Theor Appl Genet (1986) 72, 66–69

Self-compatibility in doubled haploids and their F_1 hybrids, regenerated via anther culture in self-incompatible *Solanum chacoense* Bitt.

M. Cappadocia *, **D. S. K. Cheng** ** **and R. Ludlum-Simonette**

International Plant Research Institute, 830 Bransten Road, San Carlos, CA 94070, USA

Received September 9, 1985; Accepted October 21, 1985
Communicated by H. F. Linskens

Summary. Homozygous diploid plants originating from pollen of self-incompatible *Solanum chacoense* clone IP33 were analysed genetically. Among the tested individuals, two were self-compatible. As expected, all the regenerants were compatible, as pistillate parent, with the mother clone. However, three plants also displayed compatibility in the reciprocal crosses. Abnormal S-gene behaviour was observed when some androgenetic plants were intercrossed. In addition in the F_1 hybrids between doubled haploids, the S-gene did not appear to function as expected, suggesting that some changes, possibly the generation of new S-alleles, had occurred.

Key words: Anther culture – Homozygous diploids – Self-incompatibility – S-alleles

Introduction

The production of haploids and homozygous diploid lines in species characterized by gametophytic monofactorial self-incompatibility may help in solving some important problems of the incompatibility phenomenon. The homozygous state may be particularly suitable for the analysis of constructive mutations at the S-locus. To date, new S-specificities have never been detected after mutagenic treatments but only after several cycles of obligate inbreeding (for extensive discussions, see de Nettancourt 1977).

The analysis of different S-genotypes in perfectly homozygous materials should permit the determination of whether i) somatic crossing-over within the S-locus is the possible mechanism involved in the generation of new S-specificites (Pandey 1970), ii) activation-inactivation phenomena account for this process (de Nettancourt 1971) or iii) as recently suggested by Devreux and Salamini (1984), transposable elements are involved in the phenomenon.

Pandey (1974) made available the first data on the breeding behaviour of homozygous diploids that he obtained by parthenogenesis in self-incompatible *Nicotiana forgetiana.* Subsequently, pseudogamic monoploids (later doubled by colchicine) have been produced and analysed in self-incompatible dihaploid potato (Van Breukelen et al. 1977; Van Breukelen 1981). Recently, anther culture techniques have allowed pollen-derived plants to be obtained in high numbers from self-incompatible rye (Hoffman and Wenzel 1981), *Nicotiana alata* (Khey-Pour et al. 1983), and dihaploid potato (Wenzel and Uhrig 1981). Genetic analysis of the regenerated plants, unfortunately, has been somehow limited, with the exception of the reports of Pandey (1974); Kheyr-Pour et al. (1983) and Sree Ramulu (1982). This last author has provided evidence that a high proportion of anther culture-derived regenerants in self-incompatible *Lycopersicon peruvianum* carried sexually heritable changes at the S-locus. More recently, Kheyr-Pour and Pernès (1985) also reported on the generation of new S-alleles in the progeny of pollen-derived plants.

In the framework for potato improvement using tissue culture techniques, homozygous pollen-derived plants from self-incompatible *Solanum chacoense* Bitt. ($2n=2x=24$) were produced (Cappadocia et al. 1984). The present paper reports on the compatibility behaviour of these homozygous diploid individuals and their F_1 hybrids.

Material and methods

The plant material used in the present study consisted of a self-incompatible diploid clone (IP33) of *Solanum chacoense* Bitt. noted for its useful agronomic characteristics; androgenetic ho-

---

* Present address: Institut Botanique de l'Université de Montréal, 4101, Rue Sherbrooke Est, Montréal, Québec H1X 2B2, Canada

** Present address: Sungene Inc., Palo Alto, Calif., USA

Table 1. Compatibility behaviour of *Solanum chacoense* Bitt. clone IP33 and its pollen-derived progenies[a]

♀ ♂	IP33	A7	A19	A29	A48	A89	A107	A7×A19	A7×A29	A48×A7
IP33	–	4.1	–	–	MS	44.0	13	9.0	4.0	–
A7	23.5	5.2	17.1	10.5	MS	–	–	19.1	46.5	–
A19	48.0	±	–	±	MS	±	51.0	58.6	/	/
A29	31.0	±	7.0	–	MS	/	/	/	/	/
A48	21.7	19.7	/	±	MS	/	/	/	/	/
A89	31.5	–	/	/	MS	±	–	32.5	–	29.0
A107	16.8	–	/	/	MS	–	6.3	21.1	33.0	–
A7×A19	18.3	–	/	/	MS	–	40.3	1.1	–	31.5
A7×A29	25.6	7.8	/	/	MS	11.2	14.2	36.0	28.2	–
A48×A7	18.6	24.5	/	/	MS	28.0	52.1	16.8	9.0	–

[a] The figures represent the average number of seeds per fruit
– = incompatible; ± = in these crosses pollen tubes were observed at the basis of the styles by fluorescent microscopy but no fruits developed; / = not tested; MS = male sterile

mozygous diploid plants derived from anther culture of *S. chacoense* IP33 according to the methodology previously reported (Cappadocia et al. 1984) and the diploid F_1 hybrids between androgenetic plants. The androgenetic plants were represented by single individuals, while at least 5 plants of the mother clone IP33 and of each type of F_1s were used. Since each type of F_1 originated from crosses between homozygous diploids, all the plants belonging to a given type were assumed to have identical genotypes. Nevertheless, each plant received a specific notation, although in the present paper the results of the crosses involving such plants are given in a cumulative manner. The ploidy of each plant was assessed by chromosome count on root tips as well as by chloroplast count made on stomatal guard cells (Frandsen 1968). Pollen fertility was determined on mature samples stained with 1% acetocarmine. All the plants were grown in semicontrolled greenhouses (temperature ranging from 18 to 26 °C), where self-pollinations and crosses were performed in strict isolation during the summer period under natural light conditions. When necessary, the flowers were emasculated before pollination and at least 10 flowers were used for each type of pollination. Finally, observations on pollen tube growth in styles of pollinated flowers were made for each type of cross using fluorescent microscopy (Martin 1959) and supplemented the data on fruit and seed-set.

Results

Analysis of the mother clone and the androgenetic diploids

The results on the crossing behaviour of the plants examined in the present study as assessed by seed-set and fluorescent microscopy are reported in Table 1. It can be seen that *Solanum chacoense* clone IP33, submitted to selfing, did not produce any fruit. Observations at the UV microscope confirmed the occurence of a typical reaction of incompatibility in the styles of selfed flowers. Pollen stainability was more than 90%. As expected, clone IP33 turned out to be perfectly compatible with all the androgenetic individuals in crosses where it was used as staminate parent. As far as the diploid androgenetic plants are concerned, fertility was a serious problem. Out of the plants raised up to maturity under greenhouse conditions, only a fraction flowered long and abundantly enough to be tested to some degree. The remaining plants either suffered from flower malformations or never set flowers. Pollen fertility, among the tested individuals, varied from as low as 10% in plant A19, up to 85% as in A7. Plant A48 displayed malformations of the anthers and was completely male sterile. Two androgenetic individuals turned out to be self-compatible and set seeds upon selfing, namely plants A7 and A107. Plant A89, on the other hand, never set fruit upon selfing, although observations made by fluorescent microscopy revealed the presence of several pollen tubes at the basis of the styles. These same three individuals were also found to be compatible with the mother clone even when used as staminate parents (Table 1).

When the androgenetic plants were intercrossed, the formation of seeded fruits occurred in a number of cases. Surprisingly, however, crosses such as A19 × A7, A19 × A29 and A7 × A29 failed to produce any fruit although the reciprocal crosses succeeded and observations by fluorescent microscopy revealed that pollen tube growth had proceeded regularly within the pollinated styles.

Analysis of the F_1 hybrids between androgenetic plants

Three types of progeny, resulting from crosses between A7 × A19, A7 × A29 and A48 × A7, respectively, were studied. All plants within each group were extremely similar in morphology, growth habit, leaf shape, etc., and all the plants produced pollen displaying over 60% stainability. All the crosses involving the F_1 hybrids and IP33 resulted in seed-set with the exception of the cross (A48 × A7) × IP33. Observations by fluorescent microscopy revealed that no pollen tubes had grown further than one third the length of the pollinated styles. When the three F_1s were selfed, only the progeny of A48 × A7 expressed self-incompatibility. The majority of crosses involving the F_1 hybrids and either the parents or other androgenetic plants were successful. A few cases of incompatibility (e.g. (A7 × A19) × A7 or A7 × (A48 × A7)), however, were also recorded. Finally, as shown in Table 1, when the F_1 hybrids were intercrossed, seed-set was obtained in 4 cases out of 6. Fluorescent microscopy studies confirmed a reaction of incompatibility in the crosses (A7 × A19) × (A7 × A29) and (A7 × A29) × (A48 × A7).

Discussion

In addition to its importance as a species carrying valuable agronomic traits that the breeder would like to transfer into the cultivated potato (Hawkes and Hjerting 1969), we found *Solanum chacoense* Bitt. a suitable material to study the self-incompatibility phenomenon. Such studies were made possible by the fact that doubled haploids could be obtained in clone IP33 of *S. chacoense* (Cappadocia et al. 1984) and some of them displayed enough fertility to be genetically analysed. Abnormal S-gene behaviour was observed in some of the androgenetic plants. Being that *S. chacoense* was a diploid species characterized by self-incompatibility of the gametophytic monofactorial type, the constitution of the S-locus in our clone IP33 was assumed to be for example S_1S_2. Then, pollen-derived progenies, of the type S_1S_1 and S_2S_2, are normally expected to be incompatible with the parental S_1S_2 styles in crosses of the type ♀$S_1S_2 \times S_1S_1$♂ or ♀$S_1S_2 \times S_2S_2$♂. The results obtained in the present study with the pollen-derived plants show that 3 individuals, namely A7, A89 and A107 out of the 5 homozygous diploids that were tested in reciprocal crosses with the mother clone, were compatible when used as pollen donor.

This result differs slightly from Pandey's report (1974) where all the diploid homozygous plants behaved abnormally with respect to the parent clone and displayed compatibility when used as staminate parents. This remarkable (and unexpected) feature was interpreted as a consequence of a serious disturbance of the S-locus: it being suddenly forced to operate in a perfectly homozygous genetic background (Pandey 1974). In addition, in our experiments, two plants (A7 and A107) displayed self-compatibility, while one plant (A89) did not set seeds upon selfing, although fluorescent microscopy studies excluded that a reaction of self-incompatibility was the cause of such fruit-set failure. Incidentally, this type of phenomenon was also observed when some crosses were performed between androgenetic individuals (see Table 1). In one direction the crosses succeeded, giving rise to seeded fruits, while in the reciprocal crosses no fruits developed. Investigations by fluorescent microscopy in these cases revealed the presence of pollen tubes at the basis of the style. This finding would suggest the presence of post-stylar barriers acting possibly at the time of fertilization or somewhere at the entrance of the pollen tube into the ovary. With regard to the self-compatibility of some of our plants, it should be mentioned that Wenzel and Uhrig (1981) working with double haploids in potato, also found that not all the pollen-derived plants behaved in an identical manner (being either self-compatible or self-incompatible). A similar situation has also been described by Hoffmann and Wenzel who regenerated 14 self-compatible and 4 self-incompatible homozygous individuals from anther culture in rye. Pandey (1974), on the other hand, detected only self-incompatible plants in his analysis. More recently, in the report of Kheyr-Pour et al. (1983), however, no self-compatibility was observed in pollen-derived diploids of *N. alata*. Furthermore, these authors showed that only one type of microspore, those containing the SF11 allele, were capable of regeneration. All these contrasting results, as far as the compatibility behaviour of double haploids is concerned, indicate that more work is required in order to characterize what might be species-specific differences. It should be noticed at this point that most of the reports dealing with double haploids from self-incompatible species mention the fact that many of these plants appear to suffer from variable degrees of reduction in flowering capacity, pollen fertility, flower malformations, etc. which constitute serious problems for genetic analyses (Van Breukelen et al. 1977; Hoffmann and Wenzel 1981; Kheyr-Pour et al. 1983; the present report).

In addition to the exceptional behaviour of the homozygous diploids, our genetic analysis of the F_1 hybrids between androgenetic plants also revealed a number of abnormalities with regard to incompatibility behaviour. Theoretically all these plants should have, at their S-locus, the situation present in the original clone since they are derived from crosses between plants carrying, in a homozygous condition, only one type of the two S-alleles of the mother done and producing, thereafter, only gametes of one S-type. These plants are, therefore, expected to be self-incompatible and reciprocally incompatible with the original clone. The results obtained (Table 1) show that, with few exceptions, this is not the case. In order to properly interpretate these results – that strongly suggest the presence of new S-specificities – crosses with appropriate tester stocks should be performed. In this way, the generation of new S-alleles has been detected in the progeny of pollen-derived plants (Kheyr-Pour and Pernès 1985) or anther-derived plants (possibly pollen-derived) (Sree Ramulu 1982). Unfortunately there are, at the present time, no tester stocks available in *S. chacoense* although efforts in this direction are underway (Cappadocia, in preparation).

In conclusion, the results obtained in the present study using a limited number of plants (which only allow qualitative evaluations) show that the genetic background of doubled haploids affects the proper functioning of the S-alleles and that this status of disturbance is also maintained in following generations. The analysis of the plants obtained with tester stocks of *S. chacoense* containing known S-alleles should help in evaluating the new S-specificities possibly present in the regenerated material.

References

Breukelen EWM van (1981) Pseudogamic production of dihaploids and monoploids in *Solanum tuberosum* L. and some related species. Agric Res Rep 908: 121

Breukelen EWM van, Ramanna MS, Hermsen JGT (1977) Parthenogenetic monohaploids ($2n = x = 12$) from *Solanum* *tuberosum* L. and *S. verrucosum* Schlechtd and the production of homozygous potato diploids. Euphytica 26:263–271

Cappadocia M, Cheng DSK, Ludlum R (1984) Plant regeneration from in vitro culture of anthers of *Solanum chacoense* Bitt. and interspecific diploid hybrids *S. tuberosum* L. × *S. chacoense* Bitt. Theor Appl Genet 69: 139–143

Devreux M, Salamini F (1984) Self-incompatibility and transposable elements. Incompatibility Newslett 16:2–3

Frandsen NO (1968) Die Plastidenzahl als Merkmal bei der Kartoffel. Theor Appl Genet 38: 153–167

Hawkes JG, Hjerting JP (1969) The potatoes of Argentina, Brazil, Paraguay and Uruguay. Oxford University Press, London Oxford, p 525

Hoffmann F, Wenzel G (1981) Self-compatibility in microspore-derived doubled-haploid rye lines and single grain selection for alkylresorcinol content. Theor Appl Genet 60: 129–133

Kheyr-Pour A, Dang Ha DB, Pernes J (1983) Genetics and morphology of true homozygous self-incompatible genotypes regenerated from individual microspores in *N. alata.* In: Mulcahy DL, Ottaviano E (eds) Pollen: biology and implications for plant breeding. Elsevier Biomedical, New York Amsterdam Oxford, pp 303–309

Kheyr-Pour A, Pernès J (1985) New S-allele, S-specific proteins and other data in favour of the oppositional model in the gametophytic system of *Nicotiana alata.* Incompatibility Newslett 17:70–76

Martin FW (1959) Staining and observing pollen tubes in the style by means of fluorescence. Stain Technol 34: 125–128

Nettancourt D de (1977) Incompatibility in angiosperms. Springer, Berlin Heidelberg New York, p 230

Nettancourt D de, Ecochard R, Perquin MDG, van der Drift T, Westerhof M (1971) The generation of new S-alleles at the incompatibility locus of *L. peruvianum* Mill. Theor Appl Genet 41: 120–129

Pandey KK (1970) Elements of the S-gene complex. 6. Mutations of the self-incompatibility gene, pseudo-compatibility and origin of new incompatibility alleles. Genetica 41:477–516

Pandey KK (1974) Elimination of heterozygosity and efficiency of genetic systems. Theor Appl Genet 44: 199–205

Sree Ramulu K (1982) Genetic instability at the S-locus of *Lycopersicon peruvianum* plants regenerated from in vitro culture of anthers: generation of new S-specificities and S-allele reversions. Heredity 49:319–330

Wenzel G, Uhrig H (1981) Breeding for nematode and virus resistance in potato via anther culture. Theor Appl Genet 59:333–340