

Selection of vigorous and fertile S-homo- and heterozygous tester clones from self-incompatible diploid potato, *Solanum tuberosum* L.

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Received 20 January 1997; accepted 25 May 1997

Key words: *Solanum tuberosum*, potato, gametophytic self-incompatibility, S-alleles, pseudo-compatibility, counterfeit pollination, homozygotes, embryo spot.

Summary

For the selection of diploid ($2n=2x=24$) potato (*Solanum tuberosum*) genotypes that are useful for the molecular and genetic analysis of the phenomenon of gametophytic self-incompatibility, three different types of basic populations were investigated. These populations were derived from three primary dihaploid clones, G609, G254 and B16, which possessed the S-allele combinations S1S2, S1S3 and S3S4 respectively. In order to select highly vigorous, profusely flowering, fertile and tuberising progenies, three types of populations, derived from the above mentioned diploid genotypes, were screened for performance and classified for the expression of self-incompatibility. Although the selection for well defined S-genotypes was sometimes complicated due to the occurrence of pseudo-compatibility and of a self-compatibilising factor, the use of a combination of criteria, viz., Iso Electric Focusing (IEF), pollen tube growth in the styles and the extent of berry and seed set made the selection of sufficient representatives of all six types of S-heterozygotes (S1S2, S1S3, S1S4, S2S3, S2S4 and S3S4) possible. After evaluating the strength of the self-incompatibility reaction in these heterozygotes, those with high expression were selfed, and intercrossed within their S-allele incompatibility group through the method of counterfeit pollination. In these progenies, well-performing S-homozygotes (S1S1; S2S2; S3S3; S4S4) for all four S-alleles with high expression of self-incompatibility were selected. As a result, all possible S-homo- and heterozygous genotypes with a predictable type of self-incompatibility are available and maintained both vegetatively and as botanical seed. The development of this material has paved the way for more critical analysis of molecular factors involved in self-incompatibility in diploid potato.

Introduction

The cultivated potato, *Solanum tuberosum* ($2n=4x=48$), is a self-compatible (SC) crop. Dihaploids ($2n=2x=24$) from tetraploid cultivars are usually highly sterile, less vigorous and self-incompatible (SI). Self-incompatibility in dihaploids is expected to be similar to the one-locus, multi-allelic, gametophytic system that is found in almost all other tuberous, diploid *Solanum* species. This expectation was proved to be true from the genetic analysis of self-incompatibility in male fertile genotypes that occurs rarely among dihaploids (Olsder and Hermesen, 1976; Hermesen, 1978a; Hermesen et al., 1978). These authors analysed three

fertile dihaploids, and the presumed tetraploid parent of two of the dihaploids, the cultivar Gineke, and postulated the presence of five S-alleles viz., S1, S2, S3, S4 and S5.

Through a complete diallel crossing scheme, the following genotypes were assigned to the three dihaploids that were investigated (Hermesen, 1978a): S1S2-G609; S1S3-G254 and S3S4-B16. The two latter clones, G254 and B16, possessed the necessary S-alleles for conferring self-incompatibility; nevertheless, they were self-compatible (i.e., set seed after selfing). This anomalous phenomenon was explained as due to the presence of an extra S1 allele (a duplication) in a presumably translocated segment on a different

chromosome and the authors designated this segment as “*tS1*” (Hermesen, 1978a; 1978b). This hypothesis was investigated by Thompson et al (1991), using RFLP analysis of plant material, coding for this *tS1* with the cloned stylar part of *S1*. The tri-partite structure of the *S*-locus, as proposed by Lewis (1961) had to be rejected and the translocation hypothesis for *tS1* could be maintained under the assumption that the translocation would have involved only the pollen part but none of the analyzed genomic *S1*-fragment. In dihaploids, containing such a *tS1* translocation, a fraction of the pollen grains contained the pollen parts of two different *S*-alleles instead of one, and hence inhibited self-compatibility by a competitive interaction (Crane & Lawrence, 1929; see also review, De Nettancourt, 1977) or mutual weakening as, for example, in *Brassica* (Wallace, 1979) or in polyploids (Lewis, 1943). Besides competitive interaction, also the so-called ‘pseudo-compatibility’ can occasionally bring about berry development with a few seeds in a basically otherwise self-incompatible genotype (Hermesen, 1978b).

Apart from such complications regarding self-incompatibility, the above mentioned genotypes were valuable for the characterisation of proteins that are associated with self-incompatibility in *Solanum tuberosum* (Kirch et al, 1989; Peil, 1995). As a result of this study, it was possible to correlate *S1* to *S4* alleles with specific bands of a number of polypeptides differing in their iso-electric points (Kirch et al, 1989). This observation, obviously, opened up possibilities for a more reliable method of identification of the *S*-alleles through electrophoresis, and characterisation of the self-incompatibility system in diploid potato more critically. In this context, it was essential to select defined diploid potato material, homozygous or heterozygous for particular *S*-alleles, that would be suitable for more critical genetic and molecular analysis of the incompatibility system.

Selection of diploid potato genotypes with a defined *S*-allele composition, e.g., *S*-homo- and heterozygotes, with a predictable expression of self-incompatibility is difficult for several reasons. Potato being a highly heterozygous crop, inbreeding depression is a severe problem both for selfing and for intercrossing among individuals within a small group of (diploid) genotypes. This is because the progenies in these cases are generally less vigorous, non-flowering, highly sterile, non-tuberising and frequently segregating for lethal and semi-lethal genes. In order to circumvent these difficulties, a rigid selection of diploid parents based on the performance of their progenies for some of the

important characteristics, including the typical expression of self-incompatibility, is essential.

The aims of the present investigation were: 1) to select diploid potato genotypes with highly vigorous, fertile, early and profusely flowering habit, showing good tuberisation characteristics; 2) to isolate homo- and heterozygous tester stocks for different *S*-alleles with predictable and reliable expression and 3) to produce sufficient plant material of each allelic class (seeds and tubers) for generative and vegetative maintenance.

Material and methods

Selection of the basic genotypes

Two different types of populations were screened for desirable genotypes. The first of these consisted of ‘basic’ populations derived from crosses between three dihaploid *Solanum tuberosum* ($2n=2x=24$) clones, G609 (*S1S2*), G254 (*S1S3*) and B16 (*S3S4*). The origin and the indicated genotypes of these basic clones have been described earlier (Olsder and Hermesen, 1978; Hermesen, 1978a). The progeny used for the selection of desirable genotypes had originated (see Table 1, column 2) not only from direct crosses (five original F1’s) between the dihaploid clones but also from intercrossing and selfing of progeny plants (14 populations from selfings and inbreds). Because the *S*-genotypes of each of the parents were known (column 3), it was possible to predict the expected genotypes and (in)compatibility reaction of *S*-alleles in the progeny populations (column 4). All 19 populations were screened and, with the exception of the selfing-based populations 6233 (11 plants) and 6234 (14 plants), consisted of 20-40 individuals each. All plant material was grown in the greenhouse during the growing seasons in one or more years of the period 1991-1996.

Counterfeit pollinations

The second type of populations consisted of genotypes that were selected from the basic populations mentioned in Table 1. After this selection of genotypes with predictable and well defined SI reaction, *S*-homozygous genotypes were produced through a complete diallel, involving identical *S*-genotypes with different genetic backgrounds within each of the six heterozygous incompatibility groups (*S1S2*, *S1S3*, *S1S4*, *S2S3*, *S2S4* and *S3S4*). In order to successfully self

Table 1. Basic populations (column 1) obtained from SI and SC parents (column 2) with known S-genotypes (column 3) used for the selection of well performing SI heterozygotes and SC homozygotes (column 4).

Population	Parents	Description	Obtained genotypes
Selfings			
6107	{(G254 x S1S3)20 x S1S3}8 ⊗	S1S3 ⊗	S1S3 + S3S3, SC
6108	{(G254 x S1S3)20 x S1S3}10 ⊗	S1S3 ⊗	S1S1 + S1S3 + S3S3
6233	6105-6 ⊗	S2S4 ⊗	S2S2 + S2S4 + S4S4*
6234	6105-8 ⊗	S2S3 ⊗	S2S2 + S2S3 + S3S3
Inbreds			
6101	(G254 x S1S3)20 x S1S3	S1S1 x S1S3	S1S3, SI/SC
6102	S2S3 x {(G254 x S1S3)20 x S1S3}1	S2S3 x S1S3	S1S2 + S1S3
6103	{(G254 x S1S3)20 x S1S3}1 x S2S3	S1S3 x S2S3	S1S2 + S2S3
6104	S2S3 x {(G254 x S1S3)20 ⊗ }4	S2S3 x S1S1	S1S2 + S1S3, SI/SC
6105	{(BG112 x GB61)21 x S3S4}2 x S2S3	S3S4 x S2S3	S2S3 + S2S4
6106	{(BG112 x GB61)23 ⊗ }5 x S2S3	S4S4 x S2S3	S2S4 + S3S4, SI/SC
6206	{(G254 x S1S3)20 x S1S3}9 x S2S3	S1S3 x S2S3	S1S2 + S2S3, SI/SC
6208	S2S3 x {(G254 x S1S3)20 x S1S3}2	S2S3 x S1S3	S1S2 + S1S3, SI/SC, S2S3 + S3S3, SC
6536	(GB49 x B16)17 x (GB53 x G254)41	S1S3 x S1S4	S1S4 + S3S4, SI/SC
6539	(GB66 x GB65)11 x S1S4	S1S4 x S1S4	S1S1 + S1S4 + S4S4, SI/SC
Original F1's			
6221	G254 x B16	S1S3 x S3S4	S1S4 + S3S4, SI/SC, S1S3 + S3S3, SC
6222	G254 x G609	S1S3 x S1S2	S1S2 + S2S3, SI/SC
6223	G609 x B16	S1S2 x S3S4	S1S3 + S1S4 + S2S3 + S2S4, SI/SC
6224	G609 x G254	S1S2 x S1S3	S1S3 + S2S3, SI/SC
6225	B16 x G254	S3S4 x S1S3	S1S3 + S1S4, SI/SC, S3S3 + S3S4, SC

GB = G254 x B16; BG = B16 x G254; S1S3, S2S3 and S3S4 = self-incompatible tester clones selected from GB, Gx(G x G609) and GB respectively. Numbers behind brackets indicate clone number. ⊗ = selfing, SI = self-incompatible, SC = Self-compatible due to *tSI*. Italics bold: last SC-clone used in a cross.

* S4S4 not detected.

or cross S-identical individuals, the method of ‘counterfeit pollination’ (Iwanaga et al, 1991; Singit and Hanneman, 1991; Brown and Adiwilaga, 1991) was used in addition to normal pollination. Counterfeit pollination was essential in order to promote seed set and to prevent premature fall of fruits. Premature berry fall is more likely when there is poor seed set because of fertilisation of only few or none of the egg cells. In the counterfeit pollination method, the receptive stigmas were selfed or cross pollinated as usual but after two days, pollen from one of the three IvP pollinators (*S. phureja*), viz., IvP 35, IvP48 and IvP101 were used for a second pollination. Because these IvP-pollinators are homozygous for the marker ‘embryo-spot’ (Hermsen and Verdenius, 1967), the seeds resulting from counterfeit pollination could be separated from those resulting from the first pollination. In diallel crossing, at least 10 pollinations per combination were made, using flowers from two or more inflorescences in the case of both normal and counterfeit pollination methods.

Besides normal and counterfeit pollinations, “prickle pollinations” (pollination with only IvP-pollen) were made in order to use them as controls for determining the production of spontaneous spotless seeds in such female plants. These particular spotless seeds are believed to be normally the result of diploid or haploid parthenogenesis, the latter leading to monohaploid offspring (Van Breukelen et al., 1977; Uijtewaal et al., 1987).

Statistical analysis

Data on the number of pollinations, obtained berries and number of seeds were analysed with the computer programme Statgraphics Plus v 7.1.

The number of spotless seeds after an incompatible cross plus counterfeit pollination with IvP pollen was corrected within each SI-group by subtraction of the number of spotless seeds produced after pollination with only IvP-pollen.

Selections of spotless seed samples were sown and the seedlings were tested for the accuracy of embryo spot detection, for plant performance and the expression of SI/PC/SC. The statistical analysis of the production of spotless seeds was, however, based on the determination of spotless seeds and not on seedlings without nodal band.

Simultaneous analysis of pollen and style effect was performed on “within incompatibility group” level. On total population level, the analysis of the main effects of pollinator (pollen parent) or recipient (seed parent) was performed separately. Here, pollinator or stylar effect means per clone out of the over-all analysis were consecutively added four times as covariates in an iterative approach after an initial separate analysis. The analysis on the over-all level was also performed by adding within-group means as covariate.

Classification of genotypes for S-alleles

Four criteria were used for the classification of S-allele genotypes and their SI reaction:

- 1) the S-alleles were identified by iso-electric focusing of stylar extracts with polyacrylamide gel electrophoresis (PAGE) or precast agarose gels;
- 2) PAGE results were verified through test crossing and vice versa;
- 3) the extent of pollen tube growth in the pollinated styles was monitored under a fluorescent microscope;
- 4) berry and seed set were evaluated after selfing as well as after crossing with tester genotypes.

Biochemical identification-PAGE

Iso-electric focusing of stylar extracts with PAGE was performed as described by Kirch et al. (1989) or by means of pre-cast agarose gels (Hypure gel VG 1020, Isolab inc.) following the silver staining procedure based on Tungstosilicic acid in stead of sodium permanganate, according to company specifications.

Pollen tube growth in styles

Pollen tube growth in styles was studied according to the modified technique of Martin (1959). Briefly, the technique was as follows: receptive styles were pollinated; 48 h later, they were fixed in freshly prepared 3:1 solution of ethanol acetic acid for a day or longer; macerated with 8N sodium hydroxide solution at 65 °C for

at least 8 min.; rinsed with water; stained with 0.1% aniline blue dissolved in 0.1M potassium pyrophosphate; softened styles were mounted in glycerol and observed under a fluorescent microscope (BG12/4 filter combination in Zeiss microscope).

Estimation of berry and seed set

At least 10 pollinations were made in order to determine whether a genotype was SC or SI. The genotypes that had a high percentage of pollen stainability but failed to set berries on selfing were classified as SI and those that produced berries and seeds in high numbers were considered as SC. Because a SC reaction could result either from the presence of *tS1* or be due to pseudo-compatibility, in ambiguous cases progenies of PC/SC plants were tested in order to verify whether those parents were SC or PC. Berry and seed set were estimated on the basis of seeds per berry, seeds per pollination and berries per pollination. In the case of counterfeit pollinations and the control pollinations with only IvP-pollen, the spotless seeds were separated from those with spots under a binocular microscope and counted.

Pollen stainability was estimated by mounting fresh pollen grains from three flowers, on different dates in each case, in a drop of 2% acetocarmine solution. On an average, 200 pollen grains were counted per assessment.

Results

Performance of the basic populations

Three types of basic populations consisting of selfs, inbreds and the original F1s, that were investigated in the greenhouse for performance are described in Table 1 with indication of their parents and of the genotypes obtained. In all cases, with the exception of the population 6233, the obtained S-allele genotypes of the progenies were fully concurrent with the established genotypes of the parents (Table 1). In the exceptional population 6233, only S2S2 and S2S4 genotypes could be detected, whereas the also expected S4S4 genotype was absent.

There were clear differences in performance (Table 2) among the progeny populations derived from selfs, inbreds and the basic F1s with regard to the average scores for vigour, flowering, fertility, tuberisation

Table 2. Performance of basic populations for the selection of useful heterozygous SI genotypes.

Population	# plants	Vigour	Flowering	Fertility	Tuberization	# useful plants	
						SI	SC
Selfings							
6107	10	2	3	2	1	0	(0)
6108	30	3	3	4	2	0	(−)
6233	11	4	4	4	3	1	(−)
6234	14	5	4	5	4	5	(−)
Inbreds							
6101	30	6	6	7	4	6	(6)
6102	30	5	5	5	5	7	(−)
6103	40	5	5	5	4	9	(−)
6104	30	6	6	8	7	11	(11)
6105	20	6	6	7	6	7	(−)
6106	40	6	6	6	6	7	(10)
6206	30	6	8	7	4	5	(4)
6208	40	7	8	8	3	2	(6)
6536	20	6	6	7	7	6	(6)
6539	20	5	5	7	6	8	(6)
Basic F1's							
6221	40	8	6	8	7	13	(22)
6222	40	8	7	8	7	18	(19)
6223	40	8	7	8	7	18	(15)
6224	23	8	7	8	7	9	(11)
6225	40	8	6	8	7	19	(16)

All characters were scored on an ordinal scale for each plant and the average values are presented in the columns. Ranking: 1 = lacking, 2 = present but bad, 3 = poor, 4 = insufficient, 5 = just sufficient, 6 = sufficient, 7 = satisfactory, 8 = good, 9 = very good; between brackets (): # well performing self-compatible clones, - = not segregating SC-plants

and the number of cripples (plants that were tiny, weak and brittle). In general, the progenies of the basic F1s were superior to the other two categories for all the four parameters estimated. For example, the average scores in the five basic F1 populations (6221 to 6225, Table 2) were consistently higher (with 77 useful plants) than in the 14 populations (with 74 useful plants) derived from the selfs and inbreds. Especially the frequency of useful plants after selfing was low. Because of these differences between the three population types, the progenies of the basic F1s were not only more useful for S-heterozygotes but also for the selection of the S-homozygotes using counterfeit pollination (see later).

Selection of SI and well performing S-heterozygous plants out of the basic populations

The evaluation of the basic populations proved that a majority (407/548) of the plants among them were unfit for selection of S-heterozygotes since they did not meet

the four criteria used for selection (Table 2). In a further round of selection among well performing plants of the populations, a total of 31 useful individuals from different populations were evaluated for SI-expression. All these genotypes were classified on the basis of S-allele composition through both IEF and test crossing. The expected six classes of four different alleles, i.e., S1S2, S1S3, S1S4, S2S3, S2S4 and S3S4 were found. Table 3 presents for all six expected SI classes the average scores of the selected plants for each of the four characters vigour, flowering, pollen shedding (scale 1-10) and pollen fertility (% stainable), together with their SI-expression. A notable feature was that 19 out of 31 of the genotypes that showed a typical SI-reaction (Table 3) were derived from the progeny of the five basic F1s (cf Table 1), whereas only 12 of the genotypes originated from the 10 inbred populations and none from the selfings.

In order to evaluate the strength of SI-expression, all six S-genotypes, consisting of 30 plants in total, were tested for berry and seed set after selfing (Table 3).

Table 3. Performance of selected SI-expressing S-heterozygous genotypes + key identifiers to table 8.

Plant nr.	Key	Genotype	Vigour	Flowering	Pollen fert.	Pollen shed	SI-expression		
							Seeds /	berries /	selfs
6102-16	I	S1S2	7	7	84	6	0 /	0 /	32
6104-09	2	S1S2	8	9	93	8	0 /	0 /	71
6104-21	4	S1S2	7	8	92	9	0 /	0 /	43
6222-05	15	S1S2	8	9	96	9	0 /	0 /	39
6222-39	18	S1S2	9	9	87	7	0 /	0 /	49
6101-11	1	S1S3	7	7	90	8	0 /	0 /	73
6104-19	3	S1S3	7	8	80	7	0 /	0 /	78
6104-23	5	S1S3	8	8	97	9	80 /	1 ⁺ /	148
6223-15	III	S1S3	7	8	78	7	0 /	0 /	30
6225-05	IV	S1S3	8	9	92	8		n.d. (SC)	
6225-15	V	S1S3	8	8	80	8	0 /	0 /	22
6223-40	23	S1S4	9	9	60*	9	0 /	0 /	69
6221-01	8	S1S4	8	8	78	7	0 /	0 /	92
6221-05	9	S1S4	7	9	61*	8	4 /	2 ⁺ /	64
6221-17	10	S1S4	8	8	64	7	50 /	2 /	50
6221-19	11	S1S4	8	8	64	8	42 /	4 /	62
6221-20	12	S1S4	7	9	75	9	0 /	0 /	107
6105-08	II	S2S3	7	8	50**	9	30 /	1 ⁺ /	32
6222-06	16	S2S3	7	9	80	7	150 /	3 ⁺ /	51
6222-24	17	S2S3	8	9	91	8	0 /	0 /	48
6222-40	19	S2S3	9	9	74**	9	0 /	0 /	70
6105-06	6	S2S4	7	7	72	8	61 /	5 ⁺ /	112
6105-15	7	S2S4	7	6	70	8	19 /	2 ⁺ /	52
6223-01	20	S2S4	8	7	82	6	0 /	0 /	41
6223-29	21	S2S4	9	9	79*	8	0 /	0 /	50
6223-39	22	S2S4	9	9	67*	9	0 /	0 /	51
6536-01	24	S3S4	7	9	71*	9	32 /	1 ⁺ /	55
6536-02	25	S3S4	8	9	42**	8	0 /	0 /	55
6536-09	26	S3S4	8	8	64	9	0 /	0 /	52
6221-32	13	S3S4	7	9	82	9	0 /	0 /	71
6221-37	14	S3S4	8	9	46**	8	0 /	0 /	54

Vigour and flowering impression: 1 = extremely bad, 2 = bad, 3 = poor, 4 = insufficient, 5 = nearly sufficient, 6 = sufficient, 7 = satisfactory, 8 = good, 9 = very good, n.d. = not determined; pollen fertility expressed as% acetocarmine stainable pollen; no additional mark = standard deviation between periods: 0 -5, * = 5 - 10, ** > 10. SI-expression: totals of seeds/berly/selfing in the first two years; ⁺ = caused by pollination of very young flowers; SC = self-compatible

A strict self-incompatibility reaction (no berry and seed set) was expected in all plants. However, testing the 30 plants several years, revealed that still nine of them occasionally did set (self)seed, ranging from 10 - 80 seeds per berry (compatible crosses give good berry formation and 150 - 250 seeds per berry). This could often be attributed to pollination of young flowers in which there might still have been an incompletely developed SI barrier (cf. bud-pollination). This phenomenon was considered as a less reliable SI reac-

tion. Excluding these genotypes, the strength of the SI-expression in all others was satisfactory as was evident from the absence of berry and seed set after 30 to more than 100 selfings that were made in different genotypes (Table 3). Berry and seed set in some of the S-heterozygotic plants was an indication for the persistence (genetic transmission) of pseudo-compatibility. As pointed out already in the population section, a majority of the useful S-heterozygotes (viz., serial numbers: 6221, 6222, 6223, 6225) in Table 3

was derived from three of the five populations of basic F1s mentioned in Table 1. This was reflected in the final selection of the clones to be maintained. The populations 6221 and 6225 as well as the populations 6222 and 6224 are the result of reciprocal crosses. The under-representation of the populations 6224 and 6225 does not reflect inferiority but was just a matter of random choice.

Selection of S-homozygous SI plants from the basic populations

All well performing plants in the self and inbred populations, that could have contained S-homozygous genotypes, were investigated. Among those plants, self-incompatible as well as *tS1*-based self-compatible S-homozygous genotypes could be present. Because only the S-homozygotes with self-incompatibility were essential, the basic populations were screened for such genotypes, and those with self-compatibility were discarded. In populations 6108, 6233, 6234 and 6539 (Table 1), S-homozygotes with a typical SI reaction were found. On the other hand, the S-homozygotes from populations 6107, 6208, 6221, 6225 and 6539 were all found to be self-compatible and were, therefore, discarded. Among the self-incompatible S-homozygotes, the population 6108 consisted of less vigorous individuals, and 6539 had high levels of sterility besides poor tuberization; and these were not suitable for the final selection. Only two populations, 6233 and 6234, gave rise to some desirable genotypes with valuable features (Hermesen, 1978c; Hermesen and Olsder, 1974) despite having a relatively poor general performance. Both of these populations were derived from 6105-06 and 6105-08 which were rare cases of seed set upon selfing (Table 1). This seed set was most probably the result of environment-induced PC. The success rate of this type of selfing was not predictable. The performance and S-genotypes of six of the plants selected from the 6233 and 6234 populations are presented in Table 4. Although the selected genotypes were not completely satisfactory in performance, they were typically self-incompatible, and initially useful as testers. A greater disadvantage of these successful populations was that only S2S2 and S3S3 S-homozygotes were obtained. For the selection of more vigorous S2S2 and S3S3 genotypes and of S1S1 and S4S4 homozygotes as well, a more effective method of bypassing SI, using many genotypes within an incompatibility group, was required. This was done through counterfeit pollinations.

Production of superior homozygotes from selected S-heterozygotes

All genotypes mentioned in Table 3 have been used in some way, trying to obtain more S-homozygotes. Not all the genotype combinations that were made for this purpose have been used for the screening and selection of S-homozygotes, particularly because of suspected expression of PC in the offspring or (expected) inbreeding depression. Some parental clones that have been used were excluded from the statistical analysis because of (re)appearance of SC in the selected clones or retarded growth problems, leading to bad synchronization of flowering. Five clones were excluded because of virus infection, resulting in too few observations to be of use for statistical analysis. Those genotypes were indicated by 'Roman numbers' in Table 3. Four of them gave useful S-homozygotes though (Tables 4 & 8). The PC genotypes with occasional seed set after young flower pollination were included in this experiment. Unlike the selection of S-heterozygotes, it was generally much more difficult to create S-allele homozygotes from genotypes that showed the typical SI reaction. The problem in these genotypes was to obtain seeds from selfing, or from intercrossing within an incompatibility group. These difficulties were largely overcome by making a large number of pollinations, followed by counterfeit pollinations.

For making the counterfeit pollinations, different genotypes within each S-incompatibility group were selfed and intercrossed, following the general crossing scheme exemplified for S2S3 genotypes (A, B and C) in table 5. This scheme was applied for all possible six S-heterozygous groups. One set of these crosses was carried out without counterfeit pollination (normal incompatible pollination) and the other identical set with counterfeit pollination (normal + counterfeit pollination); this means that 48 hrs after the incompatible cross, a second pollination was carried out but now with pollen from the IvP marker genotypes of *S.phureja*. In this context, approximately 4300 pollinations were made. These pollinations included three groups of within group incompatible crossing: 1) selfing a SI genotype (Self, in Table 5: bold), 2) the same genotype used as seed parent (SP, Table 5: row) with various non-self pollinators (non-bold) and 3) the same genotype used as pollen parent (PP) (Table 5: column) in non-self crosses. These pollinations yielded, approximately, a total of 1100 berries with 125000 seeds of which 9500 were without embryo-spot.

Table 4. Performance of selected SI S-homozygous genotypes derived from selfed SI clones.

Plant	Genotype	Vigour	Flowering	Pollen fert.	Pollen shed	SI		
						Seeds/	berries/	selfs
6233-11	S2S2	5	5	55**	5	0 /	0 /	70
6234-05	S2S2	5	7	75**	5	0 /	0 /	50
6234-12	S2S2	5	5	50**	5	0 /	0 /	75
6234-01	S3S3	5	5	60**	4	0 /	0 /	50
6234-08	S3S3	6	6	74**	6	0 /	0 /	90
6234-10	S3S3	6	6	41**	5	0 /	0 /	50

The scale of values is ranging from 1 (extremely bad) to 9 (very good). ** = Standard deviation > 10%. SI reaction as seeds/berries/selfed flowers (totals).

Table 5. Crossing scheme of the counterfeit pollination experiment for the production of S-homozygotes

▼ Seed parent	► Pollen parent	Normal incompatible crossing			Normal + Counterfeit pollination			Control IvP
		A	B	C	A	B	C	
A: S2S3		X	X	X	X	X	X	X
B: S2S3		X	X	X	X	X	X	X
C: S2S3		X	X	X	X	X	X	X

A, B and C belong to the same S-allele combination group, e.g. S2S3. **Bold X** = selfing. Control with IvP = pollination with IvP-pollen as in counterfeit pollination but without an incompatible first pollination.

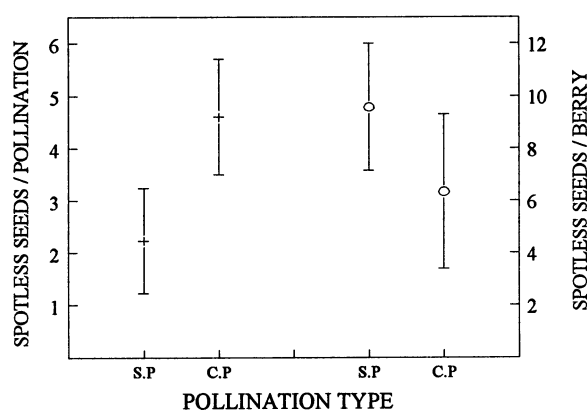


Figure 1. LSD analysis for set of spotless seeds after single normal incompatible pollination (S.P.) or counterfeit pollination assisted incompatible crossing (C.P.).

Y-axes: Left (+): spotless seeds / pollination; Right (0): spotless seeds / berry. + and 0: means. Capped error bars: 0.95% confidence intervals. Based on 473 corrected means.

In order to assess of the strength of the incompatibility reaction and to determine whether the number of spotless seeds resulting from the counterfeit pollinations was different from the number of spotless seeds obtained from the control pollinations (styles pollinated only with pollen from the marked IvP clones), a statistical analysis was performed (Table 6). The occurrence of spotless seeds from prickly pollination alone

(based on LSD-values) was statistically not significantly different from zero, but significantly different from the counterfeit pollination effect (Table 6). This was evident regardless of the consideration of parental effects. The occurrence of the number of spotless seeds after counterfeit pollination or normal single cross was calculated and analysed in two ways, viz., per pollination and per berry (Table 6). Analyses within S-genotype groups and the iterative approaches of the determination of the main effects gave basically the same (nearly identical) results. One conclusion was that the number of spotless seeds/berry was twice as high as the number of spotless seeds/pollination (Figure 1). From these calculations, it was evident that counterfeit pollinations gave rise to more seeds (spotless) that were potentially S-homozygotes than single pollinations in incompatible genotypes. The other difference was that in the case of counterfeit pollinations more berries were obtained and had to be extracted.

Despite a strong selection for typical self-incompatible genotypes, it was evident that certain individuals occasionally set seed after selfing. This was an indication for the occurrence of pseudo-compatibility due to the influence of either the male or the female parent. The genotype 6222-40, for example, was an instance of showing PC effect from the seed parent and 6222-06 from the pollen parent (Table 7). The

Table 6. Analysis of variance (ANOVA) in counterfeit pollination experiment using spotless seed production per pollination or per berry.

Spotless seeds/pollination					Spotless seeds/berry				
Main Eff	d.f.	S.S	F	γ	Main Eff	d.f	S.S	F	γ
Seedp.	24	4293.17	2.82	0.0000	Seedp.	24	13530.17	4.02	0.0000
Phu	1	558.09	7.68	0.0061	Phu	1	2149.44	15.32	0.0001
Residual	207	15039.48			Residual	207	29037.51		

Left: analysis of data set based on spotless seeds/pollination; right: ditto, on spotless seeds/berry.

Main Eff. = Main effect; Seedp. = seed parent; Phu = counterfeit/prickle pollination with IvP's; d.f = degrees of freedom; S.S= Sum of squares; F= (S.S. main effect/df)/(S.S. residual/d.f); γ = significance level.

Table 7. Strength of the SI reaction in selfings and intercrossings within incompatibility groups of selected SI-expressing S-heterozygous genotypes with or without using counterfeit pollination.

Plant	Key	Normal incompatible crossing			Normal + Counterfeit pollination		
		Self	SP	PP	Self	SP	PP
6104-09	2	9	9	9	8	8	8
6104-21	4	9	8	9	8	7	8
6222-05	15	8	9	7	5	7	9
6222-39	18	9	8	9	8	9	6
6101-11	1	9	9	6	8	9	7
6104-19	3	9	9	9	8	5	9
6104-23	5	8	7	8	8	8	8
6221-01	8	8	9	8	8	9	9
6221-05	9	7	6	8	7	7	8
6221-17	10	sc	8	sc	sc	7	sc
6221-19	11	7	8	8	8	7	8
6221-20	12	9	6	9	8	8	9
6223-40	23	9	9	5	8	9	7
6222-06	16	4	6	3	5	8	4
6222-24	17	7	6	4	7	8	6
6222-40	19	9	5	8	4	5	8
6105-06	6	8	7	8	7	7	8
6105-15	7	8	8	6	8	9	8
6223-01	20	8	9	7	8	8	9
6223-29	21	7	7	6	6	8	7
6223-39	22	8	7	7	7	7	8
6536-01	24	8	9	8	8	98	
6536-02	25	9	9	9	8	9	9
6536-09	26	9	9	9	8	8	9
6221-32	13	9	8	9	8	9	9
6221-37	14	9	8	8	8	7	8

SP = tested as seed parent; PP = tested as pollen parent. Ranking is from "1 = most PC genotype" to "9= most SI genotype", sc = self-compatible.

occurrence of pseudo-compatible genotypes occurred in the progeny in a number of cases, especially when a parental clone (e.g. 6502-38, parent of 1127-14, table 8) showed strong PC (data not shown), clearly indicates the genetic basis of this character. After

excluding such PC genotypes, it was still possible to retain a considerable number of genotypes of all S-allele homozygotes with high levels of vigour, flowering, pollen fertility, pollen shedding and SI-expression (Table 8). Those homozygotes were either hybrids

Table 8. Selected SI and SC homozygous clones derived from SI and SC clones respectively after (in)compatible pollinations using within incompatibility group pollinations or selfings in combination with or without counterfeit pollination.

Plant	Parents	Method	Genotype	Vigour	Fl.	P.F.	P.Sh.	SI-expression as totals of		
								Seeds/	Berries/	Flowers
6496-01 [^]	IV x 3	cf	S1S1	8	9	57**	9	0 /	0 /	60
6496-04 [^]	IV x 3	cf	S1S1	7	8	71**	8	0 /	0 /	64
6499-04 [^]	III x 3	cf	S1S1	7	8	96	7	0 /	0 /	73
1127-14 [^]	6502-38 x 6496-01	ii	S1S1	8	8	88	7	0 /	0 /	58
1130-03	5 x 1	cf	S1S1	7	8	92*	8	0 /	0 /	22
1136-01	12 x 23	cf	S1S1	8	7	65**	7		n.d.	
1136-02	12 x 23	cf	S1S1	7	8	74*	6			n.d.
1136-05	12 x 23	cf	S1S1	9	8	77*	7	0 /	0 /	33
1181-02	G254 x 6496-1	cf	S1S1	8	8	55**	8	0 /	0 /	25
1138-07	16 x 16	cf, se	S2S2	7	7	62**	6	n.d./	1 /	12
1138-08	16 x 16	cf, se	S2S2	7	7	69++	7	0 /	0 /	20
1139-03	17 x 19	cf	S2S2	9	7	63**	7	0 /	0 /	30
1139-05	17 x 19	cf	S2S2	8	8	70*	7	0 /	0 /	30
1140-01	6 x 22	cf	S2S2	8	8	55**	4	0 /	0 /	20
1140-02 [^]	6 x 22	cf	S2S2	7	7	60**	7	0 /	0 /	54
1140-05 [^]	6 x 22	cf	S2S2	7	7	70**	6	0 /	0 /	48
1146-02	16 x 19	cf	S2S2	8	9	90	5	0 /	0 /	22
6499-01 [^]	III x 3	cf	S3S3	8	9	88	8	0 /	0 /	55
1130-01	5 x 1	cf	S3S3	7	3	50**	3	0 /	0 /	4
1138-04	16 x 16	cf, se	S3S3	7	6	64**	7	52 /	2 /	5
1138-08	16 x 16	cf, se	S3S3	7	7	72*	7	12 /	1 /	32
1142-02 [^]	25 x 26	cf	S3S3	8	7	79	7	0 /	0 /	26
1171-01	24 x 24	cf, se	S3S3	7	7	66*	7	0 /	0 /	23
1095-04	23 x 11	ii	S4S4	7	7	69**	6	0 /	0 /	67
1095-06	23 x 11	ii	S4S4	7	7	75**	7	0 /	0 /	71
1134-01	11 x 11	se	S4S4	6	7	78*	8	n.d./	8 /	18
1147-04	23 x 11	cf	S4S4	7	6	71	6	0 /	0 /	12
6539-10[^]	see table 1	ii	S1S1-SC	8	9	99	8	2204 /	10 /	12
1132-07	12 x 10	co	S1S1-SC	5	5	51**	7		n.d	
1132-20	12 x 10	co	S1S1-SC	7	6	75*	5		n.d	

Italic-bold: SC-genotypes. Method= obtained by counterfeit pollination (cf), selfing (se), non counterfeit pollination aided intra incompatibility class pollination (ii) or by a SC-based compatible cross (co). Fl. = flowering, P.F. = pollen fertility expressed as % acetic carmine stainable pollen, P.Sh. = pollen shed. Scales ranging from 1= extremely bad to 9= very good. SI-expr. = self-incompatibility expression expressed as seeds/berries/selfed flowers. n.d = not determined. [^] = vegetatively maintained

(intercrosses within incompatibility group) or selfings. Both groups were obtained with and without the aid of counterfeit pollination. As a result of selection of well performing genotypes, most of the selfing-based genotypes (many of them showing inbreeding depression), have been excluded in favour of hybrid types. Because this population was still excessively large, it was narrowed down. The plants indicated by an asterisk (*) have been selected either on the basis of performance, scale of testing for SI expression and their value for other research topics, or have been selected at random. The S1S1 and S2S2 genotypes and to a lesser extent

the S3S3 genotypes were the most important for the molecular unravelling of the SI-system (Kirch et al, 1989, Eijlander & Ficker, in prep). The S4S4 genotypes are slightly underrepresented; this may partly be due to the low priority of obtaining this genotype, but the number was still lower than expected. Numbers are too small, however, to draw final conclusions on S-genotype-related fitness or certation.

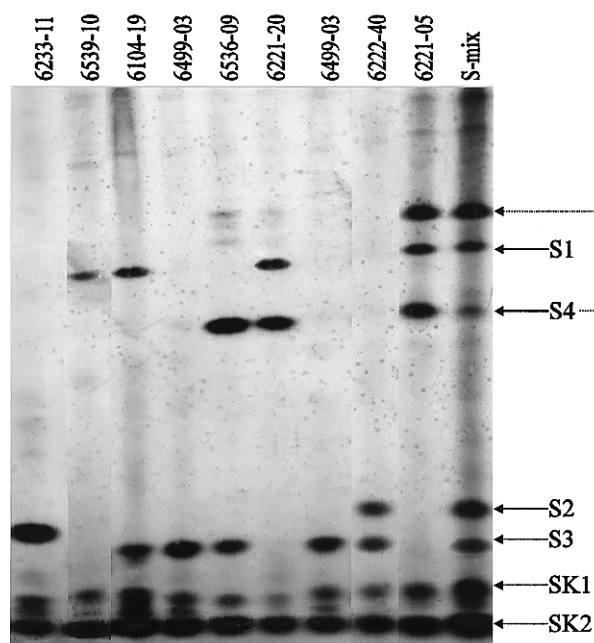


Figure 2. Iso-Electric-Focusing (IEF) pattern of stylar extracts after silver staining of 3 S-homozygotes (6233-11, 6539-19, 6499-03), 5 S-heterozygotes and a control sample. Approximately 15-25 µg protein was added to each slot. SK1 and SK2 are style specific proteins but not S-locus related. S1, S2, S3 and S4 are stylar expressed S-allele specific glycoproteins. S4 gives facultatively a secondary band.

Self-compatibility in S-homozygotes

As mentioned before (Table 1), self-compatible clones were found some of which were S-homozygous. The S2S2, S3S3 and S4S4 self-compatible clones have already been obtained on a routine basis, as is partly shown in Table 1. This was not the case with S1S1. The crosses **IV** (= **6225-05**) x 3 (= 6104-19) and **G254** x 6496-1, (in italics, bold: SC-clones; Table 8) were made with the secondary aim of testing the validity of the assumption made by Olsder and Hermesen (1976) that *tS1* does not cause mutual weakening when together in a (monohaploid) pollen grain with the complete S1-allele. Offspring populations of these crosses did not contain self-compatible S1S1 plants. Progeny of these S1S1 plants proved the absence of the SC-factor *tS1*. Plants 6539-10, 1132-07 and 1132-20, however, proved by IEF (Figure 2) and test crossing to be S1S1 homozygotes, but they were self-compatible (table 8) and capable of fertilizing other S1-expressing plants. The presence of the *tS1*-based SC-clones 1132-

07 and 1132-20 (two S1S1 SC clones out of 20 SC plants, $P(k(n=20, p=1/4) < 2) = 0.09$) proves that even S1-*tS1* pollen is not completely superseded by S4-*tS1* pollen, although a certative disadvantage is very likely.

Maintenance of the selected genotypes by means of in vitro preservation, seed tubers and true seed production

Most of the selected material is maintained in vitro, and the genotypes mentioned in Tables 3, 4 and 8 are also available in the form of seeds and tubers. Seeds were produced by crossing S-heterozygotes with S-homozygotes in order to produce 'single-class' S-heterozygotes, i.e., S1S1 x S1S2 gives rise to a single-class S1S2 heterozygote. Such genotypes are available for all six combinations with the four S-alleles. Besides these, a limited amount of single-class homozygotes (in true seed form), derived from counterfeit pollination, is available for S1S1, S2S2 and S3S3. One group of S-homozygotes, S4S4, was recently lost because of viral infection and is now only present in true seed form. The number of S-homozygous seeds from S-homozygotes with self-incompatibility was relatively small because of the difficulty of producing seed from their well functioning SI, even when counterfeit pollination was used (Table 8).

Discussion

In spite of the presence of a well defined monogenic, multiallelic, gametophytic type of self-incompatibility in diploid potato, the system was not amenable to a critical genetic and molecular analysis in the past for the following main reasons: 1) criteria for the classification of S-allele genotypes were less well defined; 2) well performing genotypes, or testers, of S-homo- and/or heterozygotes were not available and 3) potato being a highly heterozygous crop, inbreeding depression and the expression of deleterious recessive genes in the progeny were serious impediments for analyses. In the present investigation, an attempt was made to overcome the above mentioned drawbacks by the selection of well performing and well defined SI genotypes.

The classification of the genotypes of S-alleles in potato is complicated by the fact that self-compatibility often occurs either due to the so-called pseudo-compatibility or self-compatibilising factors, such as *tS1*, in an otherwise self-incompatible genotype. Clas-

sification of such genotypes in afore mentioned cases on the basis of berry and seed set, together with pollen tube growth studies (Hermsen, 1978 a, b), were relatively subjective in some cases. The identification of S-alleles through IEF (Kirch et al., 1989) combined with studies on pollen tube growth in styles as well as on test crossing was a step forward for a more reliable classification of the S-genotypes. Using a combination of the three criteria, well performing genotypes of both homo- and heterozygous genotypes for four different S-alleles have been selected in the present investigation. Accurate determination of S-allele genotypes through IEF was especially useful for the selection of self-incompatible homozygotes as well as heterozygotes and for gaining more insight into the probable inheritance of pseudo-compatibility.

Olsder and Hermsen (1976) found a complete absence of self-compatible S1S1 homozygotes. Segregation ratios displayed skewness and pollen certification or absence of mutual weakening between the S1 allele and the hypothesised *tS1* was one of the explanations.

Mutual weakening between two identical S2 alleles does not occur (crosses were made with a tetraploidised version of clone 1140-2, $\rightarrow$ S2S2S2S2, not giving a compatible reaction pattern in S2-containing styles, data not shown). This means that by applying the mutual weakening hypothesis on the occurrence of *tS1*-based SC S1S1 genotypes the self-compatibilizing factor *tS1* is *not* expressing the pollen part of the S1 allele but probably an independent gene. This was already suggested by the results obtained by Thompson et al (1991), although they did not rule out the possibility that only the pollen-part was translocated. Re-evaluation of old material of Olsder and Hermsen (1976) by IEF proved the presence of a previously undetected self-compatible S1S1 plant. S1*tS1* pollen has probably a certative disadvantage compared with the other three SC pollen types. This means that their *tS1*-hypothesis is not valid anymore, and one of the other six available hypothesis has to be accepted that was previously rejected because of the absence of self-compatible S1S1-*tS1* genotypes. The expression *tS1* is therefore actually an incorrect one. We have strong indications that self-compatibilizing factors like this '*tS1*' do occur much more frequently than is generally believed.

For producing tester genotypes, such as the S-homozygotes, showing typical SI-expression, it was essential to self the genotypes that showed strong SI-expression. In certain cases, such as 6105-06 and 6105-08 (Table 1), it was rarely possible to obtain berry set

and a limited amount of seeds and progenies. The performance of these progenies with regard to vigour, flowering and pollen fertility (Table 4), however, did frequently not reach the acceptable levels observed in the progenies generated from the basic F1s (Tables 2 and 3). Moreover, the number of really well performing progeny genotypes in the case of 6105-6 and -8 was very low. Obviously, it was necessary to produce more progenies after selfing genotypes that were well performing and showing a strong SI reaction. In other plant species such as *Petunia* and *Nicotiana*, the so-called bud pollinations are practised for producing seeds and progenies from SI genotypes (Pandey, 1963; Shivanna and Rangaswamy, 1969; Clark et al, 1990). This method, however, was not applicable in potato, because the stigma becomes receptive only during anthesis when the exudate becomes available on the stigmatic surface. Self-pollinations, using the pseudo-compatible genotypes for producing seeds and progenies, could be another option for obtaining S-homozygotes.

Flaschenriem and Ascher (1979), Dana and Ascher (1985, 1986a,b) and Liedl and Anderson (1994) investigated aspects of PC in *Petunia hybrida*. This species traces back to interspecific hybrids and displays SC or a high level of PC. They often found strong PC at levels difficult to distinguish from SC. Expression of PC could be at either the pollen or the stylar side. Nevertheless, this system may have more in common with the hybrid system of *S.tbr* x *S.ver*, (like S-locus linked SC-factors and segregation of Unilateral Incompatibility factors) than with the SI system in diploid potato (Eijlander et al, in prep.). Complications and deviations from the normal SI system in hybrids is discussed by Trognitz and Schmiediche (1993). These authors tried to integrate the incongruity hypothesis of Hoogenboom (1973) with the normal gametophytic self-incompatibility hypothesis. Because the type of pseudo-compatibility investigated here seems to be a heritable character, both from the male and the female side, the SI-expression in the progenies might be weaker and the selections will be more frequently unreliable. It has been observed as likely for several crops that PC may be polygenic and heritable (Mather, 1943; Takahashi, 1973; Henny and Ascher, 1976; Litzow and Ascher, 1983) and offers a good explanation why inbreeding can lead to an increased level of PC.

In view of this, it is essential to avoid PC through careful progeny testing in such genotypes so that completely predictable types of self-incompatible genotypes are selected. The problem with bypassing the

SI reaction in the style is that there might be a constant selection for PC expression on the pollen side. Although this is unavoidable, it might be reduced in its effect. For reducing this selection, stylar PC clones can be used when only strong SI at the pollen side is required (and vice versa). The use of PC is out of the question when pollen and style of the desired S-homozygotes are used in test crosses, unless a large progeny can be screened for reliable SI-clones. Additionally, the use of young flowers and at least one strongly SI parent is preferred for the production and selection of SI S-homozygotes. Here the counterfeit pollination with pollen from appropriate clones like IvP 35, 48 and 101 have proven to be of great help for obtaining otherwise extremely rare genotypes. This was also observed for difficult interploidy and interspecific crosses like *S.tbr* x *S.acl* and *S.sto* x *S.tbr* (Iwanaga et al, 1991; Singsit and Hanneman, 1991; Brown and Adiwalaga, 1991).

As was already detected in some of the S-heterozygous genotypes that were highly SI after selfing, there still might be a level of PC present that can only be elucidated by test crossing with other clones. This has extensively been done with the selected S1S1 and S2S2 tester clones. A pitfall with testing S-homozygotes might be that in the style two identical S-alleles will be expressed. Because the SI reaction is a quantitative one, the S-glycoprotein content might be that high that a pollen expressed PC may remain undetected. Nevertheless, even S-homozygotes may be quite PC, as has been detected in genotype 6502-38 (Table 8) and a considerable part of its offspring. Therefore, S-homozygotes have to be tested for PC in crosses on heterozygotes as well. On the other hand, various tetraploid genotypes, that were present in our collection, have been tested for their SI reaction against pollen from diploid relatives, expressing an identical S-allele. A S1S2S3S6 genotype was completely incompatible with S1, S2 and S3 pollen (data not shown), thus indicating that there can be a wide range for S-glycoprotein content in the style and/or the glycoprotein content needed for a reliable SI reaction. However, differences between expression levels of the S-alleles may reduce the efficacy of some of the S-alleles when the weakest are down-regulated, as is suggested by results of Kirch et al. (1989) and Eijlander et al. (in prep). It also indicates that S-homozygous diploids can be quite useful in testing tetraploids.

The term pseudo-compatibility has often been used in other plant species although the definition of this expression is not clear cut. In a broad sense, it has

been considered as 'leakage' of a functional incompatibility system. The criteria for considering a genotype to be pseudo-compatible are generally arbitrary. The basis is, however, the level of seed set in a self-incompatible genotype after self-pollination as compared with the mean seed set of the population, expressed as seeds/berry or the number of seed bearing berries/pollination. Such seed set can also result from a system where a self-compatibilising factor is operative (for discussion, see, Rowlands, 1964; Olsder and Hermesen, 1976; Hermesen, 1978a and 1978b). Pseudo-compatibility has also been observed in several crops after some cycles of inbreeding of self-incompatible genotypes (De Nettancourt, 1977). The mechanism of the origin of pseudo-compatibility in these crops is not clear yet. In the present investigation, as was also reported earlier (Olsder and Hermesen, 1976; Hermesen, 1978a and 1978b), there were genotypes that were difficult to be classified either as PC or SC. They were considered to be PC. Genotype 6221-17 (Table 3) is a typical example of the fact that even SC may be unreliable in its expression. It is quite possible that even this pollen expressed factor is influenced by modifier genes as has been observed for a comparable *S.verrucosum*-derived self-compatibilizing factor (Eijlander, unpublished). The level of seed set upon selfing was initially considered as an indication for a putatively useful level of PC in order to easily obtain S-homozygotes but this proved to be incorrect because of its inheritable character. In any case, all those genotypes that showed seed set upon selfing were eliminated and, as a consequence of this, only typical heterozygous SI genotypes were presumably selected. Such a careful selection was indeed effective as is evident from the fact that a large majority of the genotypes (20 out of 29, Table 3) was strictly self-incompatible after several rounds of selection during different years.

Because of the importance of plant vigour, fertility, avoidance of lethal genes and the high degree of heterozygosity required in the progeny plants, the performances of the basic populations used in this investigation deserve attention. The three original dihaploid clones, G254, G609 and B16 were known to be vigorous, profusely flowering and fertile (Olsder and Hermesen, 1976). The F1 progenies of these clones, on average, performed much better than the progenies obtained from selfings or inbreds (Table 2). Furthermore, the number of S-heterozygotes that were selected originated predominantly (19 out of 31) from the progenies of the original F1s (Table 3) which is even more striking when the ratio of SI/SC is considered.

This clearly indicates that even within a restricted number of genotypes that were used in this investigation, competent SI parents could be selected, giving rise to desirable progenies. From the point of view of the good performance of the progenies of the basic F1s and some of the inbred lines, it should be concluded that the establishment of inbred lines of diploid potato, comparable to those of maize, might be possible.

In view of the recent molecular approaches to elucidate the phenomenon of self-incompatibility in diploid potato (such as gain and loss of function analysis in genetically modified plants), genetically well defined plant material is essential. Part of the material selected in this investigation, which includes both S-homo- and heterozygous genotypes, expressing typical SI reaction, was highly valuable for this research. The two most important factors for using such selected clones as testers are the absence of PC and a good pollen fertility when used as a pollinator. This stresses the need for an extensive screening procedure as described here.

Acknowledgements

Hereby we would like to address our gratitude to Zach Sawor and Hilda Kranendonk for their assistance in the screening and counting of thousands of spotted and spotless potato seeds, to Jan, Jan, Sjaak and Teus for their assistance in the greenhouse and to Professor Dr. J.G.Th. Hermesen for critically reading this manuscript. This research was supported by the European Community 'Bridge Programme' (BIOT-CT-900172).

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