of diploid S_2 inbred progenies, their self-, ovule-, and pollen-fertilities, and segregation patterns of *S. chacoense* - specific RFLP markers in these S_2 families. The role of the *Sli* gene in producing selfed progeny and possible genetic biases associated with the *Sli* gene are discussed.

Fig. 2. Schematic representation of the *Solanum chacoense* RFLP map (after Hosaka and Hanneman 1998b) indicating the locations of the *Sli* gene (chromosome 12) and the RFLP loci studied. *S. chacoense* - specific markers are indicated in parentheses with restriction enzyme and the fragment size in base pairs: E, *Eco*RI; H, *Hind*III; and P, *Pst*I.



Materials and methods

Source of the *Sli* gene

The clone F_1-1 , an interspecific hybrid between a highly inbred clone (S_7) of *S. chacoense* (designated chc 525-3; $2n = 2x = 24$) as female and a cultivated diploid *S. phureja* clone (designated phu 1.22) as male, was used as a donor parent of the *Sli* gene. This clone is self-compatible and has the *Sli* gene in heterozygous condition (Hosaka and Hanneman 1998a).

Production of S_2 inbred progenies

Production of inbreds was started by crossing between plants of the family 93H100, and F_1-1 (Fig. 1). The family 93H100 consisted of plants raised from a mixture of seeds obtained by bulk-pollen pollinations among the cultivated diploid potatoes (the *S. stenotomum* - *S. phureja* mixed population).

In the spring of 1994, one flower each of 75 plants of the family 93H100 was pollinated with the pollen of F_1-1 . Fifty-seven berries were collected and seeds were extracted and mixed together. A family 94H87 (1125 plants) was raised from these seeds in the fall. Based on visual observations, 161 clones possessing good agronomic traits such as good, uniform tuber shape and size, fleet eyes, good tuber yield, and long dormancy were selected. Tubers from these selected clones were planted in the spring of 1995, among which 119 clones emerged and were self-pollinated. Fifty-two self-pollinated berries were formed on 19 of these clones. Seeds were extracted from these berries and 490 clones belonging to 19 S_1

Table 1. Pollen stainability and crossing behavior (berries/pollinations) in S₂ inbreds.

Inbred	Pollen stainability (%)	Bulk-pollen pollination	Selfing	Comments
96H7-1	17.6	0/2	2/9 (0)	Pseudo-compatible?
96H7-2	41.1	1/2	1/9 (15)	Selfer
96H7-3	18.6	—	0/18	
96H7-4	9.8	—	0/18	
96H7-5	0	3/9	—	Male sterile
96H7-6	0	4/6	—	Male sterile
96H7-7	35.1	0/5	0/9	Female sterile
96H7-8	28.5	0/7	1/16 (0)	Pseudo-compatible?
96H7-9	5.8	—	0/6	
96H7-10	4.9	—	—	
96H8-1	0	0/5	—	Male sterile
96H8-2	53.4	—	0/6	
96H8-3	0	0/4	—	Male sterile
96H9-1	47.5	—	0/13	
96H9-2	12.7	—	0/5	
96H9-3	41.5	2/2	3/11 (80)	Selfer
96H9-4	17.8	1/13	1/14 (20)	Selfer
96H9-5	—	—	—	No bud
96H12-1	7.7	0/14	0/10	Female sterile
96H12-2	—	—	—	Buds dropped
96H12-3	0	5/17	—	Male sterile
96H12-4	0	3/6	—	Male sterile
96H12-5	—	—	—	Buds dropped
96H12-6	0	3/6	—	Male sterile
96H12-7	2.2	4/5	5/10 (97)	Selfer
96H13-1	16.5	2/7	2/14 (30)	Selfer
96H13-2	2.3	4/7	1/4 (28)	Selfer
96H13-3	13.0	4/6	7/10 (300)	Selfer
96H13-4	61.3	2/4	1/3 (40)	Selfer
96H13-5	0	2/6	—	Male sterile
96H13-6	0	3/5	—	Male sterile
96H13-7	0	0/5	—	Male sterile
96H13-10	3.8	—	0/7	
96H13-11	0	2/6	—	Male sterile
96H14-1	0	7/10	—	Male sterile
96H14-2	13.9	—	—	
96H14-3	—	—	—	No bud
96H14-4	15.7	—	0/2	
96H14-5	12.7	—	0/6	
96H14-6	3.6	—	0/2	
96H14-7	—	—	—	No bud
96H14-9	—	—	—	No bud
96H14-10	24.6	1/3	1/3 (69)	Selfer
96H14-11	15.0	0/6	—	
96H14-12	18.3	—	—	
96H14-13	0	—	—	Male sterile
96H14-14	—	—	—	No bud
96H14-15	10.9	—	—	
96H14-16	—	—	—	No bud
96H14-17	5.0	—	—	
96H14-18	—	—	—	No bud
96H14-19	0	—	—	Male sterile
96H15-1	—	—	—	Buds dropped
96H15-2	39.8	2/6	0/9	Non-selfer
96H15-3	10.2	0/6	0/7	Female sterile
96H15-4	13.5	—	—	
96H15-5	—	—	—	Buds dropped

Table 1 (concluded).

Inbred	Pollen stainability (%)	Bulk-pollen pollination	Selfing	Comments
96H15-6	38.2	—	0/5	
96H15-7	—	—	—	Buds dropped
96H15-8	—	—	—	Buds dropped
96H16-1	36.4	3/6	0/1	
96H16-2	18.9	2/2	0/8	Non-selfer
96H16-3	8.6	1/7	0/5	Non-selfer
96H16-4	4.7	—	0/2	
96H16-5	11.2	—	—	
96H16-6	0	0/4	—	Male sterile
96H16-7	25.6	2/4	0/6	Non-selfer
96H16-8	0	5/8	—	Male sterile
96H16-9	59.1	—	0/5	
96H16-10	0	1/10	—	Male sterile
96H16-11	27.2	—	0/5	
96H16-12	0	—	—	Male sterile
96H16-13	7.2	—	0/7	
96H16-14	14.8	—	0/7	

Note: The total numbers of seeds are given in parentheses.

families (95H134–95H152) were raised in the fall of 1995. Ninety-five inbred clones belonging to 17 S_1 families were selected based on the same criteria as adopted in 1994. This cycle of selfing and selection was repeated in 1996, and 99 inbred clones belonging to 8 S_2 families were retained. Two plants each of 74 S_2 inbred clones and the parental chc 525-3, phu 1.22, and F_1 -1 were grown in the spring of 1997 and were used in the present investigation (Fig. 1).

Flowering, pollen fertility, and berry setting

Flowering was checked at weekly intervals. Pollen fertility was estimated by staining pollen grains with 1% acetocarmine and counting the percentage of well-stained pollen grains. Pollen of five flowers per inbred was studied by preparing separate slides for each flower, and counting at least 50 pollen grains. Pollen fertile inbreds having sufficient numbers of flowers were self-pollinated. Besides, the inbreds were cross-pollinated with bulked pollen collected from many inbreds. For all pollinations, the flowers were emasculated one day prior to their opening, and the pollen was applied the day after opening.

RFLP analysis

S. chacoense - specific RFLP markers were obtained previously; these were inherited from chc 525-3 to F_1 -1 and mapped in a segregating population composed of 116 clones from the cross 93H100-1 $\times$ F_1 -1 (Hosaka and Hanneman 1998b). The clone 93H100-1 was a selected clone from the family 93H100. Of the mapped markers, 46 (3–5 markers per chromosome) were used in this study (Fig. 2). These markers were chosen based on the criterion that they were detected in chc 525-3 and F_1 -1, but not in phu 1.22 and 93H100-1. Thus, the present investigation was carried out based on the assumption that the marker bands were not present in the family 93H100, although this was not tested.

DNA isolation and detection procedures for RFLP markers have been described previously (Hosaka and Hanneman 1998b). In the present investigation, however, only the presence or absence of 46 *S. chacoense* - specific RFLP markers was recorded. Observed segregation ratios for all the markers were tested to detect deviation from the expected frequencies using the chi-square test for goodness-of-fit. Based on the data obtained, Nei's (1987) genetic distances were calculated between all pairs of the S_2 inbreds, and a dendrogram using the UPGMA (unweighted pair-group mean with

arithmetic averages) method was constructed to show the relationship between the donor parent of the *Sl* gene and the S_2 progenies.

Results

Selfing in S_0 and S_1 progenies

Of the 119 clones of the family 94H87 (S_0 progeny) prepared for this study, 104 clones could be self-pollinated. The remaining 15 clones (12.6%) were not available for self-pollination due to either non-formation of flower buds, premature dropping of flower buds, or no pollen shedding. A total of 822 self-pollinations (an average of 7.9 pollinations per clone; range 1–18) were made, from which 52 berries were formed in 19 clones. Eighty-five clones did not set berries. As all berries contained normal amounts of seeds, the 19 clones (18.3%) were regarded as self-compatible. For raising S_1 progeny, 95 clones of 17 S_0 families were selected and grown, of which 33 were self-pollinated. Sixty-two clones (65.3%) could not be self-pollinated, for the same reasons as in S_0 . A total of 179 flowers were self-pollinated with an average of 5.4 pollinations per clone, ranging from 2 to 13 pollinations. Ten clones (30.3%) belonging to 6 S_1 families were self-compatible, of which 8 were used to produce S_2 families (Fig. 1).

Pollen and seed fertility in S_2 inbreds

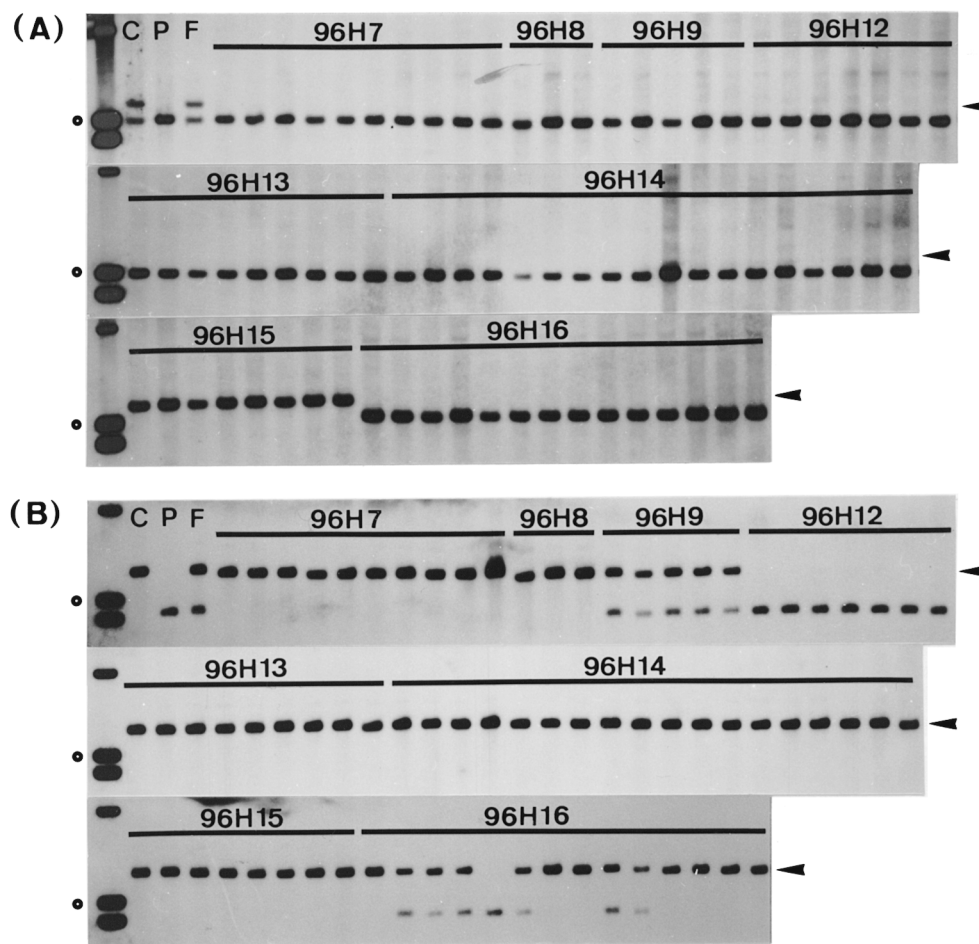
Of the 99 S_2 clones established from 6 S_1 families, 74 inbred clones belonging to 8 S_2 families were characterized for flowering, pollen fertility, and berry formation after bulk- and self-pollination (Table 1). Thirteen inbreds (17.6%) did not flower, due either to non-formation of flower buds or to premature dropping of flower buds. Eighteen inbreds (24.3%) were completely male sterile. In the remaining 43 inbreds, the pollen fertility ranged from 2.2% to 61.3%, the average being 20.4% (standard deviation, $s = 15.9$). Pollen fertility of the original parental clones was 60.8%, 42.6%, and 68.9% for chc 525-3, phu 1.22, and F_1 -1, respectively. Out of the 43 pollen fertile clones, 9 clones (20.9%) set berries with seeds on self-pollination. Two clones, 96H7-1 and

Table 2. The appearance frequencies (%) of *Solanum chacoense* - specific RFLP markers.

		Chromosome 1			Chromosome 2				Chromosome 3			Chromosome 4				Chromosome 5		
Family	No.	P218	TG71	P858	TG31	P41	P769	TG34	P435	P215	TG152	P913	P388	P1068	CT239	P140	P330	P100
96H7	10	0	0	0	0	0	0	100	80	0	0	100	80	0	0	0	100	100
96H8	3	0	100	0	67	0	67	100	0	0	0	0	0	33	33	0	0	0
96H9	5	0	0	0	0	0	0	80	0	0	0	100	100	0	0	0	100	80
96H12	7	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0	0
96H13	9	100	0	0	0	0	0	0	0	0	0	0	78	0	0	100	0	0
96H14	18	67	89	0	100	100	100	0	0	0	0	0	100	67	72	0	0	0
96H15	8	50	88	0	100	100	100	0	0	0	0	0	100	63	63	0	0	0
96H16	14	0	0	0	93	0	93	93	0	79	79	71	0	0	71	64	0	0
Overall frequency		33.8	35.1	0	55.4	35.1	55.4	40.5	10.8	14.9	14.9	33.8	71.6	24.3	39.2	33.8	20.3	18.9
Significance				***	***		***	*	***	***	***		***				*	**
		Chromosome 6				Chromosome 7			Chromosome 8				Chromosome 9					
Family		P85	P368	P263	P757	P335	P1257	P908	P808	CT88	TG510	CT265	CT68	P940	P1271	P60		
96H7		0	0	90	0	100	0	80	0	0	0	80	0	0	0	80		
96H8		0	67	0	0	0	0	0	0	100	0	0	0	0	0	0		
96H9		0	0	0	80	100	0	0	0	0	0	0	0	80	60	0		
96H12		0	57	71	86	100	0	0	0	0	71	100	0	86	71	71		
96H13		0	67	67	78	100	0	0	0	0	100	100	0	89	100	56		
96H14		89	0	0	61	78	0	72	0	0	100	100	78	0	0	0		
96H15		0	0	0	100	100	0	100	0	0	100	100	63	0	0	0		
96H16		0	0	0	79	71	0	0	0	0	0	0	0	64	57	0		
Overall frequency		21.6	16.2	27.0	63.5	85.1	0	39.2	0	4.1	54.1	68.9	25.7	36.5	33.8	24.3		
Significance		*	**		***	***	***		***	***	***	***						
		Chromosome 10					Chromosome 11				Chromosome 12							
Family		P750	P1090	TG241	P359	P1060	P537	TG497	TG44	TG46	TG393	P435	TG28	TG468	TG296			
96H7		70	70	0	0	0	0	0	0	0	0	0	100	100	100			
96H8		0	0	0	0	0	0	0	0	0	0	0	100	100	100			
96H9		0	0	0	0	0	80	0	0	0	0	0	100	100	100			
96H12		0	0	86	0	100	100	0	100	100	0	0	0	0	0			
96H13		0	0	78	0	67	56	0	0	0	0	100	100	100	100			
96H14		78	100	100	0	100	100	0	100	100	0	100	100	56	100			
96H15		0	100	100	0	0	100	0	63	0	100	100	100	88	100			
96H16		0	0	0	0	0	0	0	0	0	0	0	93	100	100			
Overall frequency		28.4	44.6	52.7	0	41.9	56.8	0	40.5	33.8	10.8	47.3	89.2	78.4	90.5			
Significance			**	***	***	*	***	***	*		***	***	***	***	***			

Note: Chi-square test was carried out for goodness-of-fit; significant at 5% (*), 1% (**), or 0.1% (***).

Fig. 3. RFLP banding patterns of chc 525-3 (C), phu 1.22 (P), F₁-1 (F), and the S₂ inbreds. (A) *Hind*III-digested genomic DNAs were probed with TG393, and (B) *Pst*I-digested genomic DNAs were probed with TG28. *Solanum chacoense* - specific markers are indicated by arrowheads. The left lane contains *Hind*III-digested lambda DNA, of which the 2.3-kbp fragment is shown by a small circle.



96H7-8, set berries containing no seeds. The number of seeds varied from 15 to 69 per berry in these selfers. When cross-pollinated with bulked pollen, berry formation occurred in 25 clones. All the berries contained normal amounts of seeds. Therefore, out of 61 clones that flowered, 27 clones (44.3%) set berries on either selfing or cross-pollination. At least 3 clones (96H7-7, 96H12-1, and 96H15-3) could be regarded as female sterile, because they failed to set berries with more than five pollinations on both selfing and cross-pollination. Also, at least 4 clones (96H15-2, 96H16-2, 96H16-3, and 96H16-7) could be regarded as self-incompatible, because they did not set berries with over five self-pollinations, although they had sufficient male and female fertility (Table 1).

Segregation of *S. chacoense* - specific RFLP markers

The genotypes of S₂ inbreds were surveyed using 46 *S. chacoense* - specific RFLP markers, which covered the whole potato genome (Figs. 2 and 3). The appearance frequency of each *S. chacoense* - specific marker (chc allele) in each S₂ family is presented in Table 2. Overall frequencies were tested against the theoretical probability of 68.75% for absence vs. 31.25% for presence. Out of the 46 markers

tested, 31 (67.4%) located on all chromosomes (except chromosome 9) showed significant distortion in segregation at the 5% level of significance; 14 were underrepresented for chc alleles, and 17 were overrepresented. Five markers, P858 (chromosome 1), P1257 (chromosome 7), P808 (chromosome 8), P359 (chromosome 10), and TG497 (chromosome 11), were completely absent in the S₂ inbreds (0% appearance). All markers on chromosome 12 were significantly overrepresented, although the 96H12 family showed 0% appearance for these markers (Fig. 3B). When segregation of these marker loci on chromosome 12 was tested against a frequency of 16.7% for absence vs. 83.3% for presence, which was expected if a chc allele was tightly linked to the *Sli* gene, only P435, the furthestmost marker from the *Sli* gene, showed significantly distorted segregation. All four markers on chromosome 2 showed high appearance frequencies, three of which were statistically significant. In contrast, all three markers on chromosome 3 were underrepresented at the 0.1% level of significance.

Relationship among inbreds

An UPGMA dendrogram was constructed to show the relationship among the S₂ inbreds, F₁-1 and chc 525-3

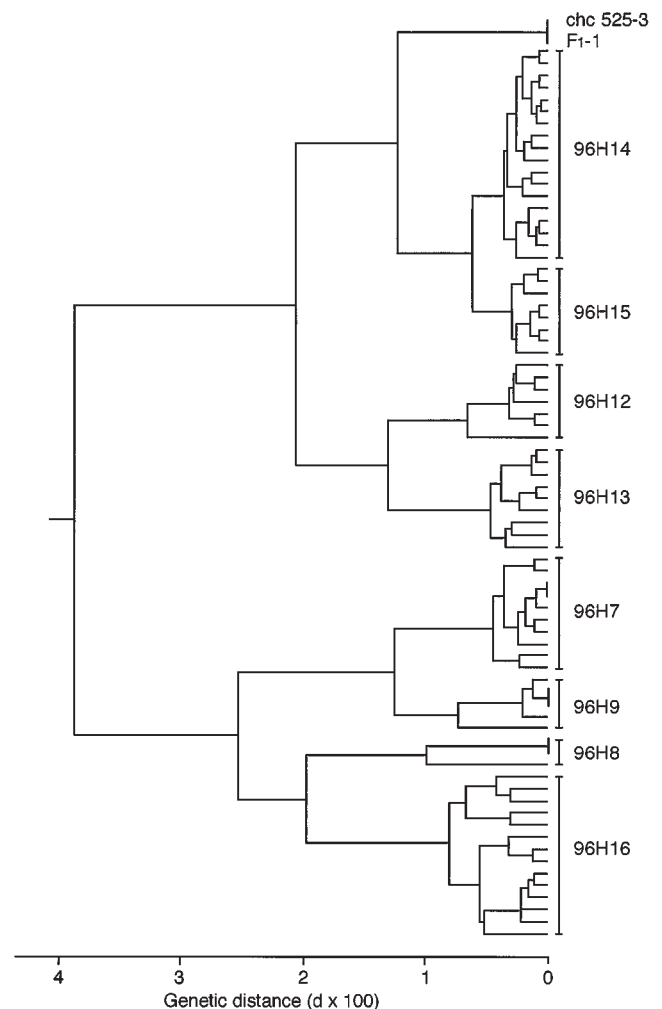
(Fig. 4). Since only chc 525-3-derived markers were used for the comparison (not all bands were compared), the dendrogram does not show the overall similarity among the materials, but shows the relationship of chc 525-3 with others. The dendrogram reflected the pedigree history of the S_2 inbreds: all S_2 inbreds within the same family made a distinctive cluster, confirming that these were derived from a single S_1 parent (Fig. 1). Two clusters of the families 96H12 and 96H13 and another two clusters of the families 96H14 and 96H15 made two separate large clusters, supporting that these were derived from the single S_0 parental clones, 94H87-15 and 94H87-16, respectively (Fig. 1). The families 96H14 and 96H15 were closer to chc 525-3 than the others, indicating that more chc alleles were retained in these S_2 families. Overall, the RFLP marker analysis perfectly reflected what would be expected from the pedigree record.

Discussion

In this investigation, we have demonstrated that *S*-locus inhibitor (*Sli*) gene was transferred to various cultivated diploid potatoes, which in turn inactivated the self-incompatibility system in these potatoes, thus making way for the generation of self-compatible segregants. Utilization of the *Sli* gene for various purposes is suggested; for example, effective genetic fixation of resistance genes as homozygotes, development of highly homozygous TPS (true potato seed) lines, and heterosis breeding of the potato. Although genetic analyses have been made for self-compatibility in different self-compatible variants of self-incompatible tuber-bearing *Solanum* species (Cipar 1964; Olsder and Hermesen 1976; Hermesen 1978a, 1978b), this is the first report that self-compatibility was introduced into the gene pool of cultivated diploid potatoes by an action of the *Sli* gene of wild species origin. The UPGMA dendrogram constructed using *S. chacoense*-specific RFLP markers (Fig. 4) perfectly corresponded with the pedigree record of the S_2 inbreds. This strongly supported that the inbreeding was proceeded as expected. Although different degrees of *S. chacoense*-specific genome were incorporated into various S_2 progenies, variation within each of the S_2 families was distinctively smaller than that between the families (Fig. 4). On continued selfing of these progenies, it is likely that highly homozygous genotypes will be developed, which will be useful as genetic stocks for various genetic analyses in potato.

A high degree of inbreeding depression was noted in this study, manifested in ways such as non-formation of flower buds, premature dropping of flower buds, no pollen shedding, or complete male sterility. Indeed, 12.6%, 65.3%, and 41.9% of the clones could not be pollinated in S_0 , S_1 , and S_2 progenies, respectively. This agrees with the observation by De Jong and Rowe (1971) that the percentage of non-flowering plants increased very sharply from S_0 (0%) to the S_1 (46%), but not from S_1 to S_2 (46%), in the course of inbreeding of self-fertile hybrids obtained from crosses between cultivated diploids and dihaploids of *S. tuberosum* subsp. *tuberosum*. Inbreeding depression was also indicated by lower pollen fertility of S_2 inbreds than parental clones and by smaller number of seeds contained in the selfed berries of S_2 inbreds in the present investigation (Table 1).

Fig. 4. An UPGMA (unweighted pair-group mean with arithmetic averages) dendrogram showing relationships between the *Sli* gene source parent, F_1 -1, and S_2 inbred progenies.



The *Sli* gene, derived from *S. chacoense*, is essential for selfing (Hosaka and Hanneman 1998a). Thus, *S. chacoense*-specific RFLP markers linked to the *Sli* gene would be selectively transmitted to the subsequent progeny. All the tested RFLP markers on chromosome 12 were significantly over-represented. The appearance frequencies of the markers fit the expected theoretical values when the marker alleles were linked to the *Sli* gene. This confirms our earlier finding that the *Sli* gene is located on chromosome 12 (Hosaka and Hanneman 1998b) and the linked loci on chromosome 12 show genetic biases associated with the *Sli* gene function. The family 96H12 lacks all *S. chacoense*-specific RFLP markers on chromosome 12. However, the family contained one selfer (96H12-7, Table 1), confirming that the family was derived not by pseudo-compatibility, but by an action of the *Sli* gene from the S_1 parental clone (95H148-7). Since 95H148-8 of the same S_1 parental family transmitted *S. chacoense*-specific RFLP markers on chromosome 12 to the S_2 progeny, recombination could have occurred somewhere on chromosome 12 between the *Sli* gene and the adjacent RFLP marker TG296 in the grandparental clone

94H87-15, so that the parental clone 95H148-7 had to be a recombinant.

The percentage of self-compatible clones in the clones actually used for self-pollination was 18.3% in S_0 , 30.3% in S_1 , and 20.9% in S_2 progenies. These proportions were much lower than the expected values of 50% in S_0 , 75% in S_1 , and 83.3% in S_2 progenies. Even if the *Sli* gene homozygotes were lethal as hypothesized previously (Hosaka and Hanneman 1998b), the expected frequency would be 50% in S_0 , 66.7% in S_1 , and 66.7% in S_2 progenies. Since TG296 (the RFLP marker most tightly linked with the *Sli* gene in our study) appeared at a frequency of 90.5% (Table 2), a high frequency of the presence of the *Sli* gene can be speculated. The reason for the lower frequency of recovering self-compatible clones seems to be the decrease in both male and female fertility due to inbreeding depression. At least 21 clones in S_2 progeny were either completely male or female sterile.

Distorted segregation was observed for 67.4% of the RFLP markers tested in the S_2 progeny. In a previous study (Hosaka and Hanneman 1998b), 43.2% of the mapped loci showed skewed segregation in a hybrid progeny between one of the clones of 93H100 family (clone 93H100-1, not used for this study) and F_1 -1. This is in agreement with the result of Rivard et al. (1996), in that a higher percentage of skewed segregation was found in selfed progenies of *S. chacoense* than in hybrid progenies between different accessions of *S. chacoense*. Most of the distorted markers in the S_2 progeny were scattered throughout the potato genome except for those on chromosomes 2 and 3, and on chromosome 12 where the *Sli* gene is located. Such distorted segregation of a whole chromosome was found for chromosomes 2 and 11 in the hybrid population of 93H100-1 $\times$ F_1 -1 (Hosaka and Hanneman 1998b). However, the markers on chromosome 2 were underrepresented with *chc* alleles in the hybrid population, whereas those were overrepresented in S_2 progeny. All the markers on chromosome 11 were underrepresented with *chc* alleles in the hybrid population, whereas those of S_2 progeny were a mixture, showing over- and underrepresented segregation. The genotypes studied in our study were highly selected in each selfing generation for good tuber characteristics, high tuber yield, long tuber dormancy, and self-fertility. These selection pressures applied might have contributed to the observed skewed segregation and the differences in distortion between the hybrid population and S_2 progeny. Other factors, such as abortion of male and female gametes, self-incompatibility, the self-incompatibility inhibitor (*Sli*) gene, and zygote selection might have caused the unequal segregation of alleles.

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