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Expression of S-locus inhibitor gene (*Sli*) in various diploid potatoes

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Summary

S-locus inhibitor gene (*Sli*), which can inhibit gametophytic self-incompatibility in diploid potatoes and alter self-incompatible to self-compatible plants, was introduced by crossing into 32 diploid genotypes as females and its expression in the F_1 and S_1 progenies was investigated. We found that the expression of self-compatibility in the F_1 hybrid progeny depended largely upon the female genotypes and partly upon the male genotypes (= *Sli* gene donor clones). Successful females produced hybrid plants, in which 67.1% of self-pollinated plants set S_1 seeds. By second selfing upon the S_1 plants, an average of 44.2% of self-pollinated plants were self-compatible. Unsuccessful females produced hybrids, most of which were self-incompatible or male-sterile. Restriction fragment patterns of chloroplast DNA (ctDNA) were able to distinguish successful females (S- or A-type ctDNA) from unsuccessful females (W- or T-type ctDNA). A ctDNA high-resolution marker analysis using seven microsatellites and H3 marker supported a higher degree of differentiation between the two

groups of ctDNA types and implied a possible interaction between the cytoplasm and *Sli* gene function. However, it has been known that the cytoplasm having T-type ctDNA and that derived from *Solanum demissum* (haplotype 26 of W-type ctDNA) cause male sterility, and the present case with unsuccessful females were likely caused by male sterility rather than the failure of *Sli* gene function.

Introduction

Gametophytic self-incompatibility controlled by *S* locus with multi-allelic *S* genes prevents self-fertilization in diploid potatoes (Pushkarnath, 1942; Pandey, 1962; Cipar et al., 1964). Recently, we found a dominant gene, named *S*-locus inhibitor gene (*Sli*), which is sporophytically expressed to inhibit a function of *S*-allele in pollen, and alters self-incompatible plants to self-compatible ones (Hosaka & Hanneman, 1998). The *Sli* gene may induce selfed progeny and continuous selfings may produce highly homozygous lines, which are attractive in genetic analyses, genetic fixation of traits, production of

TPS (true potato seed) varieties, and heterosis breeding of the potato (Birhman & Hosaka, 2000).

Birhman & Hosaka (2000) introduced the *Sli* gene by crossing into cultivated diploid potatoes, which expressed self-compatibility and were selfed up to S₂ generation. Subsequent continuous selfings successfully produced S₆ progeny (Phumichai et al., in press).

Nevertheless, use of *Sli* gene has been very much limited to only our laboratory. To enhance a wide application of *Sli* gene in potato breeding and genetics, its expression with various genetic backgrounds is reported and possible causes for failure in acquiring selfed progeny are discussed.

Materials and methods

Plant material

An initial source of *Sli* gene was a self-compatible variant of a wild Argentine diploid species *Solanum chacoense* Bitter (clone chc 525-3), which was pollinated as female with pollen of a cultivated diploid species *S. phureja* Juz. et Buk. (clone 1.22) (Hosaka & Hanneman,

1998). One of the F_1 hybrids (clone F_1 -1) was crossed with a *S. stenotomum*-*S. phureja* bulk population (93H100 family). The seeds obtained were bulked and planted as a family 94H87. One plant (clone 94H87-16) of this family derived three progeny clones (97H32-6, -7 and -14). The clone F_1 -1 was also crossed with one plant (clone 93H100-1) of the 93H100 family and derived three hybrid clones (94H89-43, -97 and -124). These F_1 -1 and its six derived clones were used as *Sli* gene donors (Fig. 1). Upon request, superior *Sli* gene donors will be available for distribution from the Potato Introduction Station (NRSP-6), Sturgeon Bay, Wisconsin, USA, in near future. Thirty-two diploid genotypes, which set abundant berries and produced ample pollen, were used as female (Table 1), with which one to four of seven *Sli* gene donors were crossed as pollen parents (Table 2).

Self-compatibility

F_1 hybrids (S_0) and their selfed progenies (S_1) were evaluated for self-compatibility by selfing with an ordinary manner (Hosaka & Hanneman, 1998).

Chloroplast DNA analysis

Chloroplast DNA (ctDNA) from selected clones was analyzed using *Bam*HI, *Hind*III or *Pvu*II restriction fragment patterns (Hosaka & Hanneman, 1988), seven ctDNA microsatellite markers (NTCP6-9, 12, 14 and 18, all developed by Provan et al., 1999) and H3 marker (developed by Hosaka, 2003). All detection procedures were described in Sukhotu et al. (2004).

The ctDNA phenotypes were compared with those reported by Sukhotu et al. (2004) and an un-weighted pair-group method with arithmetic means (UPGMA) dendrogram was constructed using PAUP 4.0b10 (Sinauer Associates, Inc.).

Results

Evaluation of female genotypes

Thirty-two genotypes with various origins (clone no. 2-33) were pollinated with pollen of *Sli* gene donors (mean 1.4, range 1-4 *Sli* gene donors). The performance upon selfing in these F₁ hybrids and the following S₁ plants is shown in Table 1. The clone chc 525-3 (an initial source of *Sli* gene) was also included in Table 1, which was

crossed as female with pollen of a non-*Sli*-gene donor (*S. phureja* clone 1.22). There were two distinct hybrid groups; one successfully produced selfed progeny (clones 1-18) and the other was unsuccessful (clones 19-33).

In the successful hybrid group, an average of 76.3% (standard deviation SD=23.24) of hybrids were self-pollinated. The remaining 23.7% of them were not self-pollinated due to non-formation of flower buds, premature dropping of flower buds, or no pollen shedding. Upon selfing, 67.1% (SD=16.91) of self-pollinated F₁ hybrids were self-compatible. By second selfing upon the S₁ plants, the number of self-pollinated plants was significantly decreased to an average of 32.0% (SD=11.83). The frequencies of selfers were also decreased in most progenies with an average of 44.2% (SD=11.56).

In the unsuccessful hybrid group, two types of hybrids were recognized. The F₁ hybrids from clones 19-31 were self-pollinated with an average frequency of 79.1% (SD=12.16), which was approximately similar to that of the successful female group. Nevertheless, an average of only 1.6% (SD=3.18) was self-compatible, and all of the S₁ plants failed to set S₂ seeds by selfing. In contrast, F₁ hybrids from clones 32 and 33 showed extremely poor pollen shedding.

Only 6.3% (SD=2.90) of them were self-pollinated with much less number of pollen grains, resulting in complete failure on selfing.

Evaluation of Sli gene donors

The *Sli* gene donors themselves were self-compatible and transmit the *Sli* gene to the successful females, resulting in self-compatible hybrids with frequencies of 26.7-87.6% (Table 2). In contrast, nearly all *Sli* gene donors failed to induce self-compatible hybrids with unsuccessful females.

Sli gene expression in a backcross family

The clone W794215-H33 (clone 23) fairly produced aceto-carminestainable pollen, but had never been successful in crossing as pollen parent (data not shown). A hybrid family 96H17 ($W794215-H33 \times F_1-1$) consisted of plants whose pollen grains were stainable with aceto-carmines, but not functioned when crossed as bulk-pollen pollinators (Table 3). All F_1 hybrids were self-incompatible as summarized in Table 1 (clone 23), whereas some were crossable with pollen of F_1-1 (Table 3), resulting in five backcross families ($W794215-H33 \times F_1-1$) $\times$

F₁-1. These backcross families, sharing the cytoplasm of W794215-H33 and 75% of chromosomal information of F₁-1, did not produce any self-compatible plants (see Table 1, clones 26-30).

Association with ctDNA difference

ctDNA types were determined for all female and male parents (Tables 1 and 2). Most of successful females were descended maternally from *S. phureja* and had S-type ctDNA in common, and two successful female clones had A-type ctDNA. All female clones with W- or T-type ctDNA except chc 525-3 were unsuccessful in selfing. The *Sli* gene donors had W- or S-type ctDNA, showing no obvious association with the percentage selfers (Table 2).

Since A-, S- and T-type ctDNA have been known as single ctDNA haplotypes by further evaluation with microsatellite and H3 markers (Sukhotu et al., 2004), only selected clones for these ctDNA were investigated by these high-resolution markers (W6522195-19 for A-type, 93H100-1 and W822229-5 for S-type, and 98H20-5 for T-type ctDNA). As the W-type was a general type for various wild species (Sukhotu et al., 2004), three clones descended from different maternal sources were examined (chc 525-3, W862207-1 and W794215-H33).

As expected, two S-type ctDNA clones showed the same phenotypes with each other in all markers, which corresponded to the haplotype 2 of Sukhotu et al. (2004) (Fig. 2, Table 4). W862207-1 and W794215-H33 showed a new NTCP9 fragment with approximately 310 base pairs in size (Fig. 2), whose ctDNA was distinguished as a new haplotype 26 from the any other haplotypes reported by Sukhotu et al. (2004). The fragment size using NTCP9 was previously reported to be 279 base pairs for *S. chacoense* (chc 525-3) and *S. vernei* Bitt. et Wittm. (PI 473308) (Sukhotu et al., 2004), but was corrected to 278 base pairs in this study. In total, five ctDNA haplotypes were identified (Table 4), which were able to compare with our previous ctDNA data (Fig. 3). The haplotypes 1 and 2 of successful females were clearly separated from haplotypes 6 and 26 of unsuccessful females. The haplotype 25 of chc 525-3 was rather closer to those of unsuccessful females but was successful in selfing (Fig. 3).

Discussion

We found that the expression of self-compatibility in the F₁ hybrid progeny depended largely upon the female parent. Further investigations indicated that the difference of ctDNA was clearly associated with the expression of *Sli* gene. The ctDNA of haplotype 2 (= S-type ctDNA, which was defined by Hosaka (1986) based on restriction fragment patterns only), haplotype 1 (= A type) or haplotype 25 (= W type) was associated with successful induction of self-compatibility, whereas that of haplotype 26 (= W type) or haplotype 6 (= T type) was with unsuccessful induction (Table 1). The F₁ hybrids and its backcross progeny to a self-compatible parent (sharing ctDNA haplotype 26) failed to induce a self-compatible plant (Table 2), implying that the ctDNA haplotype 26 prevented the function of *Sli* gene. As shown in Fig. 3, haplotypes 1 and 2 were the most common ctDNA among cultivated potatoes in the Andean highlands, and were clearly separated from haplotypes 6 and 26. Thus, the difference of ctDNA might relate in some way to the function of *Sli* gene. This assumption may be opposed to the case of the haplotype 25, which was rather closer to haplotypes 6 and 26 (Fig. 3), but was the ctDNA of chc 525-3 and associated with self-compatibility in chc 525-3 and F₁-1 (Hosaka & Hanneman, 1988).

Cytoplasmic male-sterility has been well known in potato, in which the cytoplasm of the common potato (*S. tuberosum* L. ssp. *tuberosum*) ($2n=4x=48$) (mostly having T-type ctDNA) is interacting with at least six different dominant nuclear genes *ASF*, *In*, *Sm*, *Sp*, *TA* and *VSA* and causes different types of male sterility (Grun, 1979; Grun & Staub, 1979; Ross 1986). Thus, most of haploid plants ($2n=2x=24$) from commercial varieties are pollen-sterile, even when they have been produced from pollen-fertile varieties (Peloquin & Hougas, 1960; Ross et al., 1964; Howard, 1970). The two haploid clones (clones 32 and 33) used in this study were considerably female and male fertile, but most of the resulted hybrids were no-pollen-shedding plants, suggesting that the above type of cytoplasmic male-sterility was functioning. Clones 19-31 had W-type ctDNA and likely haplotype 26 according to the parentage relationship (Table 1). Our old record indicated that clones 19-22 were all maternally descended from a haploid of *S. tuberosum* L. ssp. *andigena* Hawkes (stock no. 217, cv. Yurrac-pacco), which, however, did not show any maternal relationship with clones 23-31. The clones 23-31 were relatively new lines bred from a haploid plant of a German cultivar Tunika, whose cytoplasm was descended from a so-called W-race (a German breeding line derived from a

Mexican hexaploid wild species *S. demissum* Lindl.) (Ross, 1986). *Solanum demissum* was extensively used in a German breeding program for introduction of late blight resistance (Ross, 1966, 1986). Two accessions of this species had W-type ctDNA (Hosaka, 1986; Nakagawa & Hosaka, 2002), but not known for the ctDNA haplotype. In the backcrossing of *S. demissum* (6x) by *S. tuberosum* (4x), successful crosses were made only from *S. demissum* as female, and the resulted pentaploid hybrid as female due to cytoplasmic male-sterility (Dionne, 1961). Thus, the *S. demissum* cytoplasm was preferentially transmitted to the bred varieties and the present materials. Dionne (1961) reported that many hybrid derivatives of *S. demissum* as female parent produced high percentages of stainable pollen but this pollen was non-functional. We observed the same phenomenon for the clones 19-31. Thus, the failure to obtain selfers in the hybrids using clones 19-33 as females is likely due to cytoplasmic male-sterility rather than the interaction between ctDNA and *Sli* gene. In fact, some selfers were obtained, though with extremely lower frequencies, from hybrids with three females, suggesting the *Sli* gene itself was functioning even under these ctDNA (Table 1).

The percentage selfers also depended upon the *Sli* gene donors used. The *Sli* gene donors have been estimated to be a heterozygote (*Sli*/-) for F₁-1 (Hosaka & Hanneman, 1988) and a homozygote (*Sli*/*Sli*) for 97H32-6 (Phumichai et al., 2004). Thus, it is suggested that the percentage selfers in the hybrid progeny highly depend on the *Sli* gene genotype of pollen parent. Our recommended *Sli* gene donors are 97H32-6 and 97H32-14, because they have more cultivated-type genetic background, and show profuse pollen production, relatively short tuber-dormancy, and good tuber yields under any day-length conditions.

Phumichai et al. (in press) reported that the percentage selfers in its selfing generations from 97H32-6 (the best *Sli* gene donor) decreased steadily from 82.5% in S₁ to 26.7% in S₄, although those from clones 5 and 6 fluctuated largely between generations with averages of 38.3% from S₁ to S₅. In the present study, an overall percentage selfers in F₁ was 61.7%, and that of S₁ was 44.1%. Thus, the reduction of the percentage selfers through selfing generations could be a general rule, which is probably due to reduction of general fertility by inbreeding depression.

In conclusion, the cytoplasm must be taken into account for successful use of *Sli* gene. Unfortunately, however, preferred diploid

clones in most of breeding programs would be haploid clones of the common potato, which is predominated mostly by T-type ctDNA and partly by W-type ctDNA of *S. demissum* (Hosaka & Hanneman, 1988; Douches et al., 1991; Hosaka, 1993; Powell et al., 1993; Provan et al., 1999; Lössl et al., 2000). Thus, cytoplasmic male-sterility would be a perpetual problem likewise with that encountered in using haploid × wild species hybrids for diploid parental breeding (Hougas & Peloquin, 1962; Yerk & Peloquin 1990). As proposed for the case of using haploid × wild species hybrids (Ross et al., 1964), the use of *Sli* gene donors as female could be alternative (not tested).

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Figure legends

Figure 1. Pedigrees of the *Sli* gene donor clones.

Figure 2. Banding patterns of chloroplast DNA microsatellites (NTCP markers) and H3 marker. The most left lane contained 10 base ladder markers for microsatellites and *Hind*III digests of lambda DNA for H3 marker. Lane 1, W6522195-19; lane 2, 93H100-1; lane 3, W822229-5; lane 4, chc 525-3; lane 5, W862207-1; lane 6, W794215-H33; lane 7, 98H20-5.

Figure 3. UPGMA dendrogram showing similarity among chloroplast DNA haplotypes 1, 2, 6, 25 and 26. All other haplotype data were cited from Sukhotu et al. (2004). Corresponding ctDNA types and sample names are denoted with haplotype numbers. Cultivated species are shown by abbreviation and the number of accessions prefixed. adg, *S. tuberosum* ssp. *andigena*; adj, *S. ajanhuiri*; cha, *S. chaucha*; cur, *S. curtilobum*; juz, *S. juzepczukii*; phu, *S. phureja*, stn, *S. stenotomum*; tbr, *S. tuberosum* ssp. *tuberosum*.

Table 1. Expression of *Sli* gene on the F₁(=S₀) and S₁ plants

Female parent of the F ₁ (=S ₀) ¹		First selfing ²	Second selfing ³
Clone	ctDNA		
Successful female			
1. chc 525-3 (chc)	W	15/15(250)/7	2/387/212(4266)/116
2. W6522195-19 (unknown)	A	156/68(333)/50	8/81/34(168)/17
3. WB902207-4 (W6522195-19)	A	78/71(415)/41	8/82/17(66)/9
4. 93H100-1 (phu-stn)	S	116/116(1274)/66	4/74/38(195)/7
5. WB922236-2 (114, phu)	S	87/50(325)/34	34/485/81(265)/31
6. WB922236-3 (114, phu)	S	122/40(253)/26	26/346/105(447)/36
7. W822229-5 (114, phu)	S	62/42(171)/14	4/45/13(68)/5
8. W872210-15 (114, phu)	S	94/61(285)/46	8/72/20(64)/14
9. W882204-3 (114, phu)	S	106/79(404)/59	8/83/28(101)/9
10. WB902205-1 (114, phu)	S	105/103(612)/96	8/66/35(156)/17
11. W892209-11 (461, phu)	S	103/86(377)/77	8/65/18(58)/9
12. W892209-13 (461, phu)	S	106/90(405)/69	8/68/17(58)/7
13. W872208-14 (P10173-5)	S	82/68(309)/33	8/75/20(73)/10
14. W872208-36 (P10173-5)	S	153/146(575)/93	8/96/26(80)/14
15. W872209-33 (276, phu)	S	237/221(993)/138	8/76/27(103)/8
16. W872209-36 (276, phu)	S	83/79(354)/64	8/91/24(91)/10
17. W872209-51 (276, phu)	S	103/85(522)/80	8/62/22(84)/10
18. 96H4-11 (unknown)	S	208/52(436)/25	10/260/33(142)/15
Unsuccessful female			
19. W862207-1 (2x 217, adg)	W	113/97(411)/0	
20. WB902241-9 (2x 217, adg)	W	104/89(499)/4	2/6/3(11)/0

21. WB902241-12 (2x 217, <i>adg</i>)	W	71/66(355)/6	
22. WB902241-24 (2x 217, <i>adg</i>)	W	64/58(310)/0	
23. W794215-H33 (2x Tunika, <i>dms</i>)	W	27/24(342)/0	
24. W794215-H34 (2x Tunika, <i>dms</i>)	W	43/32(141)/0	
25. W794215-H35 (2x Tunika, <i>dms</i>)	W	57/49(203)/0	
26. 96H17-5 (W794215-H33)	W	6/3(77)/0	
27. 96H17-19 (W794215-H33)	W	29/18(206)/0	
28. 96H17-29 (W794215-H33)	W	30/25(211)/0	
29. 96H17-43 (W794215-H33)	W	14/11(82)/0	
30. 96H17-59 (W794215-H33)	W	22/16(129)/0	
31. W882201-3 (W794215-H33)	W	89/69(334)/5	2/5/2(6)/0
32. MTD88-50 (2x Atlantic, <i>tbr</i>)	T	71/3(24)/0	
33. 98H20-5 (2x Konafubuki, <i>tbr</i>)	T	72/6(41)/0	

¹ In parenthesis, an immediate maternal parent, or a maternal parent descended as much as possible (in *Italic*), is presented. *adg*, *S. tuberosum* ssp. *andigena*; *chc*, *S. chacoense*; *stn-phu*, a clone from *S. stenotomum* and *S. phureja* bulked population via open-pollination; *dms*, *S. demissum*; *phu*, *S. phureja*; *tbr*, *S. tuberosum* ssp. *tuberosum*.

² No. of plants / no. of pollinated plants (total flowers) / no. of selfers.

³ No. of families / no. of plants / no. of pollinated plants (total flowers) / no. of selfers.

Table 2. Self-pollinations of F₁ hybrids derived with different males (=Sli gene donors)

Male parent of the F ₁ (=S ₀)		Selfing ¹	Percentage selfers
Clone	ctDNA		
With successful females			
F ₁ -1	W	186(1616)/93	50.0%
97H32-6	S	621(3233)/544	87.6%
97H32-7	S	224(1056)/131	58.5%
97H32-14	S	374(1702)/218	58.3%
94H89-43	S	19(158)/11	57.9%
94H89-97	S	18(126)/10	55.6%
94H89-124	S	15(152)/4	26.7%
With unsuccessful females			
F ₁ -1	W	100(1071)/0	0.0%
97H32-6	S	89(499)/4	4.5%
97H32-7	S	98(496)/6	6.1%
97H32-14	S	273(1258)/5	1.8%
94H89-43	S	3(22)/0	0.0%
94H89-97	S	0(-)/-	-
94H89-124	S	3(19)/0	0.0%

¹ No. of pollinated plants (total flowers) / no. of selfers.

Table 3. Fertility in 96H17 family (W794215-H33 $\times$ F₁-1)

Clone	Pollen stainability ¹	Pollinated with pollen of ²		
		Self	Bulk ³	F ₁ -1
96H17-1	-	0/18	0/9	
96H17-5	-	0/22		1/9
96H17-6	+	0/12		
96H17-10	?	0/5		
96H17-12	+	0/7		
96H17-15	?	0/21		
96H17-16	?	0/16	0/6	
96H17-19	+	0/11		3/4
96H17-21	?	0/9		
96H17-25	+	0/15	0/11	0/5
96H17-29	+	0/18	0/10	7/7
96H17-34	?	0/16	0/7	
96H17-35	+	0/13		
96H17-38	+	0/5		
96H17-39	?	0/4		
96H17-43	+	0/12	0/5	2/5
96H17-52	+	0/24	0/4	
96H17-53	+	0/14	0/11	0/10
96H17-55	+	0/24	0/11	0/6
96H17-57	+	0/19	0/9	
96H17-59	+	0/21	0/8	5/9
96H17-60	+	0/15	0/4	
96H17-65	+	0/11	0/2	
96H17-67	?	0/10		

¹ Pollen stainability with aceto-carmin. +, fairly stainable; -, very poor stainability; ?, not examined.

² No. of berries / no. of flowers pollinated.

³ Bulk pollen collected from 96H17 family.

Table 4. Characterization of ctDNA

Sample	ctDNA type	NTCP phenotype in base pairs ¹							H3 type	Haplo- type ²
		6	7	8	9	12	14	18		
W6522195-19	A	174	174	250	289	237	151	186	2	1
93H100-1	S	127	173	251	289	239	150	186	2	2
W822229-5	S	127	173	251	289	239	150	186	2	2
98H20-5	T	173	173	252	279	235	149	188	1	6
chc 525-3	W	175	174	252	278	234	152	187	1	25
W862207-1	W	174	174	253	310	235	151	188	1	26
W794215-H33	W	174	174	253	310	235	151	188	1	26

¹ The size of the 310 base pair fragment in NTCP9 was approximate.

² Haplotype identity numbers correspond to those of Sukhotu et al. (2004), although the haplotype 26 was new.

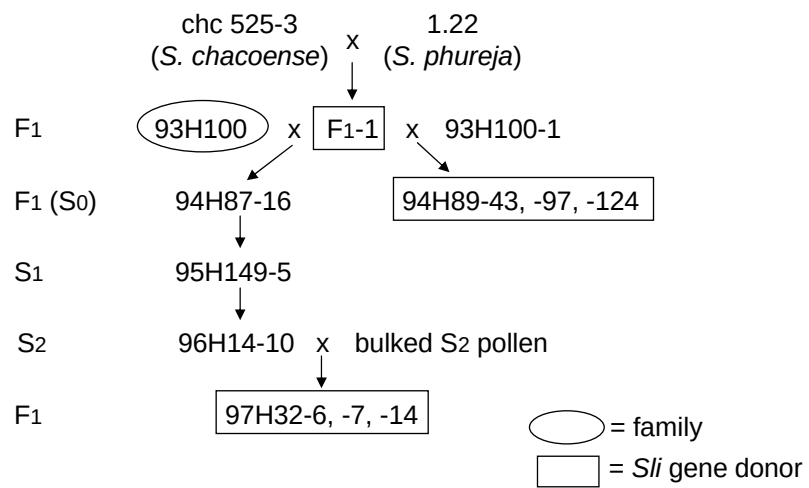


Figure 1

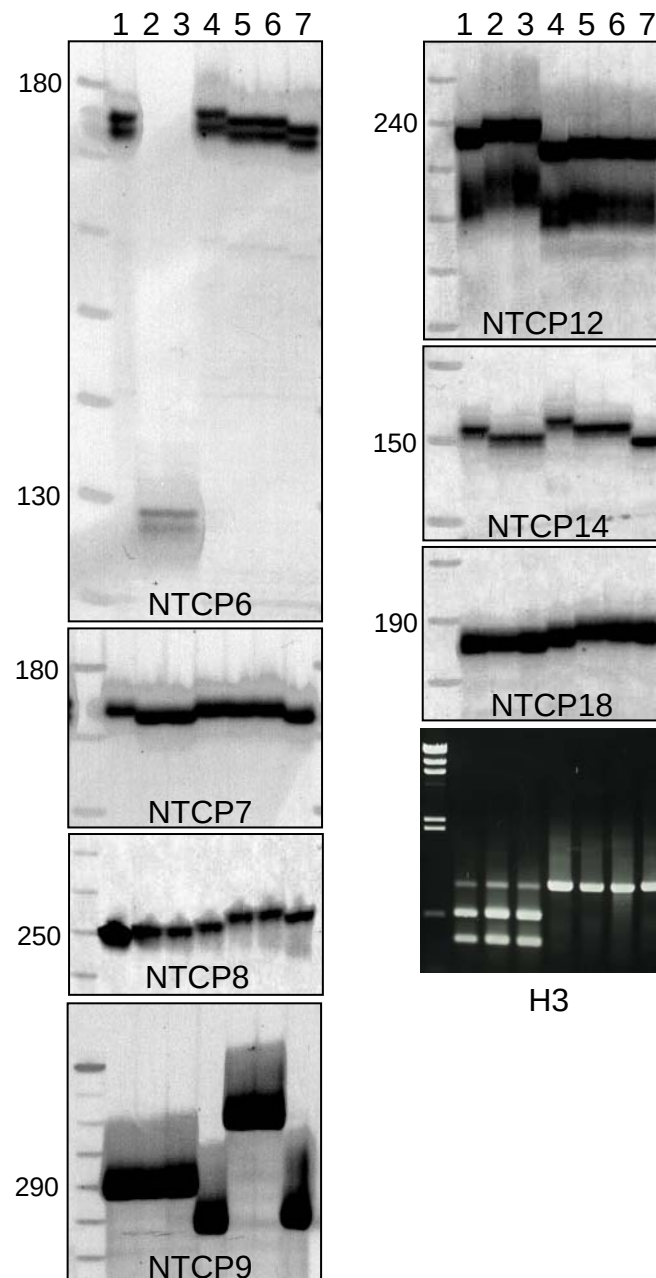


Figure 2

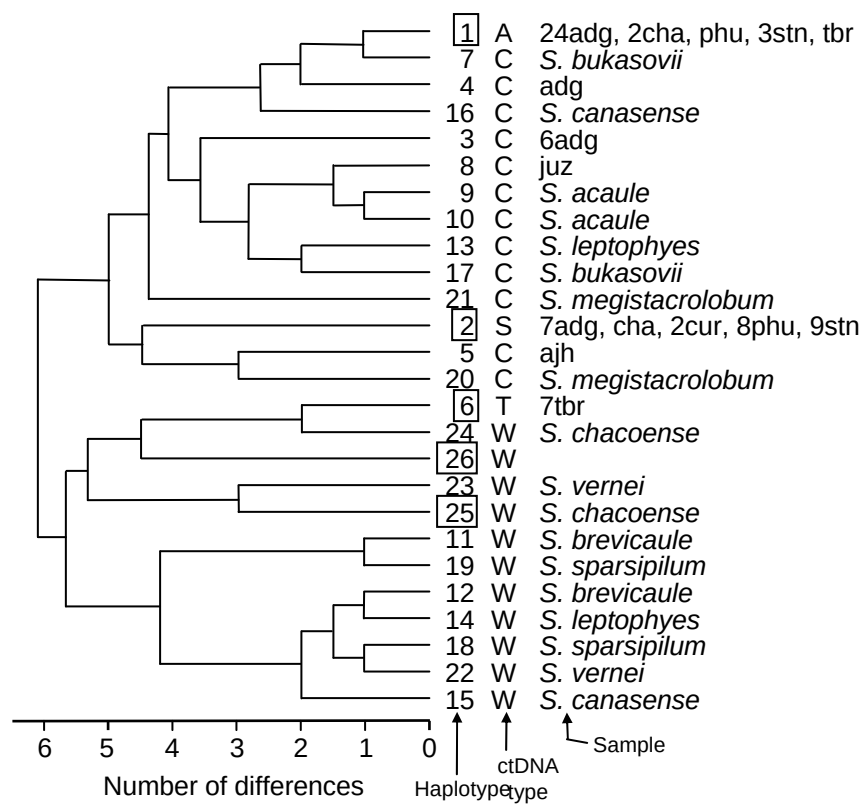


Figure 3