Mutability of the self-incompatibility locus and identification of the S-bearing chromosome in Nicotiana alata

A.J.G. van Gastel

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Dit proefschrift met stellingen van Antonius Johannes Gerardus van Gastel, landbouwkundig ingenieur, geboren te Gorssel op 20 februari 1945, is goedgekeurd door de promotoren, dr.ir. J.H. van der Veen, hoogleraar in de erfelijkheidsleer en dr. D. de Nettancourt, maître de conférances aan de Université Catholique de Louvain.

De Rector Magnificus van de Landbouwhogeschool,
J.P.H. van der Want

Wageningen, 8 maart 1976

A.J.G. van Gastel

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr.ir. J.P.H. van der Want, hoogleraar in de virologie,
in het openbaar te verdedigen
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Abstract

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Also: Doctoral thesis, Wageningen.

γ rays, X rays, fast neutrons and ethyl methanesulfonate (EMS) were used for inducing mutations at the self-incompatibility locus of *Nicotiana alata*.

Chronic gamma irradiation and EMS treatment neither induced self-compatibility mutations nor led to changes from one S allele to another. X rays and fast neutrons induced many self-compatibility mutations, but did not generate new self-incompatibility alleles.

Triploid individuals were male sterile.

Tri(S)allelic aneuploid plants were self-incompatible because heterogenic di(S)allelic pollen grains are not functional.

Self-compatibility in pollen-part mutants with and without a centric fragment was explained by complementation of the mutant S allele by a fragment or duplication. Deviations of expected segregation ratios were explained by lethality of S homozygotes.

It was shown that the longest unsatellited acrocentric chromosome is the S-bearing chromosome.

Keywords: self-compatibility mutations, ionizing radiation, ethyl methanesulfonate, triploids, aneuploids, complementation.

This thesis will also be published as Agricultural Research Reports 852.

Stellingen

1. Men kan niet verwachten dat, door gebruik te maken van "competitive interaction", het chromosoom waarop de S-locus ligt kan worden geïdentificeerd.

   Dit proefschrift.

2. De complementatietheorie geeft de beste verklaring voor de zelf-compatibiliteit van "pollen-part" mutanten.

   Dit proefschrift.

3. Er moet meer aandacht besteed worden aan het ontstaan en de inductie van nieuwe incompatibiliteitsallelen.

   D. de Nettancourt (1972). Genet. agrar. 26: 163-216.
   Dit proefschrift.

4. In individuele planten van een inteeltpopulatie, ontstaan door zelfbevruchting van een diploïde zelf-incompatibele plant, kunnen soms meer dan twee S-allelen tot expressie komen.

   A.J.G. van Gastel en D. de Nettancourt (1975). Incompatibility Newsletter 6: 66-69.

5. Recente onderzoekingen hebben niet kunnen bevestigen dat elk S-allel een specifiek peroxydase-isoënzympatroon geeft.

   K.K. Pandey (1967). Nature 213: 669-672.
   G.M.M. Bredemeijer (1976). Voordracht, Workshop on the use of ionizing radiation in agriculture, Wageningen.

6. Bij de genetische bestrijding van de uievlieg dient naast het gebruik van translocaties en de steriele insectentechniek aandacht besteed te worden aan andere methoden.

7. De adventiefspruitmethode dient ook gebruikt te worden bij de mutatieveredeling van generatief vermeerderde gewassen.

8. Alvorens eigenschappen van de nucellus ook aan de integumenten toe te kunnen schrijven moet meer onderzoek gedaan worden.

M.S. Ramanna and M.C.A. Mutsaerts (1971). Euphytica 20: 145-151.
H.J. Wilms (1974). In: H.F. Linskens (ed.), Fertilization in higher plants, North-Holland publishing company, Amsterdam, p. 155-160.

9. De uitspraak, dat genetische transformatie bij hogere planten een eenvoudig en snel middel is voor de verbetering van rassen, berust op onvoldoende wetenschappelijke gronden.

K.K. Pandey (1975). Incompatibility Newsletter 6: 91-121.

10. Dat de verhoging van de prijs van bijensuiker de nekslag voor de Nederlandse imkerij zou zijn, is niet juist en wordt alleen geponeerd door imkers die hun hobby op te commerciële basis bedrijven.

A.J.G. van Castel
Mutability of the self-incompatibility locus and identification of the S-bearing chromosome in Nicotiana alata

aan hen die zich over het verschijnen van dit proefschrift verheugen

Voorwoord

In de eerste plaats wil ik mijn ouders bedanken die mij in staat hebben gesteld een opleiding aan de Landbouwhogeschool te volgen.

Mijn promotor prof. J.H. van der Veen wil ik dankzeggen voor de stimulerende belangstelling tijdens het onderzoek. Vele uren heeft U besteed aan het doornemen en bewerken van de talrijke manuscripten die aan dit proefschrift vooraf gingen. Uw kritische op- en aanmerkingen hebben bijgedragen tot het tot stand komen van het proefschrift in de huidige vorm.

Mijn promotor dr. D. de Nettancourt ben ik veel dank verschuldigd voor de geweldige begeleiding van het onderzoek. De eerste twee jaar hebben we zeer nauw samengewerkt. Jij hebt mij ingewijd in de kneepjes van het wetenschappelijk onderzoek. Tijdens je verblijf in Rome en later in Brussel hebben we altijd erg goede contacten onderhouden. Altijd was je bereid om resultaten te bespreken, manuscripten te bewerken en suggesties voor verder onderzoek te geven. Het is dan ook niet overdreven om te zeggen dat zonder jou dit proefschrift niet tot stand zou zijn gekomen.

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Dr. G.M.M. Bredemeijer wil ik noemen als een goede wetenschappelijke buur. Het geduld waarmee je mijn genetische verhalen over de S-locus aanhoorde en de wijze waarop je de talrijke manuscripten doornam die aan dit proefschrift voorafgingen, heb ik altijd erg op prijs gesteld.

De studenten Martien Beek, Loek van Soest en Jan Simonsma hebben in de vorm van een doctoraal onderzoek hun steen(tje) bijgedragen.

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Ook wil ik niet nalaten om Wim den Dunnen, Adri Kooyman en Hans Schouten te bedanken voor de goede verzorging van de soms moeilijk te kweken tabaksplanten; Rie 't Hart-Versteeg, Wil van Marion-Noorlander en Riet Coenen voor het typen en hertypen van vele manuscripten.

De contacten met Pudoc waren plezierig en leerzaam; ik ben deze instantie zeer erkentelijk voor de correctie van het Engels en de overige hulp bij de uitgave van dit proefschrift.

Last, but not least, wil ik mijn vrouw Elly bedanken voor het ontwerpen van de omslag.

Curriculum vitae

De auteur werd op 20 februari 1945 te Gorssel geboren en bezocht de HBS aan het Stedelijk Lyceum te Zutphen. Hij begon in 1963 met de studie aan de Landbouwhogeschool te Wageningen. In 1970 behaalde hij het ingenieursdiploma in de richting plantenveredeling, met als specialisaties erfelijkheidsleer (verzwaard) en algemene plantenziektenkunde. Sinds oktober 1970 is hij als wetenschappelijk medewerker in dienst van het Instituut voor Toepassing van Atoomenergie in de Landbouw te Wageningen.

Contents

Parts of this work have been published before in the following papers:

D. de Nettancourt, P. Dijkhuis, A.J.G. van Gastel & C. Broertjes, 1971. The combined use of leaf irradiation and of adventitious bud technique for inducing and detecting polyploidy marker mutations and self-compatibility in clonal populations of *Nicotiana alata* Link and Otto. Euphytica 20: 508-520.

A.J.G. van Gastel & D. de Nettancourt, 1974. The effects of different mutagens on self-incompatibility in *Nicotiana alata* Link and Otto. I. Chronic gamma irradiation. Radiat. Bot. 14: 43-50.

A.J.G. van Gastel & D. de Nettancourt, 1975. The sensitivity of the pollen and stylar component of the self-incompatibility reaction to chronic gamma irradiation. Radiat. Bot. 15: 445-447.

A.J.G. van Gastel & D. de Nettancourt, 1975. The effects of different mutagens on self-incompatibility in *Nicotiana alata* Link and Otto. II. Acute irradiations with X-rays and fast neutrons. Heredity 34: 381-392.

1 General introduction

1.1 DEFINITION OF INCOMPATIBILITY

Incompatibility occurs in more than 3000 species (250 genera, 70 families) and is genetically controlled by one (S locus) or more loci. It has been defined as the inability of a fertile seed-plant to produce zygotes after self-pollination (self-incompatibility) or after cross-pollination (cross-incompatibility).

Recently Hogenboom (1973) and Hermsen et al. (1974) suggested that in crosses between individuals of two different species, the role of incompatibility is secondary or absent, but that non-function results from a lack of genetic information in one or both parents (incongruity). On the other hand, several authors have shown that interspecific incompatibility is a function of the S locus (e.g. Pandey, 1973; de Nettancourt et al., 1974).

In view of the controversy on the possible involvement of the self-incompatibility locus in interspecific incompatibility, the term incompatibility is here restricted to intraspecific incompatibility.

1.2 CLASSIFICATION AND IMPORTANCE OF INCOMPATIBILITY SYSTEMS

One can distinguish between heteromorphic and homomorphic systems and between gametophytic and sporophytic systems. Heteromorphic systems are characterized by differences in the morphology of the flowers of different plants (e.g. stylar length, anther height, pollen and stigma morphology). These differences are generally controlled by the same gene that controls the incompatibility reaction or by genes which are linked to the S gene (Ernst, Lewis, Baker, quoted by Dulberger, 1975). In heteromorphic systems, incompatibility phenotypes of the pollen and of the pistil are determined sporophytically, that is to say by the diploid genotype of the maternal and paternal plant.

In homomorphic systems, differences in floral morphology (if any) do not accompany incompatibility. The incompatibility phenotype of the pistil is governed by relationships of dominance of the S alleles in the somatic cells of the pistil. The pollen phenotype in such homomorphic systems depends on the haploid genotype of the pollen grain itself (gametophytic incompatibility) or on the diploid genotype of the paternal plant (sporophytic incompatibility). In the latter case relationships of dominance of the S alleles occur.

Extensive surveys of the features characterizing these different systems have been given by numerous authors (e.g. East, 1929; Stout, 1938; Lewis, 1949b; Bateman, 1952; Linskens, 1965; Lundqvist, 1965; Arasu, 1968; de Nettancourt, 1972).

Self-incompatibility is not only important for its wide occurrence in higher plants, but also because it may complicate selection and breeding. The findings of several authors on the distribution of self-incompatibility can be summarized as follows (de Nettancourt, 1972).

- Self-incompatibility is widespread in all families that contribute species to agriculture.
- Leguminosae and Solanaceae are usually characterized by a gametophytic monofactorial system of self-incompatibility.
- Gramineae generally display a bifactorial gametophytic system.
- Cruciferae and Compositae most often exhibit a system that is sporophytic and homomorphic.
- Linaceae, Primulaceae and many Rubiaceae are sporophytic and heteromorphic.

1.3 STRUCTURE AND FUNCTION OF THE S LOCUS IN MONOFACTORIAL GAMETOPHYTIC INCOMPATIBILITY

Lewis (1960) clearly demonstrated, for the monofactorial gametophytic system, the tripartite structure of the S locus (Fig. 1) with two activity parts controlling the reaction in the pollen and in the style, respectively, and one specificity part determining the specificity and common to both pollen and style. The main arguments for such a tripartite structure of the S locus have been reviewed by de Nettancourt (1972).

That the S locus prevents inbreeding and consequently promotes outbreeding is clearly illustrated by the inhibition of pollen tubes by identity of S specificities in pollen and in pistil.

Different theories have been formulated to explain self-incompatibility. The antigen-antibody theory (East, quoted by Linskens & Kroh, 1967) assumes

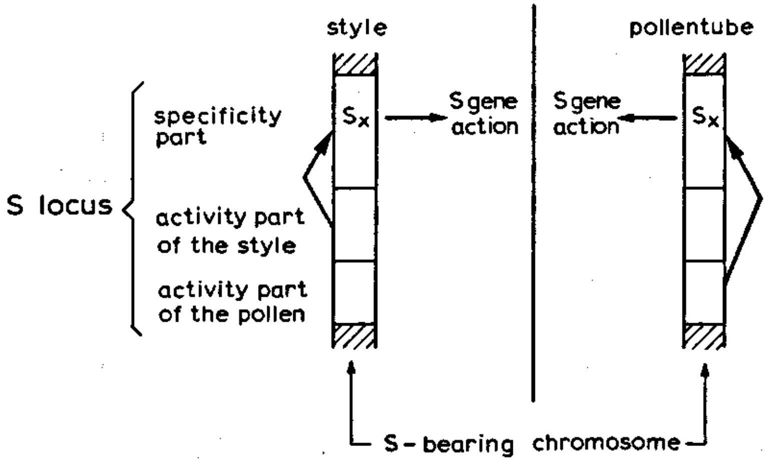


Fig. 1. Structure of the S locus.

that an S allele specific antigen from the pollen tube combines with an S allele specific antibody from the style to produce a complex which directly or indirectly inhibits pollen tube growth. This hypothesis supposes that different incompatibility compounds in pollen and style participate, during selfing, in the incompatibility reaction.

Since one would expect a self-rejection process to be based on the recognition of similar substances in pollen and style, Lewis (1965), Ascher (1966) and Linskens (1968) postulated that identical proteins from pollen and style form a dimer (or tetramer), which acts as an inducer or repressor of a pollen tube growth system.

Incompatibility can also be considered to represent the absence of growing capacity. Hence the enzyme theory of Kroes (1973) assumes that pollen grains that contain a certain S allele, lack an S allele specific enzyme needed by the tube to penetrate the style and to take up essential stylar nutrients. The theory of Kroes (1973) is less likely than the dimer hypothesis because several implications can hardly be accepted (e.g. impossibility to explain self-compatibility mutations as genetic losses or to account for the promotion of self-compatibility by inhibitors).

The incompatibility reaction has recently been subdivided into two phases (see Linskens, 1974):

- the recognition reaction during the early stage of contact with the stigma. This phase involves interaction between pollen proteins and proteins on the stigma (van der Donk, 1974; Heslop-Harrison, 1975), and
- the rejection reaction involving physiological and biochemical processes

resulting in inhibition of pollen tube growth (van der Donk, 1974; Bredemeijer & Blaas, 1975). The evidence of such a two-step process is that even where pollen tubes are inhibited several hours after pollination, a reaction between pollen and pistil can be detected biochemically and microscopically during the first minutes after contact between pollen and stigma.

1.4 MUTATION SPECTRUM AT THE S LOCUS

Because of the complexity of the structure of the S locus and of the incompatibility reaction itself, many genetic and nongenetic modifications can be induced and recorded. In fact, the self-incompatibility locus in monofactorial gametophytic systems is unique for analysis of different types of mutations and physiological changes. These modifications are, when they lead to a breakdown of the incompatibility relationship, automatically screened by the stylar barrier, and selected, out of millions of individual cells, for transmission to the next generation. Because of the many different S alleles segregating at the S locus genetic polymorphism and evolution of incompatibility substances can be studied.

The literature (reviewed by de Nettancourt, 1969, 1972) and my work show that the modifications which can be induced or recovered in the breeding system of self-incompatible plants are of different types. They correspond to the generation of new self-incompatibility alleles, to self-compatibility mutations (stylar-part mutations, pollen-part mutations, revertible mutations), and to pseudo-compatibility (Fig. 2).

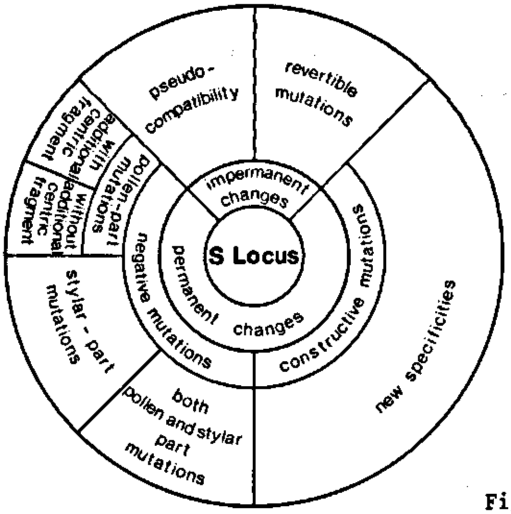


Fig. 2. Mutation spectrum of the S locus.

New S alleles (specificities) result from a constructive (positive) modification in the specificity part of the S locus. They have never been induced by irradiation but can be recovered in inbred progenies (Denward, 1963a, 1963b; de Nettancourt & Ecochard, 1969; Pandey, 1970a, 1970c, 1972; de Nettancourt et al., 1971b, 1975; Hogenboom, 1972b; Anderson et al., 1974; van Gastel & de Nettancourt, 1975). Pollen tubes with such a new specificity can, after self-pollination, penetrate the style and fertilization occurs. In the next generation the new specificity will be active in the pollen and in the style; thus the plants will be self-incompatible (SI). As can be seen from the diagram below, any pollen grain bearing a new S allele is accepted by the style of the plant generating it and is transmitted to the following generation.

S_2S_3 x S_a pollen → S_2S_a + S_3S_a
♀ SI SI

The resulting plants (S_2S_a and S_3S_a) are reciprocally cross-compatible with the original S_2S_3 genotype.

A stylar-part mutation results from a negative change (deletion or inactivation) in the stylar-activity part of the S locus. A style carrying a stylar-part mutation in one of its two S alleles (S_2 or S_3) will no longer be able to inhibit the growth of pollen tubes with the same mutated S allele or with the initial wild-type allele from which the mutation originated.

S_2S_{3sp} x S_2S_{3sp} → S_2S_{3sp} + $S_{3sp}S_{3sp}$
♀ ♂ SC SC

S_2S_{3sp} x S_2S_3 → S_2S_3 + $S_{3sp}S_3$
♀ ♂ SI SI

Of course, a pollen grain carrying a stylar-part mutation at the S locus is incompatible.

S_2S_3 x S_2S_{3sp} is incompatible
♀ ♂

Stylar-part mutations have been reported by Lewis (1951), Lewis & Crowe (1954a), Pandey (1956, 1967, 1970a), Hoffmann (1971) and Hogenboom (1972b).

A pollen-part mutation results from a change in the pollen-activity part of the S locus. Two types of pollen-part mutations have been recorded. One which is associated with the presence of an additional centric fragment in the compatible pollen grain and one which does not display such a fragment. Pollen-part mutations have been recorded by several authors (Lewis, 1946, 1949a, 1951, 1961; Lewis & Crowe, 1954a; Pandey, 1956, 1965, 1967, 1969a, 1969b, 1970a; Brewbaker & Emery, 1960; Brewbaker & Natarajan, 1960; Hoffmann, 1971; Hogenboom, 1972b; Hermsen, 1972).

Part of the pollen produced by artificial tetraploids of species with a gametophytic self-incompatibility system, is self-compatible (Stout & Chandler, 1942; Crane & Lewis, 1942; Lewis & Modlibowska, 1942; Lewis, 1943, 1947; Atwood, 1944; Atwood & Brewbaker, 1953; Brewbaker, 1954, 1958; Emery et al., 1960; Pandey, 1968). It behaves in the same manner as pollen that carries a pollen-part mutation.

A pollen grain carrying a pollen-part mutation is accepted by all plants, including the one that produces it and those carrying the original wild-type allele.

$$\begin{array}{ccccc} S_2S_{3pp}\otimes & \rightarrow & S_2S_{3pp} & + & S_{3pp}S_{3pp} \\ & & SC & & SC \end{array}$$

$$\begin{array}{ccccccc} S_2S_3 & x & S_2S_{3pp} & \rightarrow & S_2S_{3pp} & + & S_3S_{3pp} \\ \female & & \male & & SC & & SC \end{array}$$

A style carrying a pollen-part mutation at the S locus is incompatible with pollen grains from the original plant.

$$\begin{array}{cccl} S_2S_{3pp} & x & S_2S_3 & \text{is incompatible.} \\ \female & & \male & \end{array}$$

Plants combining within one S allele a mutation of the pollen and stylar activity part of the S locus, accumulate the phenotype of these two types of mutations. They have been found by Pandey (1956, 1967) and Lewis & Crowe (1954a). Such plants cannot be distinguished from plants that have lost their specificity segment.

Revertible mutations are, according to Lewis (1951), Lewis & Crowe (1953, 1954a), Pandey (1956, 1959, 1967) and Davies & Wall (1961), temporary mutations of the pollen-activity part which revert back to their original state after one or more cell divisions. Pollen tubes containing a revertible mutation are self-compatible in all styles, including those carrying the original S allele. If so, the activity is usually restored at some stage between fertilization and formation of pollen mother cells in the next generation.

$$\begin{array}{ccccccc} S_2S_3 & \times & S_2S_{3R} & \rightarrow & S_2S_{3R} & + & S_3S_{3R} \\ & & & & \downarrow & & \downarrow \\ \text{♀} & & \text{♂} & & S_2S_3 & & S_3S_3 \\ & & & & \mathrm{SI} & & \mathrm{SI} \end{array}$$

The resulting plants (S_2S_3 and S_3S_3) carry the same specificities (S_2 or S_3) as the original genotype (S_2S_3).

Pseudo-compatibility can be defined as a tendency, which is not due to mutations at the S locus, to produce seeds after incompatible pollinations. The phenomenon is thus basically different from that of revertible mutations. The progenies of pseudo-compatible plants are not necessarily pseudo-compatible and break up into the usual groups of S genotypes which can be expected to occur after selfing.

$$\begin{array}{cccccc} S_2S_3 \otimes & \rightarrow & S_2S_2 & + \; S_2S_3 & + & S_3S_3 \\ & & \mathrm{SI} & \;\;\; \mathrm{SI} & & \mathrm{SI} \end{array}$$

The resulting plants carry the same alleles as the original genotype and both classes of S homozygotes are present.

According to Pandey (1959), pseudo-compatibility should be classified in three groups.

Environmental pseudo-compatibility is caused by external (e.g. temperature) and internal (age) environments of the plant. Delayed pollinations and pollinations at the end of the season, which lead to seed setting (*Nicotiana*: East & Park, 1918; *Brassica*: Kakizaki, 1930; *Lilium*: Ascher & Peloquin, 1966a; *Petunia*: Shivanna & Rangaswamy, 1969), are examples of environmental pseudo-compatibility.

Artificial pseudo-compatibility can be brought about by mechanical, chemical or environmental treatment of plants, flowers or styles. The increase in seed set upon selfing after exposure of plants, flowers or styles to heat shocks (for references see Section 3.4.1), irradiation treatment (Section 3.4.1), CO_2 (Nakanishi et al., 1969; Nakanishi & Hinata, 1973, 1975), hormones, RNA or protein inhibitors (Emsweller et al., 1960; Perquin & de Nettancourt, 1970; Ascher, 1971; Matsubara, 1973; Henny & Ascher, 1975) is a result of artificial pseudo-compatibility. Furthermore, pseudo-compatibility can be induced by bud pollination (e.g. Kakizaki, 1930; Pandey, 1963; Shivanna & Rangaswamy, 1969), electric aided pollination (Roggen et al., 1972; Roggen & van Dijk, 1973), steel-brush pollination (Roggen & van Dijk, 1972), intra-ovarian pollination (Bosio, quoted by Maheshwari, 1950; Kanta, 1960; Maheshwari & Kanta, 1961; Kanta & Maheshwari, 1963; Rangaswamy & Shivanna, 1971) and pollination of decapitated styles (Linskens & Kroh, 1967).

Genetic pseudo-compatibility is governed by genes that influence the activity of the S locus and the strength of the incompatibility reaction (modifiers of the S locus: Lewis, 1943; Mather, 1943; Denward, 1963a, 1963b; Pandey, 1956, 1970a; Townsend, 1966, 1970; Hogenboom, 1968, 1972a, 1972b; Thompson & Taylor, 1971).

Moreover, pseudo-compatibility may be the result of cytoplasmic mutations which affect the products of the S gene (Pandey, 1970a). After segregation of these cytoplasmic units during cell division, expression of the pseudo-compatibility character will be erratic.

1.5 THE S LOCUS IN BASIC AND APPLIED STUDIES

The structure, function and mutation spectrum make the monofactorial gametophytic system of self-incompatibility in higher plants an outstanding material for fundamental research in fields not directly related to self-incompatibility. Interest in the S system in genetics, mutagenesis, radiobiology, radioprotection, biochemistry, evolution and the possibilities of applied research with self-incompatible species that may benefit the plant breeder have been reviewed by de Nettancourt (1972).

1.6 AIMS OF THE INVESTIGATION

This study had the following aims.

- To use the screening system provided by the S locus to compare different mutagens for their capacity to induce mutations at the S locus.
- To test different mutagens for their capacity to induce changes from one

allele to another (new specificities).

- To establish the nature of the self-compatibility in pollen-part mutants.
- To establish cross relationships in triploid and aneuploid individuals.
- To identify the S-bearing chromosome.

Such analyses should contribute to our understanding of the self-incompatibility system, enable us to modify the breeding system and allow us to use the S system as a model for radiation and mutation breeding experiments.

2 S mutations in leaf-propagated material

2.1 INTRODUCTION

De Nettancourt et al. (1971a), Broertjes (1972) and van Gastel et al. (1972) have found that the vegetative propagation technique by adventitious bud formation on detached leaves may induce genetic and nongenetic changes. Because this propagation method was used for producing the experimental plants, it was necessary to establish whether or not S mutations are present among the plants derived by means of this propagation method and, if mutations were present, to determine the type of the mutation.

2.2 MATERIAL AND METHODS

2.2.1 Test species, propagation technique and growing conditions

Nicotiana alata Link et Otto (2n=2x=18) was chosen as test plant (Fig. 3) because its self-incompatibility system is monofactorial and gametophytic (East & Mangelsdorf, 1925) and because its capacity to mutate towards self-compatibility has been clearly demonstrated (Pandey, 1965, 1967, 1969a, 1970a).

In order to deal with a genetically homogeneous population, a clone was established, from a single S_2S_3 plant, by the stem-cutting technique. The seed was provided by Dr K.K. Pandey (Grasslands Division, DSIR, Palmerston North, New Zealand). The material used in the experimental series was obtained by leaf propagation (Fig. 4) (Broertjes et al., 1968).

The homozygous tester clones (S_2S_2 and S_3S_3) needed for identification of S mutations were obtained by bud pollination (Pandey, 1963) of one S_2S_3 flower and multiplied by the stem-cutting technique.

Vegetative propagation was carried out in an air-conditioned greenhouse (temperature 22°C, day; 22°C, night; relative humidity about 95%; natural light conditions). After propagation all plants were transferred to another greenhouse (temperature 22°C, day; 17°C, night; relative humidity 60-70%; natural light conditions).

Fig. 3. The test plant: *Nicotiana alata* Link et Otto.

Fig. 4. The development of roots and plantlets on detached leaves of *Nicotiana alata* (leaf-propagation method).

2.2.2 Pollination procedures and detection of S mutations

To detect changes in the self-incompatibility character, self-pollinations were carried out (3-5 flowers per individual) on 535 plants derived from leaves.

To distinguish between pollen and stylar part mutations, self-compatible plants were crossed reciprocally with the original mother plant (three flowers per plant). Moreover, testcrosses with S_2S_2 and S_3S_3 testers as male parents were carried out. This procedure allows the detection of the allele which carries a stylar-part mutation. However, as it is not possible to detect the mutated allele (S_2 or S_3) of a pollen-part mutant in this way, the inbred progenies of pollen-part mutants were analysed. The S allele which is in homozygous condition in such inbred progenies, is the allele carrying the pollen-part mutation.

To detect changes in S specificity (constructive mutations) 365 self-incompatible plants were crossed reciprocally with the initial mother clone.

2.2.3 Chromosome counts and stomata measurements

Since leaf propagation sometimes produces tetraploid plants (Broertjes, pers. comm.), chromosome counts were made for all self-compatible individuals and for a number of self-incompatible individuals taken at random from the population. Root tips were treated for 30 min in a saturated aqueous solution of alpha-bromonaphthalene, 6 min hydrolysed in 1 N HCl at 59°C and stored in 70% alcohol. Counts were made from squashes in 1% orcein (in 45% acetic acid). The same cytological technique was used for ascertaining the presence of an additional centric fragment in diploid self-compatible mutants.

The length of guard cells of ten stomata per plant was measured to identify tetraploids in the population. These measurements were carried out in all self-compatible individuals and in a number of self-incompatible plants taken at random from the population.

2.3 RESULTS

As can be seen in Table 1 the majority of the plants (492 out of 535), obtained by leaf propagation, were clearly self-incompatible. Cytological analysis showed that such plants displayed 18 chromosomes in their root tip cells and were, thus, diploids ($2n=2x=18$). Their average stomata size was 25 µm with a range of 12 to 34 µm (Fig. 5).

Table 1. Type of compatibility and ploidy level in 535 plants of *Nicotiana alata* obtained by leaf propagation.

Type of compatibility	Ploidy level	Number of plants
Self-incompatible	2x	492
Stylar compatible	2x	2
Pollen compatible	2x	3
Self-compatible	4x	37
Self-incompatible	2x/4x[1]	1

[1] Only epidermal cells are 4x.

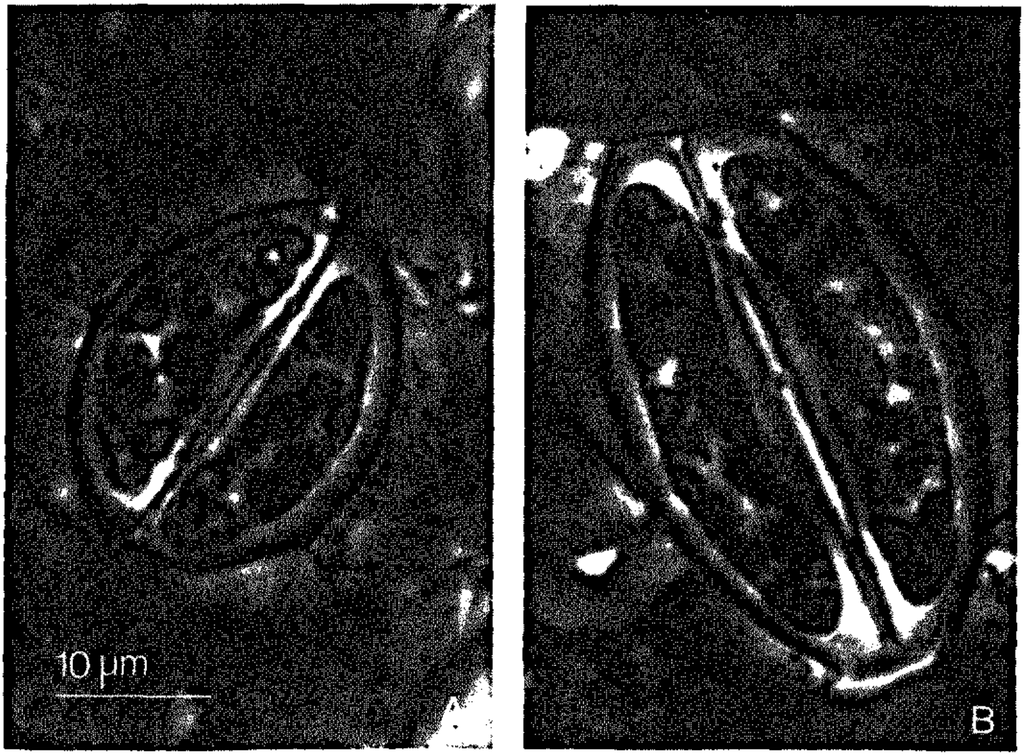


Fig. 5. Stomata size in leaves of (A) diploid and (B) tetraploid plants.

2.3.1 Self-compatibility due to tetraploidy

Forty-two plants were self-compatible. Most of these plants were characterized by thicker stems, broader leaves and larger flowers (Fig. 6) and displayed in the meristematic cells of their root tips 36 chromosomes (2n=4x=36). The stomata size was larger than that of the diploid self-incompatible individuals (Fig. 5) being 39 μm with a range of 29 to 49 μm It proved to be possible to screen the entire population for tetraploidy

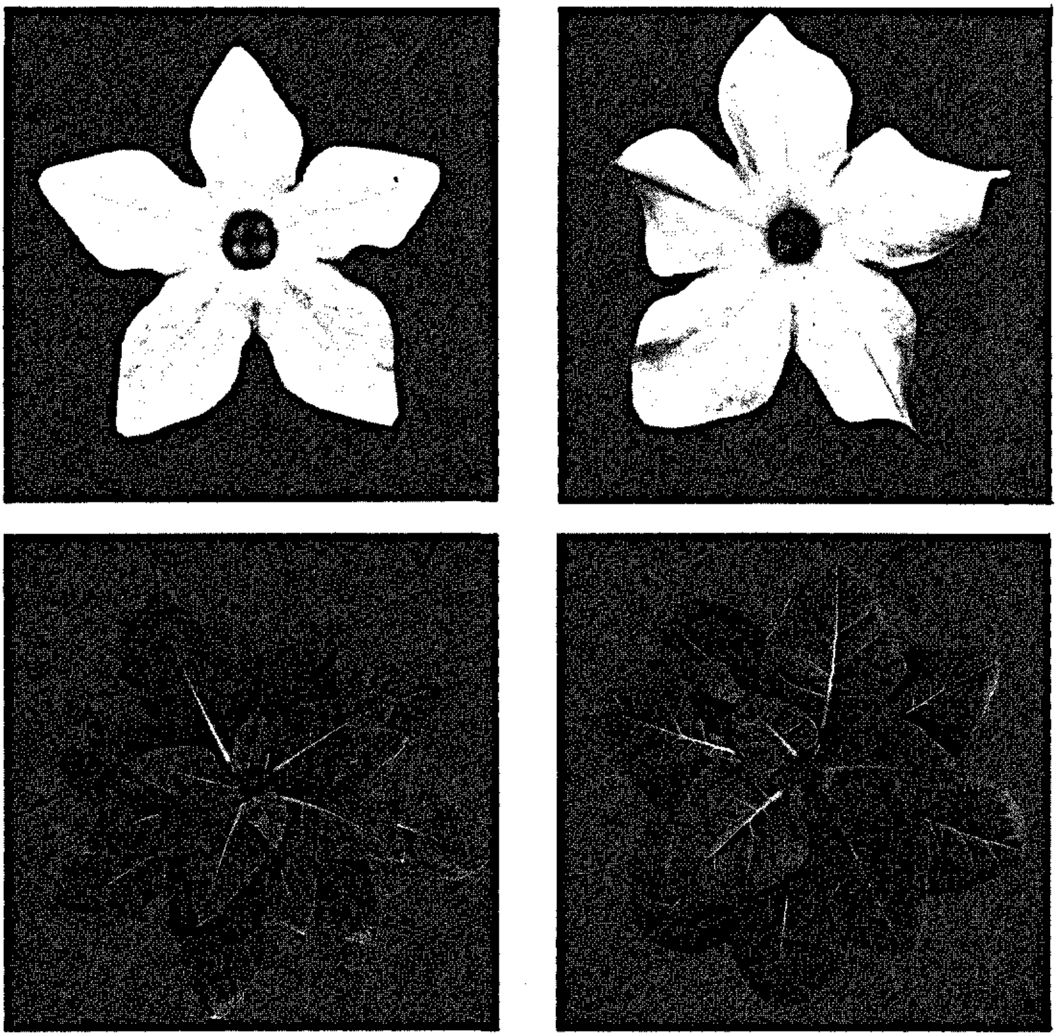


Fig. 6. Diploid (A) and tetraploid (B) plant and flower.

by a simple estimate of leaf and flower size rather than by a cytological investigation.

In reciprocal crosses with the mother clone the 37 tetraploid individuals were cross-compatible only if they were used as males. In other words, they maintain in the style the presence of the original S_2 and S_3 specificities, but they produce self-compatible pollen. All self-compatible plants with the tetraploid phenotype expressed such a type of compatibility.

One individual which clearly displayed the tetraploid phenotype was self-incompatible. This plant was tetraploid in its epidermal cells only, all other tissue investigated (roots and pollen) being normally diploid. The plant represents an interesting example of cytochimerism resulting from leaf propagation.

2.3.2 Self-compatibility in diploid plants

The testcrosses performed on the five diploid self-compatible individuals (Table 2) clearly showed that two of these (SP-1 and SP-2) maintained in the pollen the presence of S_2 and S_3 specificities but that the style had lost the capacity to reject S_2 pollen. Therefore, one can conclude that SP-1 and SP-2 carried a stylar-part mutation associated with the S_2 allele ($S_{2sp}S_3$). Results in advanced progenies confirmed this conclusion.

The three other plants (PP-1, PP-2 and PP-3) maintained the two original specificities in the style but produced compatible pollen. In other words PP-1, PP-2 and PP-3 resulted from a mutation of the pollen-activity part of

Table 2. Results of testcrosses to identify the type of self-compatibility mutation involved. sp = stylar-part mutation, pp = pollen-part mutation.

Mutant	Self-pollination	S_2S_3		S_2S_2		S_3S_3		S genotype
		♂	♀	♂	♀	♂	♀	
SP-1	+	+	−	+	+	−	+	$S_{2sp}S_3$
SP-2	+	+	−	+	+	−	+	$S_{2sp}S_3$
PP-1	+	−	+	−	+	−	+	S_2S_{3pp}
PP-2	+	−	+	−	+	−	+	$S_{2pp}S_3$ or S_2S_{3pp}[1]
PP-3	+	−	+	−	+	−	+	S_2S_{3pp}
Nonmutant	−	−	−	−	+	−	+	S_2S_3

[1] No progeny tests have been carried out to distinguish between these two genotypes.

Fig. 7. Karyotype of a self-compatible mutant with a centric fragment.

the S locus (pollen-part mutation). Testcrosses in advanced progenies showed that the mutation was associated with the S_3 allele in PP-1 and PP-3 (S_2S_{3pp}). The identity of the mutated allele was not determined for PP-2. Cytological analysis of plants in the advanced progenies of the pollen-part mutants indicated that two of these mutants carried an additional centric fragment (Fig. 7).

The five diploid self-compatible mutants represent 0.9% of the total population. In other words 0.9% of the plants derived from leaves carried an S mutation. However, this figure is reduced to 0.4% if one takes into account that not a single S mutation was found during screening of more than 900 plants needed for other experiments.

2.3.3 Constructive mutations

The reciprocal crosses between the initial clone (S_2S_3) and 365 self-incompatible experimental plants, obtained by leaf propagation, did not result in seed set. Therefore, no constructive mutations were generated by leaf propagation.

2.4 DISCUSSION

The fact that only one mutant displayed a chimeric structure provides additional evidence that the apex of each adventitious plantlet can be traced back to cells ultimately derived from a single epidermal cell (Broertjes, 1972). One would certainly have expected many more examples of chimerism if several stem cells in the leaf had participated in the organogenesis of each plantlet or if the mutations had occurred at later developmental stages.

2.4.1 Tetraploidy and self-compatibility

The finding that all complete tetraploid plants produced self-compatible pollen is not unexpected since several authors (Stout & Chandler, 1942; Crane & Lewis, 1942; Lewis & Modlibowska, 1942; Lewis, 1943, 1947; Atwood, 1944; Atwood & Brewbaker, 1953; Brewbaker, 1954, 1958; Emery et al., 1960; Pandey, 1968) have shown that diploid pollen with two different S alleles often behaves as compatible pollen. Such a phenomenon is believed to result from competitive interaction, in the pollen grain, between two different S alleles, which require the same substrate to produce the necessary incompatibility substances.

It is interesting to note that Pandey (1968) found large fluctuations in response between colchicine-induced tetraploid plants of *N. alata* which had identical S genotypes. My results on tetraploid plants (not produced by colchicine but by leaf propagation of the S_2S_3 clone) do not show such variability and confirm Pandey's (1968) conclusion that the variations in self-compatibility which he observed among S heterozygous tetraploids were due to differences in the genetic background or, possibly, to disturbances induced by the colchicine treatment.

2.4.2 S mutations in diploid plants

In addition to the self-compatible tetraploid individuals five diploid self-compatible plants were found (Table 1). Since a comprehensive discussion on pollen-part mutants will be given in Chapter 7 it is sufficient to state here that such pollen-part mutants occur spontaneously in *Oenothera organensis* (Lewis, 1948, 1951), *Prunus avium* (Lewis & Crowe, 1954a), *Trifolium repens* and *T. pratense* (Pandey, 1956), *Nicotiana bonariensis* (Pandey, 1969b) and *N. alata* (Pandey, 1970a). In *N. alata* Pandey (1970a) observed that spontaneous pollen-part mutants do not usually display a centric fragment. This was not observed in my study because two (out of three) pollen-part mutants screened had a centric fragment. The role of such a fragment in the manifestation of self-compatibility is extensively discussed by several authors (Brewbaker & Emery, 1960; Brewbaker & Natarajan, 1960; Lewis, 1961; Pandey, 1965, 1967) and in Chapter 7.

Several authors reported the occurrence of stylar-part mutations in various species such as *Oenothera organensis* (Lewis, 1951), *Prunus avium* (Lewis & Crowe, 1954a), *Trifolium repens* and *T. pratense* (Pandey, 1956), *Nicotiana alata* (Pandey, 1967, 1970a) and *Lycopersicon peruvianum* (Hoffmann, 1971; Hogenboom, 1972b). Most of these mutations have been induced artificially.

It is likely that, not only the tetraploids, but also the pollen and stylar part mutants resulted from the leaf-propagation technique which appears to be mutagenic.

It is most interesting to observe that, in a situation where no selective screening restricts the detection of mutations to pollen-part alterations, both types (2sp and 3pp) were found in a group of five. This indicates that the mutability of the stylar-activity part of the S locus is approximately the same as that of the pollen-activity part. Such an indication could not have been reached by the conventional screening method which scores pollen-part mutations and ignores stylar-part mutations unless they are associated with a change in the pollen-activity part of the same S allele. The detection of stylar-part mutations is further complicated by the fact that, even if they are transmitted to the next generation, they will be expressed only in S heterozygotes ($S_{2sp}S_3$). An S homozygous plant with one allele bearing a stylar-part mutation ($S_{2sp}S_2$) does not express self-compatibility because the other unmutated allele maintains in the style the capacity to reject self-pollen.

In conclusion to this discussion on the occurrence of S changes in leaf-propagated material, the results obtained show that leaf-propagated plants cannot be used in experiments aiming at the induction of S mutations before a selection has been carried out to discard the tetraploids and the self-compatible diploids which appear in clonal populations after leaf propagation. This selection procedure is of a simple nature because all tetraploids can easily be detected on the basis of leaf and flower phenotype, whereas self-compatible diploids can be identified after self-pollination.

Finally it should be noted that the leaf-propagation method is of interest for inducing genomic changes.

3 Chronic gamma irradiation

3.1 INTRODUCTION

As was stressed in Chapter 1 the gametophytic system of self-incompatibility is an outstanding material for testing the relative efficiencies of different mutagenic agents and for classifying mutagens with regard to their capacity to induce self-compatibility mutations and constructive mutations (new S alleles which, so far, have never been induced by means of mutagenic treatments).

Five different types of permanent genetic modifications (see Fig. 2) can occur at the tripartite locus of self-incompatibility. These, with the exception of stylar-part mutations, are automatically screened, at the haploid level, by the stylar barrier. They may be classified, in the subsequent generation, by relatively simple crossing procedures and cytological observations.

This chapter is restricted to an analysis of the effects of low doses of chronic gamma irradiation. Pandey (1970a) suggested that chronic exposure to low dose rates of gamma rays would be ideal for producing labile mutations which may, under favourable genetic background, produce mutations of a constructive nature.

3.2 MATERIAL AND METHODS

3.2.1 Test material and growing conditions

The irradiated and the control series belong to a vegetatively propagated clone derived by leaf propagation (Broertjes et al., 1968). The leaf-propagation method has been found to generate self-compatibility (see Chapter 2) and morphological changes (de Nettancourt et al., 1971a; van Gastel et al., 1972). Tetraploids and visible "mutations" were detected by phenotypic selection, whereas self-compatible diploids were identified after self-pollination. These self-pollinations were carried out on the first flower, one day before the beginning of the irradiation. If these self-pollinations did not result in seed set, the plants were considered to be self-incompatible. The plant material ultimately maintained in the experimental series consisted only of diploid, strictly self-incompatible, individuals.

Temperature in the irradiation greenhouse was maintained at 22°C during the day and 17°C at night, relative humidity remained constant throughout the experiment (60-70%), whereas light conditions were natural. At the dose rates 7.0 and 14.3 rad/h additional light was provided.

3.2.2 Irradiation

Plants of the S_2S_3 clone were arranged in rows in the gamma greenhouse (360 Ci ^{137}Cs) at eight different distances from the sources which correspond to dose rates of 14.3, 7.0, 4.0, 2.5, 1.8, 1.0, 0.6 and 0.3 rad/h. Although the two sources (each corresponding to 180 Ci ^{137}Cs) were spaced one above the other to ensure a homogeneous dose distribution in a vertical direction, between 30 and 130 cm and at any distance greater than one meter from the sources, the inflorescences were held as much as possible at the same height.

All plants were introduced in the greenhouse at the "one flower stage". Control plants were placed behind the concrete wall which shields the sources and where the background was 0.07 rad/h. Except for a daily interruption from 9:00 to 12:00, irradiation was continuous and lasted for three months.

In a later experiment, which was carried out to distinguish between the effects of irradiation on the pollen and effects on the pistil, S_2S_3 plants, reaching the flowering stage, were placed at the following dose rates: 14.3, 7.0, 2.5, 1.0 and 0.07 rad/h. The irradiation lasted for one month.

3.2.3 Pollination procedures and detection of S mutations

To detect possible modifications in the self-incompatibility character of the irradiated plants (five per dose rate) and for transmitting any induced S mutation to the following generation, self-pollinations (50 flowers per plant) were carried out daily in the irradiated series and in the control population. To decrease flower dropping, which might prevent mutated pollen tubes from reaching the ovule before abscission, and to induce swelling of the ovary, so that fruits with low number of seeds can be recovered, pollinated flowers were smeared around the calyx, with 1% naphthalene acetamide in lanolin (Pandey, 1967).

After harvest of the fruits, the seeds (or some of them) present in each fruit were sown and the resulting plants self-pollinated and crossed reciprocally with the original mother clone and with homozygous tester stocks (S_2S_2 and S_3S_3). This procedure, which permits an accurate check on the nature of most S mutations, was repeated three times for each individual.

In order to test the reproducibility of the results and to distinguish between effects on the pollen and effects on the pistil, the second series of experiments was conducted which involved reciprocal crosses between control plants and individuals exposed to chronic irradiation.

Ten plants were exposed at each dose rate and during the irradiation period about ten stigmas per plant were pollinated with pollen harvested from unirradiated S_2S_3 individuals. Reciprocally, pollen from ten flowers of each irradiated individual was collected and used for pollination of unirradiated flowers, the pollen of each irradiated flower being distributed to the stigmas of four unirradiated flowers. As in the selfing experiment, 1% naphthalene acetamide was used to decrease flower dropping and to induce swelling of the ovary.

In this second experiment no attempts were made to determine the nature of the induced seed set because observations (see Section 3.3.2) indicated that, in such cases, the majority of the seeds resulted from impermanent genetic changes (see Section 3.4.1).

3.2.4 Pollen stainability

Although not necessarily identical to pollen fertility, pollen stainability is, from a practical point of view, the easiest method for estimating damages inflicted to pollen grains (pollen abortion ≈ nonstainable pollen). Pollen stainability in the control plants and in the irradiated individuals was estimated in flowers which reached anthesis three weeks after the beginning of the experiment, by staining tests with iodine (Eriksson, 1962). All counts were based on 200 grains per flower and three random flowers of each plant.

Material and methods not described here are presented in Chapter 2.

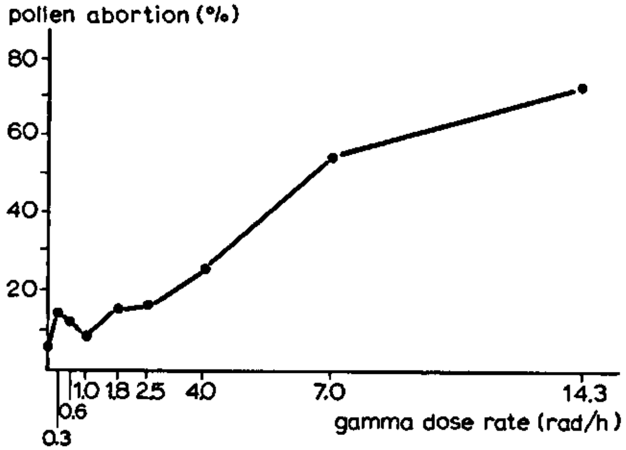


Fig. 8. Relationship between pollen abortion and gamma dose rate. P value with Spearman rank correlation test: < 0.01; alternative hypothesis: increase with dose.

3.3 RESULTS

3.3.1 Irradiation effects in the treated generation

The relationship between pollen abortion and dose rate is presented in Fig. 8. It can be seen that abortion increases significantly with increasing dose rate. At the highest dose rate abortion amounted to 73.1% compared with 5.7% in the control.

Irradiation did not result in a clear decrease in plant height (Table 3). At the highest dose rates flowering capacity was reduced. As a consequence of this reduction, the time required for each plant to produce a total of 50 flowers increased.

The effects of irradiation on fruit and seed yields were particularly striking (Table 4 and Fig. 9) and showed that chronic gamma treatment induced the production of a higher number of seeded fruits per plant. A similar trend was observed in seeds per plant. At the highest dose rates the number of seeds per plant decreased, probably as a result of increased ovule sterility.

3.3.2 Analysis of inbred progenies

In several progenies no flowering plants were obtained or the number of plants tested was rather low (Fig. 9). This was due to the low number of seeds that produced a flowering plant (19.6%).

The results of the testcrosses which were performed on each individual, raised from the seeds obtained after self-pollination of irradiated plants, are presented in Table 5 and Fig. 9. Only one clear S mutant could be

Table 3. Effects of chronic gamma irradiation in the treated generation.

Dose rate (rad/h)	Number of plants	Increase in plant height per plant (cm)	Number of inflorescences per plant	Days needed to produce 50 flowers per plant
0.07 (control)	5	31.0	6.2	35.6
0.3	5	32.0	7.0	34.6
0.6	5	31.0	5.4	36.4
1.0	4	30.0	8.0	35.8
1.8	5	28.0	6.4	36.4
2.5	5	32.0	5.8	36.0
4.0	5	29.0	4.6	35.8
7.0	5	28.0	3.6	39.8
14.3	5	28.0	2.6	43.2
P value with Spearman rank correlation test		0.06	<0.01	0.01
Alternative hypothesis		decrease with dose	decrease with dose	increase with dose

Table 4. Effects of chronic gamma irradiation on fruit and seed yield after self-pollination. (A): seeded fruits per plant, (B): seeds per plant.

	Dose rate	Plant I	Plant II	Plant III	Plant IV	Plant V	$\bar{x}$[1]
A	0.07	1	1	0	3	1	1.2
	0.3	3	3	3	0	0	1.8
	0.6	0	0	2	2	3	1.4
	1.0	-	2	6	4	3	3.8
	1.8	1	14	5	1	0	4.2
	2.5	4	14	3	1	1	4.6
	4.0	3	9	3	8	9	6.4
	7.0	3	9	2	6	2	4.4
	14.3	3	5	1	4	14	5.4
B	0.07	1	3	0	3	1	1.6
	0.3	12	18	4	0	0	6.8
	0.6	0	0	2	2	3	1.4
	1.0	-	4	953	5	5	241.8
	1.8	2	1822	5	1	0	366.0
	2.5	5	3099	4	1	1	622.0
	4.0	3	11	6	1378	1418	563.2
	7.0	3	22	6	7	2	8.0
	14.3	5	5	1	5	576	118.4

[1] For both parameters, P value with Spearman rank correlation test: < 0.01; alternative hypothesis: increase with dose.

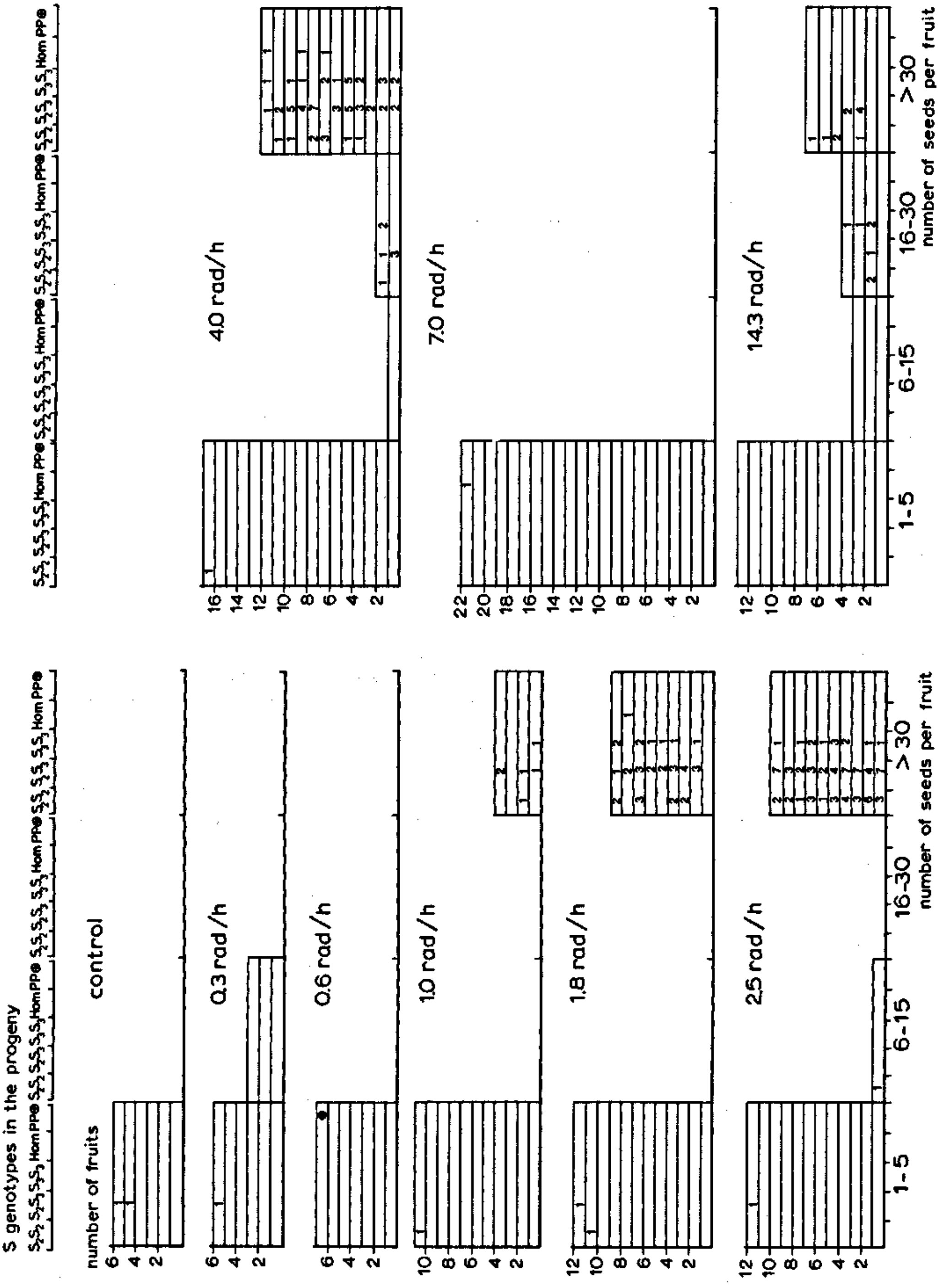


Fig. 9. Distribution of fruit and seed set after self-pollination of gamma irradiated S_2S_3 plants and distribution of S genotypes in the progenies. Plants with erratic behaviour are not included. HOM = S homozygous individual, pp+ = pollen-part with an additional centric fragment (●).

Table 5. Classification of S genotypes in the inbred progenies of gamma irradiated S_2S_3 individuals. HOM = S homozygote, pp = pollen-part mutant.

Dose rate	Number of plants tested	S_2S_2	S_2S_3	S_3S_3	HOM[1]	pp	Erratic behaviour
0.07	2	0	2	0	0	0	0
0.3	1	0	1	0	0	0	0
0.6	1	0	0	0	0	1	0
1.0	8	2	4	1	0	0	1
1.8	41	10	21	8	1	0	1
2.5	91	29	47	12	0	0	3
4.0	74	11	40	20	3	0	0
7.0	1	0	0	0	1	0	0
14.3	18	7	7	4	0	0	0

1. No testcrosses have been carried out to distinguish between S_2S_2 and S_3S_3.

detected in the progenies of the irradiated series (0.6 rad/h). This mutant, which produced more than 100 seeds per fruit after selfing, was cross-compatible as staminate parent with the original clone and the two tester stocks (S_2S_2, S_3S_3) and rejected as pistillate partner, S_2 and S_3 pollen. In other words the plant is an S heterozygote which carries a pollen-part mutation.

Cytological analysis showed that the mutant displayed an additional centric fragment similar to the ones previously described by Pandey (1965, 1967, 1969a) in pollen-part mutants of *N. alata* (see Fig. 7).

In addition, several individuals (entered in the body of Table 5) occasionally produced seeds after selfing. The amount of seeds per fruit was, however, low and most probably resulted from pseudo-compatibility (Pandey, 1959, 1970a). Repeated self-pollinations and testcrosses revealed that such plants were indeed normal self-incompatible S homozygotes or S heterozygotes. Further tests in the advanced progenies confirmed this conclusion.

Finally, five individuals (Table 5) responded erratically to self-pollination and/or to testcrosses and could not be classified accurately. Progeny testing showed that such plants did not carry a permanent mutation of the S locus.

Except for these five plants, the self-compatible pollen-part mutant and five homozygous plants (Table 5), the inbred progenies segregated in 59 S_2S_2 : 122 S_2S_3 : 45 S_3S_3 which does not deviate significantly from the expected 1 : 2 : 1 ratio ($\chi^2_2 = 3.2$; $P \approx 0.20$).

3.3.3 Fruit and seed set after reciprocal crosses between control plants and individuals exposed to chronic gamma irradiation

The data obtained for fruit and seed yield in the second experiment, which was meant to distinguish between effects on the pollen and on the pistil are presented in Table 6. The radio-induction of seed production results from an effect on the pistil component of the self-incompatibility reaction, because in crosses between irradiated pistillate partners and unirradiated males, the increase in seed set is significantly correlated to dose rate and reaches values which correspond to a total yield of more than 30 seeds per plant (14.3 rad/h).

After crosses between unirradiated pistillate parents and irradiated pollen donors, on the other hand, a slight increase in seed set was observed in some experimental series. The absence of seeds at the highest dose rate (14.3 rad/h) cannot be due to pollen sterility, because at this dose rate sufficient fertile pollen was present (see Fig. 8).

Table 6. Effects of chronic gamma irradiation on seed and fruit yield after crosses between (A) gamma irradiated S_2S_3 ♀ and unirradiated S_2S_3 ♂ and (B) unirradiated S_2S_3 ♀ and irradiated S_2S_3 ♂.

Dose rate	Seeded fruits per plant	Seeds per plant
A 0.07	0	0
1.0	0	0
2.5	0.1	0.2
7.0	0.3	3.8
14.3	2.0	34.4
P value with Spearman rank correlation test[1]	< 0.01	< 0.01
B 0.07	0	0
1.0	0	0
2.5	0.3	3.0
7.0	0.2	3.4
14.3	0	0
P value with Spearman rank correlation test[1]	0.22	0.22

[1] Alternative hypothesis; increase with dose.

3.4 DISCUSSION

3.4.1 Irradiation effects in the treated generation

Irradiation had practically no effect on plant height. This is not surprising since all plants were already in the flowering stage at the beginning of treatment. The reduced number of inflorescences and delayed flowering can be understood as resulting from meristematic damage. Similar observations have been reported several times in the literature (e.g. Yamakawa & Sparrow, 1965; Yamakawa, 1966).

The increase of pollen abortion at higher dose rates was expected from the work of many different authors (e.g. Yamakawa, 1966; Yamakawa & Sparrow, 1966).

Since practically no fruits with seeds were obtained in the control series the increase in the number of seeded fruits per plant at the higher dose rates cannot be attributed solely to the hormone treatment. The increase in seed set per plant after selfing is, in contrast to the experiments of de Nettancourt and Ecochard (1968), not only due to an increase in the number of fruits produced per plant but also to an increase in the number of seeds per fruit. Therefore, the phenomenon does not result from an inhibition of floral abscission but rather from a direct effect on either the pollen or the stylar component of the self-incompatibility reaction.

Such an effect, if it results from an action on the stylar component, must be attributed to an inactivation of the S locus in the style or to an inhibition of the incompatibility reaction. It is also possible that the high seed set obtained after self-pollination in the irradiated series originated from radiation-induced impermanent changes in the pollen.

The results of crosses between irradiated females and unirradiated males, and between unirradiated females and irradiated males showed that it is the style that is involved in the induction of pseudo-compatibility by low dose rates of chronic irradiation.

A similar effect on the stylar component of the self-incompatibility reaction has been obtained with a wide range of different treatments consisting of acute X irradiation of mature styles (Linskens et al., 1960; Hopper & Peloquin, 1968), application of hot water before pollination (Bali, 1963; Hecht, 1964; Hopper et al., 1967; Günther et al., 1968) and of slight increases in temperature several days before and after pollination (el Murabaa, 1957; Leffel, 1963; Townsend, 1965; Ascher & Peloquin, 1966b, 1970; Kendall & Taylor, 1969; Chen & Gibson, 1973; Ronald & Ascher, 1975). Although all these treatments, including the chronic exposure to low irradiation doses which was analysed in my study, lead to the same end point (pseudo-compatibility), it is possible, in view of their extreme differences in intensity and application time, that they affect different steps of the incompatibility reaction. During floral development long-term treatments with low irradiation doses or temperature increases probably interfere with the production of incompatibility substances in the style, while acute irradiation doses to open flowers or heat shocks from hot water applications before pollination, most likely inactivate in the mature style one or several stylar substances which participate in the incompatibility reaction (Hopper & Peloquin, 1968; Hodgdon & Hecht, 1975).

If one assumes that irradiation effects are essentially deleterious and inhibitory, the one conclusion which emerges from my study and from those of Linskens et al.(1960) and of Hopper & Peloquin (1968) is that self-incompatibility is not a passive phenomenon resulting from the absence of a growth component but an active process involving specific metabolic activity. A similar conclusion has been reached, from an electron microscopical study (de Nettancourt et al., 1973): although a general inhibition of protein synthesis in incompatible pollen tubes probably constituted one of the main features of the self-incompatibility reaction the entire rejection process could not be attributed to a mere cessation of pollen tube growth.

3.4.2 The efficiency of low dose rates of chronic irradiation for inducing mutations at the S locus

The fact that only one S mutation was found in the progenies analysed shows that chronic exposure is not an effective treatment for inducing mutations at the S locus. The results indicate that one self-compatible individual was obtained following 1950 self-pollinations in the irradiated series.

The low efficiency of chronic irradiation suggests that either repair processes are very active during exposure to low dose rates, or that the sensitive period is so short that the total dose accumulated during this period is almost negligible (de Nettancourt & Devreux, 1969).

Concerning the low number of S mutations recovered, one remark should be made. The possibility exists that more mutations were induced but they are not recovered because only a low number of seeds produced a flowering plant (see Fig. 9). Furthermore, S mutations are usually detected in fruits with a low number of seeds (see Fig. 13). Nevertheless the results indicate that after chronic irradiation the number of S mutants obtained is much lower than after acute treatments (see Chapter 4 and Pandey, 1967).

Pandey (1970a) has suggested that constructive mutations might possibly be induced by low doses of chronic irradiation. Yet, not a single plant was found with a new S allele. This finding strongly suggests that chronic irradiation is as ineffective as acute treatment for producing new alleles at the self-incompatibility locus of higher plants.

4 Acute irradiations with X rays and fast neutrons

4.1 INTRODUCTION

The mutagenic analyses carried out so far have shown that X rays (Lewis, 1946, 1949a, 1951, 1961; Lewis & Crowe, 1954a, 1954b; Pandey, 1956, 1965, 1967, 1969a, 1970a; Brewbaker & Shapiro, 1959; Brewbaker & Emery, 1960; Brewbaker & Natarajan, 1960; Hoffmann, 1971) are considerably more efficient than chronic gamma irradiation (see Chapter 3 and de Nettancourt & Ecochard, 1968) for inducing self-compatibility mutations. However, neither acute nor chronic exposure to X rays or γ rays have the capacity to reconstruct, within the S locus, a new functional self-incompatibility allele (for a review see de Nettancourt, 1972). In other words, ionizing radiation is a useful agent for transforming a self-incompatible plant into a self-compatible one, but seems to be of no use as far as the generation of new specificities is concerned.

As a logical continuation of these investigations, the present study aimed at comparing the mutation spectrum induced by X rays at the S locus to that of fast neutrons, a mutagen with a high RBE (relative biological effectiveness) (Contant et al., 1971) and a specific mutagenicity (Lundqvist & von Wettstein, 1962).

4.2 MATERIAL AND METHODS

4.2.1 Irradiation sources and irradiation doses

X irradiation was applied (Fig. 10) at a dose rate of 20 rad/min with a Philips 250/25 deep-therapy apparatus, operating at 250 kV and 15 mA, with an additional 0.5 Cu, 1.0 Al filter.

The source of fast neutrons was the BARN (Biological Agricultural Reactor Netherlands). Absorbed doses were calculated from the equation $D_p = 1.25 \times D_{N(CH)}$, where D_p is the absorbed dose in rad by the irradiated plant tissue, 1.25 is the correction factor taking into account the

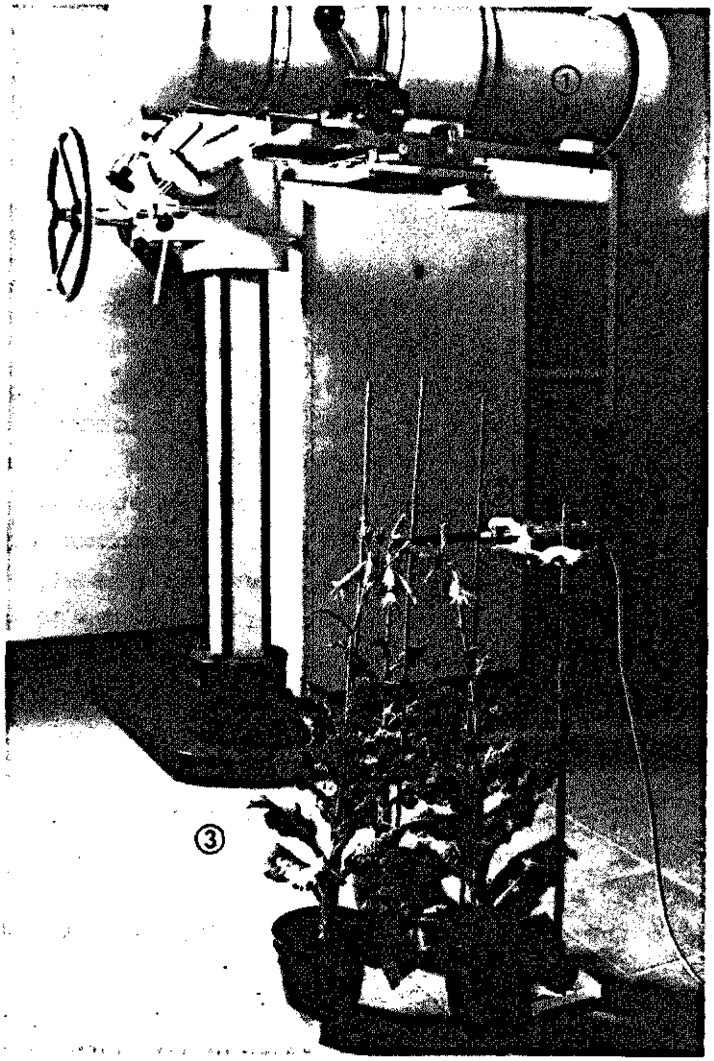


Fig. 10. Experimental set-up for X irradiation. 1 = X ray apparatus, 2 = dosimeter, 3 = irradiated plants.

elementary composition of flower buds in the related species *Lycopersicon esculentum* (Contant et al., 1971) and $D_{N(CH)}$ is the measured dose in ethylene (in rad). The gamma contamination of the fast neutron beam did not exceed 10% of the total absorbed dose. Plants were always placed at the same distance from the source. The exposure time was 33 minutes.

To compare the mutagenic effects of X rays and fast neutrons at similar levels of induced pollen sterility, doses were determined, in a preliminary experiment, which led to 20, 50 and 75% of pollen abortion after irradiation of pollen mother cells (PMC). In this preliminary experiment the following doses were chosen: X rays, 100, 300, 500, 700, 900, 1100, 1300 and 1500 rad; fast neutrons, 25, 50, 100, 200, 300, 400 and 550 rad.

4.2.2 Determination of meiotic stage

Since the meiotic stage is the most sensitive stage for the induction of S mutations and because S mutations induced after the tetrad stage are too late to express themselves in the pollen (Lewis, 1949a; Pandey, 1970b), all irradiations were carried out on buds with PMCs at the meiotic stage.

As meiosis is also the most sensitive stage for the induction of pollen abortion (Yamakawa, 1966; Yamakawa & Sparrow, 1966), the meiotic stage was determined by establishing the relationship between pollen abortion and the number of days that irradiated buds needed to reach anthesis (van Gastel, unpublished). The highest increase in pollen abortion was observed 9 - 10 days after X irradiation and 11 - 12 days after fast neutron irradiation. Therefore, it was assumed that meiosis, the most sensitive stage for the induction of S mutations, took place 9 - 10 days and 11 - 12 days before flower opening after X rays and fast neutrons, respectively.

4.2.3 Pollination procedures and detection of irradiation effects

A total of 167 and 99 flowering plants (S_2S_3), were submitted to X rays and fast neutrons, respectively. The control series consisted of 65 plants.

To transmit modifications in the self-incompatibility character, mature pollen from flowers which had been in meiosis at the time of irradiation, was collected and deposited on the stigmas of two unirradiated flowers from the same S_2S_3 clone. Intentionally, no self-pollinations were made because mutations induced in the egg cells are not transmitted to the next generation unless the style is also mutated (or pseudo-compatible). Moreover, ovular damage occurs in irradiated flowers. Self-pollination will only have a nuisance value because effects induced in the maternal tissue will overlap the effects induced in the pollen. Cross-pollinations between irradiated males (PMC irradiation) and unirradiated females only allow changes induced in the pollen to be transmitted. To prevent abscission of flowers or of young capsules and to induce swelling of the ovary, 1% naphthalene acetamide in lanolin was applied around the calyx of the flowers immediately after

Table 7. Consequences of pollinations with pollen from irradiated plants (PMC irradiation) and unirradiated plants.

	Control	X ray dose (rad)			Fast neutron dose (rad)		
		300	600	825	50	175	300
Pollinations	487	572	472	420	257	247	157
Seeded fruits	11	7	13	9	20	11	8
Seeds	154	22	391	54	217	35	311
Seeds sown	129	22	247	54	107	35	110
Seeds germinated	53	3	36	10	38	13	59
Plants tested	36	2	25	9	24	6	49
Pollen-part mutants	2	1	1	9	7	6	2
Stylar-part mutants	0	0	3	0	0	0	1

pollination.

After fruit harvest, the seeds (or some of them) present in each fruit were sown (Table 7) and the resulting plants self-pollinated and testcrossed with the original mother clone and with homozygous tester stocks (S_2S_2 and S_3S_3).

4.2.4 Estimation of mutation rates

A factor which must be taken into consideration for the calculation of the mutation rates is the time the S mutation was induced.
Since irradiation was applied to meiotic buds, more than one self-compatible pollen grain may have resulted from a single mutational event. To overcome this complication, the mutation rates were first calculated in two different ways.

- By estimating the number of self-compatible individuals in the progenies per pollination. This assumes that each mutation gives rise to only one mutant and, therefore, probably represents an overestimate of the mutation rate.
- By estimating the number of fruits containing mutated seeds per pollination. This assumes that each self-compatibility mutation gives rise to a cluster of seeds and probably represents an underestimate of the mutation rate.

As the results obtained in my study showed that most mutants appeared in different progenies, the best method for estimating mutation frequencies was the one which took into account the number of self-compatible individuals per pollination. Accordingly the values calculated by this method are given and considered for discussion.

As the number of S mutants detected in progeny tests only represents a fraction of the number of mutations induced, I tried to determine the total number of mutations by assuming that all mutations occurred independently of other damage leading to sterility and lethality. This estimate of all induced S mutations was obtained by corrections which took into account the number of S mutations supposedly eliminated through a casual association with pollen abortion, failure of seed germination or flowering inhibition (Table 7). However, an analysis of the results at the end of my experiments showed that these corrections did not modify the general trends expressed by the uncorrected data and were, therefore, superfluous. They are consequently omitted here, where the uncorrected values are dealt with.

4.2.5 Pollen stainability

To establish the doses which led to 20, 50 and 75% of induced pollen sterility after PMC irradiation, pollen stainability was estimated as soon as meiotic buds had evolved into mature flowers (200 grains per flower).

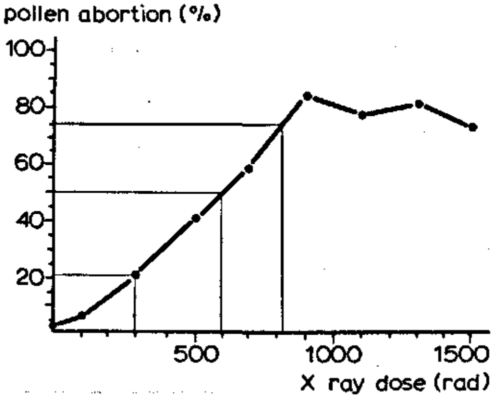
Material and methods not described here are given in Chapters 2 and 3.

4.3 RESULTS

4.3.1 Irradiation effects on pollen stainability and on fruit and seed set

The relationship between pollen abortion after irradiation of meiotic buds and irradiation dose is presented in Figs. 11 and 12. Both X rays and fast neutron irradiation increased pollen abortion. After X irradiation a maximum of 85% of pollen abortion was reached at 900 rad; at the dose of 300 rad fast neutrons, 75% of the pollen was aborted. Higher neutron doses resulted in a considerable amount of flower dropping. The doses which led to 20, 50 and 75% of induced pollen sterility after PMC irradiation are: X rays, 300, 600 and 825 rad; fast neutrons, 50, 175 and 300 rad.

The effects of irradiation on fruit and seed yield after crosses between irradiated pollen donors and unirradiated recipients are presented in Tables 7 and 8. X rays had no effect on the number of seeded fruits per pollination. However, after fast neutron irradiation the number of seeded fruits per pollination was two to three times higher than in the control series. With regard to seed set no clear dose-response relationship could be established as only certain doses resulted in an increase in seed production.



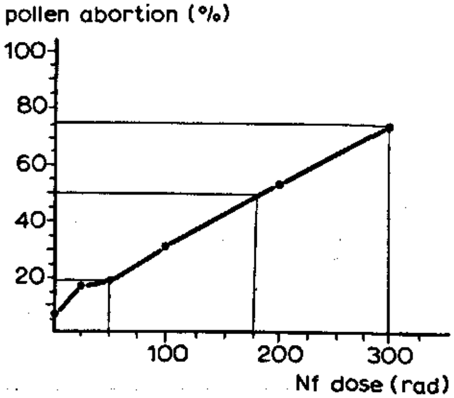


Fig. 11 (left). Relationship between pollen abortion after irradiation of meiotic buds and X ray dose. P value with Spearman rank correlation test: < 0.01; alternative hypothesis: increase with dose.
Fig. 12 (right). Relationship between pollen abortion after irradiation of meiotic buds and fast neutron (Nf) dose. P value with Spearman rank correlation test: < 0.01; alternative hypothesis: increase with dose.

Table 8. Effects of X rays and fast neutrons on fruit yield, seed yield and mutation rate after crosses between unirradiated S_2S_3 ♀ and irradiated S_2S_3 ♂ (PMC irradiation).

	Control	X ray dose (rad)			Fast neutron dose (rad)		
		300	600	825	50	175	300
Seeded fruits per pollination	0.023	0.012	0.028	0.021	0.078	0.045	0.051
Seeds per pollination	0.32	0.04	0.83	0.13	0.84	0.14	1.98
Pollen-part mutants per pollination	0.004	0.002	0.002	0.021	0.027	0.024	0.013
Stylar-part mutants per pollination[1]	0	0	0.010	0	0	0	0.018

1. The number of mutants obtained are corrected for the fraction of seeds which have been sown.

Table 9. Classification of S genotypes in the progenies of crosses between unirradiated S_2S_3 ♀ and irradiated S_2S_3 ♂ (PMC irradiation). In brackets number of plants analysed. pp = pollen-part mutation, sp = stylar-part mutation, HOM = S homozygote, HET = S heterozygote.

	Control	X ray dose (rad)			Fast neutron dose (rad)		
		300	600	825	50	175	300
	(36)	(2)	(25)	(9)	(24)	(6)	(49)
Nonmutants							
S_2S_2	9	0	2	0	4	0	16
S_2S_3	19	0	9	0	8	0	24
S_3S_3	6	1	10	0	5	0	6
Mutants							
pp-HOM	1	0	0	2	1	0	1
pp-HET	1	1^{1}	1^{1}	7	6	6	1
sp	0	0	3^{2}	0	0	0	1^{2}

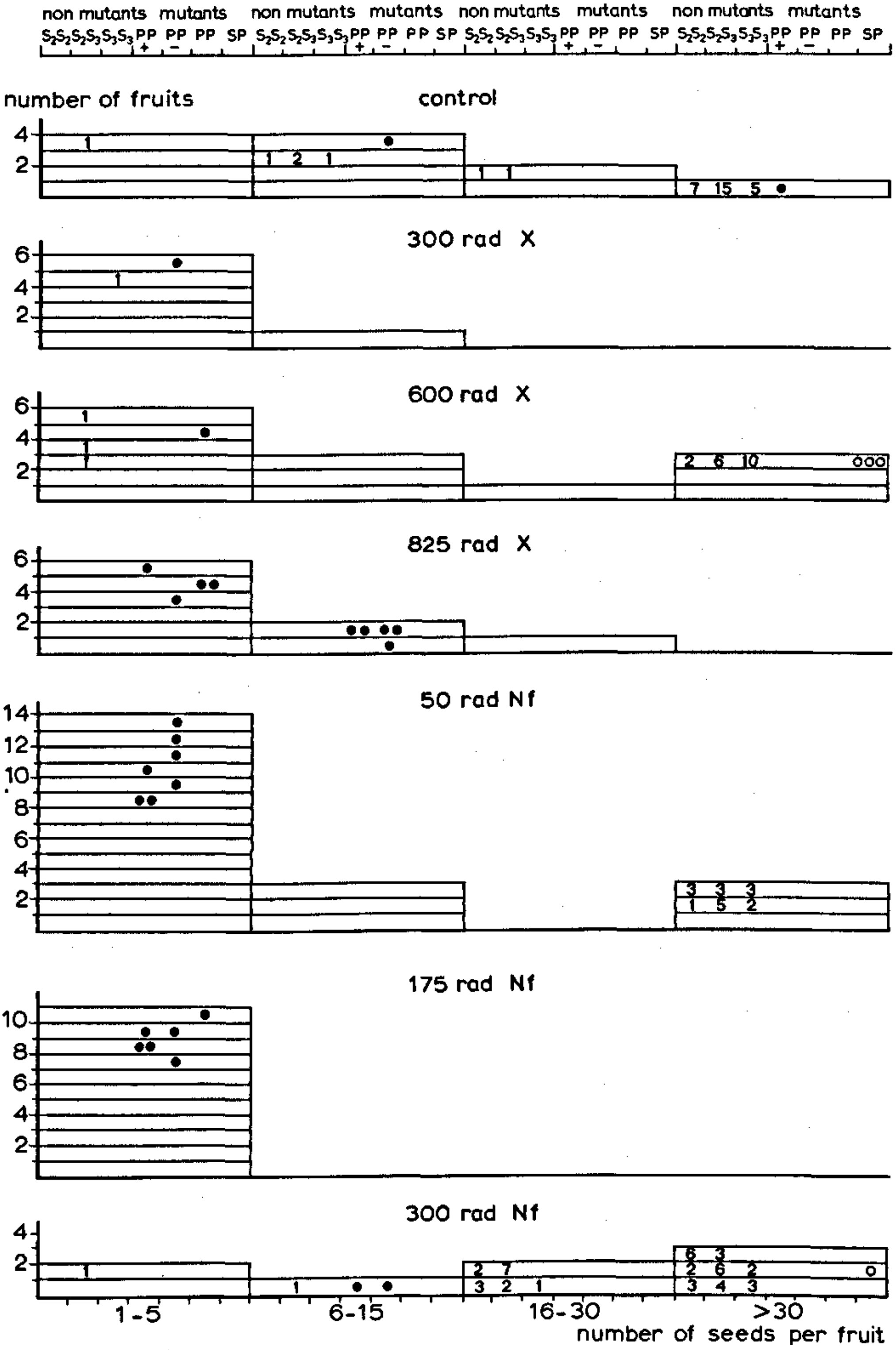
1. Classification based on results in advanced progenies.
2. Partial mutants.

4.3.2 *Progeny testing for S mutations*

Due to the low number of seeds which produced a flowering plant (Table 7) the number of plants tested in some experimental series was rather low (Fig. 13). The testcrosses for detecting S mutants among the progenies revealed that 32 individuals (14 in the X ray series, 16 after fast neutrons and two in the control) were self-compatible mutants (Table 9). As can be seen in Fig. 13, the majority of the mutants was detected in different progenies and usually arose from fruits which only contained few seeds. Sometimes when more than one mutant was detected in the same fruit the mutants often belonged to different classes (with and without the centric fragment).

Except for the 32 self-compatible individuals all progenies expressed self-incompatibility and segregated in 31 S_2S_2 : 60 S_2S_3 : 28 S_3S_3, a ratio which does not deviate significantly from the expected 1 : 2 : 1 ratio ($\chi^2_2 = 0.16$; $0.90 < P < 0.95$). The self-incompatible individuals were usually

Fig. 13. Distribution of fruit and seed set after crosses between unirradiated S_2S_3 ♀ and irradiated S_2S_3 ♂ (PMC irradiation) and distribution of mutated and unmutated individuals in the progenies. pp+ = pollen-part mutant with an additional centric fragment (•), pp- = pollen-part mutant without an additional centric fragment (•), pp = pollen-part mutant not analysed cytologically (•), sp = stylar-part mutant (o).


non mutants mutants
S2S2 S2S3 S3S3 PP + PP − PP SP
number of fruits
control
300 rad X
600 rad X
825 rad X
50 rad Nf
175 rad Nf
300 rad Nf
1-5
6-15
16-30
>30
number of seeds per fruit

detected in clusters, that is to say within few fruits. Such fruits usually contained more than 30 seeds (Fig. 13).

In the 26 stable self-compatible mutants the self-compatibility character only involved the capacity of the pollen to grow through normally incompatible styles (pollen-part mutants). As can be seen in Table 9, five of the 26 self-compatible mutants were S homozygotes (reciprocally compatible with the original S_2S_3 mother clone, but incompatible as females, with one of the homozygous tester stocks). The shortage of S homozygotes is probably due to lethality (see Chapter 7). The testcrosses with homozygous tester clones revealed that four of these plants were S_3 homozygotes (pp-HOM$_3$), while the fifth was homozygous for S_2 (pp-HOM$_2$).

The remaining 21 self-compatible plants were cross-compatible as staminate parents with the original clone and with the two tester stocks, but rejected, as pistillate partners, S_2 and S_3 pollen. These plants are S heterozygotes which carry a pollen-part mutation (pp-HET).

The five pp-HOM plants (4 pp-HOM$_3$ and 1 pp-HOM$_2$) and 19 of the pp-HET plants were analysed cytologically (Table 10). Eleven mutants displayed a centric fragment (Fig. 7) in addition to the normal chromosome complement. Such a fragment was also found by Brewbaker & Emery (1960), Brewbaker & Natarajan (1960), Pandey (1965, 1967, 1969b) and by Hogenboom (1972b). Among these 11 mutants, eight were pp-HET, one was pp-HOM$_2$ and two were pp-HOM$_3$. Fragment and nonfragment self-compatibility mutations were obtained in equal numbers.

Table 10. Description (cytology and S genotype) of the self-compatible pollen-part mutants. + = mutant displays a centric fragment, - = mutant does not display a centric fragment, pp = pollen-part mutation, HET = S heterozygote, HOM$_2$ = S homozygote (S_2).

	Number of mutants analysed	Control		X rays		Fast neutrons	
		+	-	+	-	+	-
pp-HET	19	0	1	2	4	6	6
pp-HOM$_2$	1	0	0	1	0	0	0
pp-HOM$_3$	4	1	0	0	1	1	1
Plants with a fragment	11	1		3		7	
Plants without a fragment	13		1		5		7

Six plants responded erratically to the testcrosses (Table 9). An analysis of the advanced progenies of these plants revealed that a pollen-part mutation segregated in two progenies. The situation was not clear in the other four cases but part of the pollen produced by the four erratic mutants carried a stylar-part mutation.

4.3.3 Mutation rates

The mutation rates (number of self-compatible individuals per pollination) are shown in Table 8. Both after X rays and fast neutrons no clear relationship between doses and responses was present. Roughly it can be said that the highest dose of X rays and all doses of fast neutrons increased the number of pollen-part mutants per pollination by a factor of three or more as compared with the spontaneous mutation rate in the control.

Stylar-part mutants were only detected at two doses (600 rad X rays; 300 rad fast neutrons).

4.4 DISCUSSION

4.4.1 Irradiation effects on pollen stainability and on fruit and seed set

The increase of pollen abortion with increasing dose in both the fast neutron and the X ray series was expected (see Section 3.3.1). The RBE values, (X ray dose to induce a certain amount of pollen abortion) / (neutron dose to induce the same amount), calculated for a number of X ray doses (Table 11) decreased with increasing X ray dose in a manner similar to the one reported by several authors (see Kellerer & Rossi, 1972).

Table 11. Dose dependence of RBE (relative biological effectiveness) for pollen abortion.

Pollen abortion (%)	X ray dose (rad)	RBE	Pollen abortion (%)	X ray dose (rad)	RBE
10	160	20.0	45	540	3.5
15	225	11.2	50	600	3.3
20	295	5.9	55	650	3.2
25	345	4.9	60	710	3.0
30	395	4.4	65	745	3.0
35	440	4.0	70	785	2.9
40	490	3.6	75	820	2.8

An increase in fruit set and seed set (see Table 8) was also reported after chronic exposure of self-incompatible species (see Chapter 3 and de Nettancourt & Ecochard, 1968) and attributed to an inhibition of floral abscission or to an alteration of metabolic activity in the style, but was never found to occur to the same extent after acute treatment of PMCs. Since all pollinations were carried out on unirradiated plants, the stimulation of fruit set and seed set must have resulted from modifications induced in the pollen. These modifications were, in many instances of an impermanent genetic nature, as the number of S mutations recorded in the progenies is relatively low (for a further discussion, see Section 4.4.5).

4.4.2 The efficiency of fast neutrons for inducing self-compatibility mutations

The mutation rates were slightly higher after fast neutron irradiation than in the X ray series (see Table 8). Although no relationship could be established between neutron doses and mutation rates, it is clear that the RBE (now defined as X ray dose to induce a certain number of S mutations / neutron dose to induce the same number) of fast neutrons exceeds one.

The best advice which may be given to the plant breeder, who is anxious to recover a maximum of self-compatible mutants by a minimum of labour and of greenhouse space is to use fast neutron irradiation (which produces the highest number of mutations per pollination) and to score for mutations only among those progenies arising from fruits with low seed numbers (less than 15 seeds per fruit).

The very large quantitative differences which were observed between X rays and γ rays (see Chapter 3) probably resulted from the fact that X rays were applied at a high dose rate (20 rad/min), whereas gamma irradiation was chronic at dose rates which did not exceed 14.3 rad/h.

4.4.3 Types of permanent self-compatibility mutations induced by X rays and fast neutrons

As could be expected from the fact that only pollen grains are submitted to the screening system provided by an incompatible style, all 26 stable self-compatibility mutants recorded in the progeny tests were pollen-part mutants.

Among the 24 plants analysed cytologically, 11 displayed a centric fragment whereas the remaining 13 plants did not exhibit any visible addition or clear modification in their karyotypes. It is as far as I know the first time that the latter type of mutation has been induced in such high frequency. Lewis (1961) concluded on the basis of segregation data, that pollen-part mutations without a fragment occurred after irradiation and that, therefore, the induced self-compatibility character in pollen-part mutants of *Oenothera* did not depend on the presence of additional fragments in the pollen grain. Pandey (1967) found only one mutant without a fragment out of 61 mutants analysed cytologically.

Since in my work, the two classes of mutants were equally distributed among the X ray and the fast neutron series, there is no difference in the mutation spectra of the two mutagens and the discrepancy with the data of Pandey (1967) cannot be attributed to a specific property of fast neutron irradiation. The difference in results may perhaps be attributed to the higher dose (1000 rad) used by Pandey (1967) or to variations in the genetic constitution of the *Nicotiana* clones, which were used as test material.

The exact nature of the self-compatibility in the two types of mutants (with and without a centric fragment) is a matter of controversy and will be discussed in Chapter 7.

4.4.4 Erratic stylar-part mutants

The fact that four erratic stylar-part mutants (three in the same fruit) were found shows, as in the experiments of Pandey (1967), that it is also possible to mutate the stylar-activity part of the S locus.

Because stylar-part mutations do not confer self-compatibility on the pollen grains, these mutations must have been associated with a phenomenon which enabled the pollen to overcome the incompatibility barrier. Since the stylar-part mutants were only detected in progenies where a high number of nonmutants were present (see Fig. 13), it is proposed that such stylar-part mutant pollen grains were able to fertilize the eggs as a result of pseudo-compatibility. Since S homozygotes with a stylar-part mutation in one of the two S-bearing chromosomes cannot express a self-compatible phenotype, it is possible that larger numbers of stylar-mutants were present in the progenies.

4.4.5 Revertible mutations

Many workers (Lewis, 1951; Lewis & Crowe, 1953, 1954a; Pandey, 1956, 1959, 1967; Davies & Wall, 1961) have found that, as in my study, some of the pollen which is produced by irradiated plants seems to have lost its specificity; the pollen tubes were able to penetrate an incompatible style and fertilization occurred. However, in the following generation self-compatibility is no longer observable and all plants behave as regular self-incompatible individuals. According to Lewis (1951) four different arguments suggest that the nature of such a temporary phenomenon is genetic.

- The low frequency of occurrence.
- The low number of seeds when setting occurs.
- The increase in frequency by X rays.
- The absence of simultaneous effects on both alleles.

Of the four possible explanations (mutation of a modifier, mutation in the vegetative nucleus, mutation of a cytoplasmic component and revertible mutations), Lewis (1951) considered that of revertible mutations as most likely. Lewis (1951) and Pandey (1956, 1959, 1965) considered that such mutations result from labile premutations which subsequently revert, after a certain number of cell divisions to the original allelomorph.

I tried to find out whether the numerous examples of temporary compatibility observed in my study, could be explained solely by revertible mutations or whether pseudo-compatibility was also involved. The fact that most of the unmutated offspring were detected in the progeny of fruits containing high numbers of seeds (see Fig. 13) is certainly not compatible with the ideas of Lewis (1951) who considered that revertible mutations occur at low frequency and that a low number of seeds will be obtained if setting occurs. It can be calculated (Tables 7 and 9) that the frequency of unmutated offspring did not clearly increase after irradiation; only certain doses resulted in a sharp increase in the number of self-incompatible plants per pollination. Moreover, almost all progenies, where the number of individuals was sufficient, segregated for the two classes of S homozygotes and the S heterozygous class (see Fig. 13). This segregation also indicates that revertible mutations are not involved because they would lead to a lack of one S homozygous class in the progeny.

The remaining progenies, which failed to segregate for the two S homozygous classes (see Fig. 13), may be interpreted as the result of revertible mutations. The number of individuals tested in these progenies is, however, so low that the absence of one class of homozygous plants is not surprising. Therefore, my results are in contradiction with the conclusions of Lewis (1951) and Pandey (1956, 1959), because they assumed that all self-incompatible individuals in the progeny of unirradiated females and irradiated males were the result of revertible mutations. Pandey (1967) showed that such individuals could also result from permanent stylar-part mutations.

The data I obtained indicate that, at best, only a small number of revertible mutations occurred and that the majority of self-incompatible individuals in the progenies of crosses between irradiated males and unirradiated recipients was due to pseudo-compatibility processes.

4.4.6 Constructive mutations

Spontaneous generation of new functional S alleles has been reported in inbred populations (Denward, 1963a, 1963b; de Nettancourt & Ecochard, 1969; Pandey, 1970a, 1970c, 1972; de Nettancourt et al., 1971b, 1975; Hogenboom, 1972b; Anderson et al., 1974; van Gastel & de Nettancourt, 1975). My work (see Chapters 3 and 4) and that of Lewis (1951) and Pandey (1967) showed that mutagenic treatment does not induce changes from one S allele to another. Since new specificities are first generated in the style (see de Nettancourt et al., 1971b), it is possible that they cannot be expressed or transmitted in experiments where only mutations are scored in the pollen.

4.4.7 Implication for plant breeding

The utility for the plant breeder of induced or spontaneous self-compatible mutants of the same type as those which were obtained in my study has been reviewed by de Nettancourt (1972). He summarized three main applications.

- The production in homozygous condition of valuable combinations of genes and the elimination of sublethal combinations of genes.
- The maintenance of a high level of self-fertilization (within clone) in fruit crops.
- The production of inbred lines for synthetic varieties and F_1 hybrid seeds.

With respect to the production of inbred lines it has always been taken for granted that the self-compatibility character to be used should be of a temporary nature and ought not to involve the participation of a permanent mutation at the S locus. However, I would like to point out a possible use

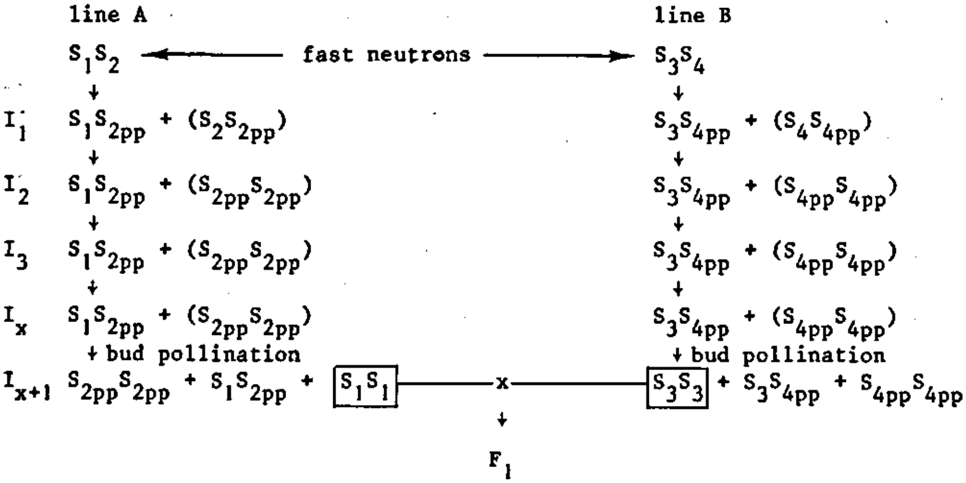


Fig. 14. Inbreeding scheme of self-compatible S heterozygotes and production of self-incompatible S homozygotes for constitution of F_1 hybrid seeds. pp = pollen-part mutation.

of permanent self-compatible mutants for the production of F_1 hybrid seed. The system I propose is outlined in Fig. 14 and replaces almost entirely bud pollination by the simple self-pollination of self-compatible S heterozygotes. Such self-compatible S heterozygous mutants need to be selfed repeatedly for several generations and their progenies sorted out for S heterozygosity in each generation until the desired degree of homozygosity has been reached. Selected S heterozygotes are then submitted to forced selfing (e.g. bud pollination) and their progenies screened for self-incompatible S homozygous plants, which can be used for the production of F_1 hybrid seeds.

Such a scheme is simple and will not even require any testcrossing for detection of S heterozygotes if the self-compatibility character is based on the presence of a competition-fragment which only leads to the formation of S heterozygotes.

5 Ethyl methanesulfonate treatment

5.1 INTRODUCTION

Because EMS is known to be a powerful mutagen (e.g. Ehrenberg et al., 1961; Gaul, 1962; Lundqvist & von Wettstein, 1962; Röbbelen, 1968; van der Veen & Wirtz, 1968), it was decided to use this chemical for inducing mutations at the S locus. After EMS treatment of seeds of *Arabidopsis thaliana* van der Veen (pers. comm.) has collected for a number of different phenotypic classes, single gene recessives which were all of independent origin. Tests of allelism (noncomplementation) were done by a diallel cross within each phenotypic class. The results were: 6 mutations at in total 5 loci (genic male steriles), 8 mutations at 6 loci (late flowering), 5 mutations at 4 loci (seed coat colour), 4 mutations at 3 loci (vital chlorophylls), 4 mutations at 3 loci (long hypocotyl). These show that at a given locus it is possible to find a second mutant. So, it is not excluded a priori that, given the S locus, some mutants will be recovered after EMS treatment of a limited number of seeds.

A first series of experiments was carried out by soaking seeds in different concentrations of the chemical. Because the embryonic apical meristem is multicellular and composed of three layers (L_1, L_2, L_3) such treatment were, of course, expected to result in chimerism, if mutations could be induced. Since gametes are derived from the L_2 layer, S mutations induced in L_2 are transmitted to the next generation if they confer compatibility on the pollen. Satina (1944) found for *Datura stramonium* (also a solanaceous species) that the conducting tissue in the style is derived, by periclinal divisions from the L_1. Therefore, a stylar-part mutation in L_1, may allow pollen tube growth, but the mutation is not transmitted. It was also considered that chimerism within an ontogenetic layer (mericlinal chimerism) may be progressively lost during development, e.g. by somatic drift (Balkema, 1972) and that one can expect a flower to be nonchimeric for a given ontogenetic layer.

Experiments were also carried out with EMS applied to flower buds. Here each induced S mutation in a pollen grain can be normally selected by the stylar sieve.

5.2 MATERIAL AND METHODS

5.2.1 Seed treatment

S_2S_3 seeds obtained from a cross between an S_3S_3 and an S_2S_3 plant, were treated with 64 or 128 mM EMS (not buffered) for 6½ h at 20°C in the dark and, thereafter, rinsed in water. A preliminary experiment had shown that these concentrations hardly reduced germination and survival (as compared with the 0-mM control), and did not lead to a high degree of sterility (as measured by reciprocal crosses with a compatible clone). A third concentration (256 mM) tested in the preliminary experiment was found to severely reduce seedling survival and was not used in further experiments.

The experimental material consisted of 15, 49 and 39 plants in the control and in the two EMS series, respectively.

Ten to fifteen flowers, distributed throughout the inflorescence of each experimental plant, were pollinated by the pollen from S_2S_3 plants, and reciprocally the pollen of each of these flowers was used to pollinate two flowers from the untreated S_2S_3 plants. In addition 3 - 5 flowers of each control and treated plant were selfed.

The exact nature of the suspected cases of S mutations was established in the M_2 progeny by subjecting each M_2 plant to reciprocal crossing with the S_2S_3 tester stocks and, when necessary, with S_2S_2 and S_3S_3 tester clones.

5.2.2 Treatment of inflorescences

The inflorescences of *N. alata* plants (obtained by leaf propagation) were treated by the tongue-slit method (Oehlkers, 1946; Linskens, 1959; Röbbelen, 1968) with 16 mM (60 plants) and 32 mM (60 plants) for 24 h (EMS not buffered). Control plants (10) were treated with water (Fig. 15).

Buds with PMCs at the meiotic stage reached anthesis 9-12 days after irradiation (see Section 4.2.2.). Because we assumed that EMS-treated buds needed the same length of time to reach anthesis, and since the meiosis is the most sensitive stage for the induction of S mutations, pollen from flowers, which reached anthesis 9-12 days after EMS treatment were used to pollinate two untreated S_2S_3 flowers. Since bud development may be retarded, pollen

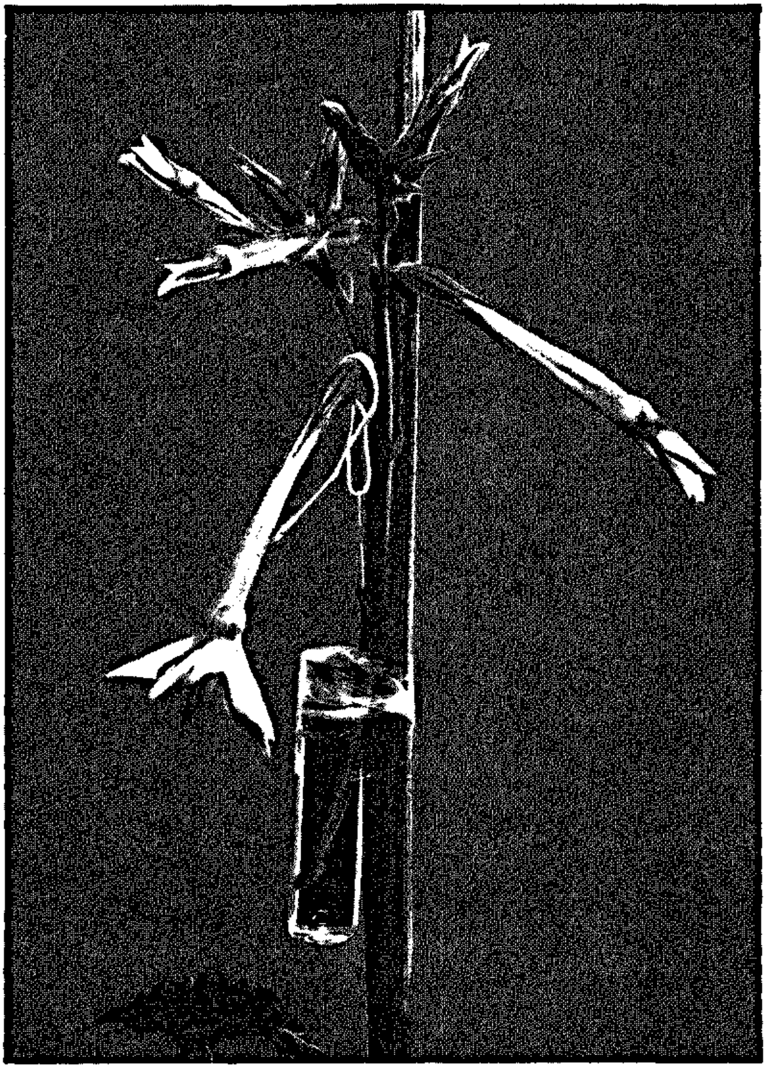


Fig. 15. Method of application of ethyl methanesulfonate (tongue-slit method).

from flowers which reached anthesis 13-18 days after EMS treatment were also used for pollination.

To detect a possible influence on the stylar component of the incompatibility reaction, flowers from treated plants were crossed with the pollen from control flowers (S_2S_3 in genotype). To prevent flower abscission and to induce swelling of the ovaries, 1% naphthalene acetamide in lanolin was applied around the calyx of pollinated flowers.

After fruit harvest, the seeds (or some of them) present in each fruit were sown and the resulting plants were testcrossed. After crosses between EMS-treated plants and untreated S_2S_3 individuals the number of fruits was so large that the seeds from only a limited number of fruits could be sown.

As will be seen in the Section on Results, the pollen grains submitted to mutation screening had been collected from flowers which originated from buds that did not contain PMCs at the meiotic stage at the same time the EMS was applied; that is to say after the sensitive period. To determine the sensitive stage an additional experiment was carried out which established the relationship between the time of treatment and the greatest amount of induced pollen abortion. For this purpose ten plants (S_2S_3 in genotype) were treated by the tongue-slit method with 16 mM EMS. Control plants (10) were treated with water.

Pollen stainability was estimated for each flower as soon as the treated buds had evolved into mature flowers.

5.2.3 Growing conditions

All research with EMS, except the research described in the last part of Section 5.2.2, was carried out in a greenhouse of the Department of Genetics, Agricultural University, Wageningen. Temperature ranged from 21 °C to 27 °C (occasionally values above 30 °C were reached) and relative humidity from 80 to 90%. Light conditions were natural.

Material and methods not described here are given in Chapters 2, 3 and 4.

5.3 RESULTS

5.3.1 Seed treatment

Fruit and seed set after crosses with plants raised from EMS-treated seeds are presented in Table 12 and Fig. 16. None of the control plants were found to produce fruits after selfing or in crosses. The plants raised from the EMS-treated seeds also proved to be self-incompatible. In crosses with the original mother clone used as male, eight plants treated with 64 mM produced a total of 16 fruits and five plants treated with 128 mM EMS yielded ten fruits. The number of seeds in these fruits ranged from 9 - 191 and 8 - 95, respectively. When the plants raised from the EMS-treated seeds

Table 12. Effects of EMS (Ethyl methanesulfonate) treatment of seeds on fruit and seed yield in the M_1 generation. In brackets number of plants obtained.

	Concentration EMS (mM)		
	0 (15)	64 (49)	128 (39)
Self-pollination			
pollinations	75	224	137
seeded fruits	0	0	0
Treated ♀ x S_2S_3			
pollinations	225	588	466
seeded fruits	0	16	10
seeds per fruit	-	63.5	28.1
S_2S_3 x treated ♂			
pollinations	450	1176	932
seeded fruits	0	1	0
seeds per fruit	-	9	-

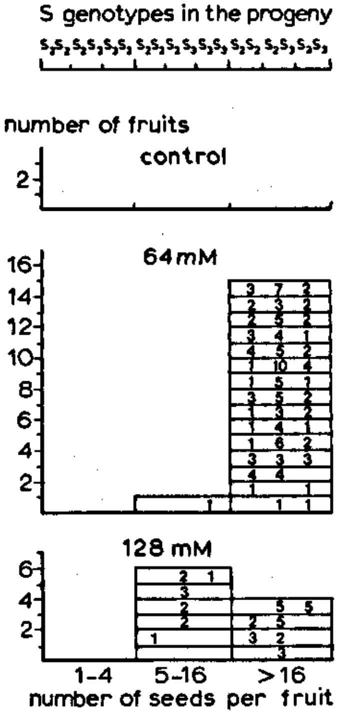


Fig. 16. Distribution of fruit and seed set after crosses between plants raised from EMS (ethyl methanesulfonate) treated S_2S_3 seeds (♀) and control S_2S_3 plants (♂) and S genotypes in the M_2 progenies. Plants with erratic behaviour are not included.

Table 13. Classification of S genotypes in the M_2 progenies of crosses between plants raised from EMS-treated seeds (♀) and plants raised from untreated S_2S_3 seeds (♂). In brackets number of M_2 plants analysed.

	Concentration EMS (mM)		
	0 (0)	64 (127)	128 (37)
S_2S_2	-	30	6
S_2S_3	-	65	24
S_3S_3	-	27	6
Erratic behaviour	-	5	1

were used as male parents only one fruit containing nine seeds was produced.

Due to the fact that only 12.6% of the seeds produced a flowering plant only a total of 164 plants have been tested. The results of testcrosses on the M_2 individuals are presented in Table 13 and Fig. 16. Except for six plants with erratic behaviour all offspring were self-incompatible. The progenies segregated in 36 S_2S_2, 89 S_2S_3 and 33 S_3S_3 genotypes. This ratio does not deviate significantly from a 1 : 2 : 1 ratio ($\chi_2^2 = 2.6$; $0.2 < P < 0.3$). The single progeny obtained from the crosses between S_2S_3 and the plants raised from EMS-treated seeds segregated into one S_3S_3 and two S_2S_3 genotypes (not entered in Table 13). From the results in the M_3 generation it was concluded that the M_2 plants with erratic behaviour did not carry any S mutation.

5.3.2 Treatment of inflorescences; effects of EMS on the pollen component of the incompatibility reaction

No significant increase in fruit and seed set was obtained in the crosses between untreated pistillate plants and EMS-treated staminate parents (Table 14, Fig. 17). The majority of the fruits (85%) produced in the control and the treated series contained from one to four seeds. It is possible that the capacity of the plants to produce seeds resulted in part from a physiological effect of the tongue-slit method or from an effect of the relative high temperature which occurred incidentally at noon.

The percentage of germinating seeds was very low: the exact percentage was not established. The results of the testcrosses on each plant in the progenies of matings between untreated S_2S_3 females and EMS-treated males are summarized in Table 15 and Fig. 17. Except for seven self-compatible mutants,

Table 14. Fruit and seed yield after crosses between untreated S_2S_3 ♀ and EMS-treated S_2S_3 ♂ (treatment of inflorescences). In brackets number of plants treated.

	Concentration EMS (mM)		
	0 (10)	16 (60)	32 (60)
Pollinations	60	358	284
Seeded fruits per 100 pollinations	6.7	8.1	4.6
Fruits with 1- 4 seeds per 100 pollinations	3.3	6.7	4.6
5-16 seeds per 100 pollinations	3.3	0.8	0
> 16 seeds per 100 pollinations	0	0.6	0

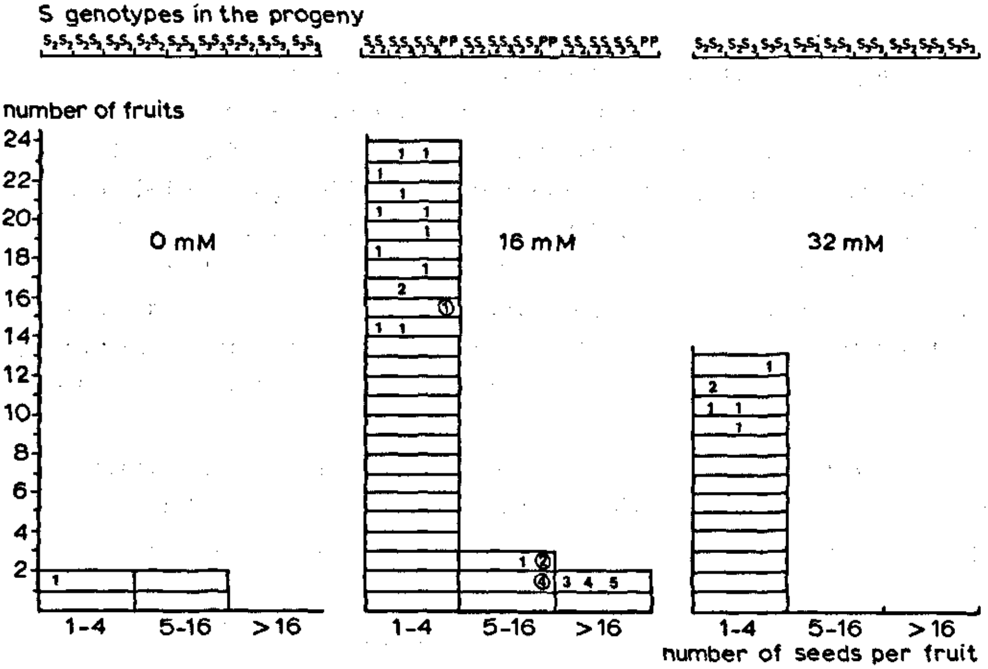


Fig. 17. Distribution of fruit and seed set after crosses between untreated S_2S_3 ♀ and EMS-treated S_2S_3 ♂ (treatment of inflorescences) and S genotypes in the progenies. pp = pollen-part mutant (O)

all individuals in the progenies constantly expressed self-incompatibility and segregated in 11 S_2S_2 : 11 S_2S_3 : 11 S_3S_3, which does not deviate significantly from the expected 1 : 2 : 1 ratio ($\chi^2_2 = 3.7$; $0.1 < P < 0.2$).

The seven self-compatible plants originated from three different fruits.

Table 15. Classification of S genotypes in the progenies of crosses between untreated S_2S_3 ♀ and EMS-treated S_2S_3 ♂ (treatment of inflorescences). In brackets number of plants analysed.

	Concentration EMS (mM)		
	0 (1)	16 (33)	32 (6)
S_2S_2	1	7	3
S_2S_3	0	9	2
S_3S_3	0	10	1
Mutated plants	0	7	0

Two progenies had the same staminate parent. The testcrosses on these seven plants and the analysis of the advanced progenies revealed that they all carried a pollen-part mutation; two of these plants were S_2 homozygotes (pp-HOM_2) and one was homozygote for S_3 (pp-HOM_3). The remaining four plants were S heterozygotes (2 individuals pp-HET_2 and 2 pp-HET_3).

The mutation rate, expressed as number of self-compatible individuals per pollination amounts to 0.02 for the experimental series at 16 mM EMS. However, such a figure is reduced to 0.008 if one considers that the mutated seeds present in a same fruit (see Fig. 17) arose from a single mutational event and then defines the mutation rate as the number of fruits containing mutated seeds per pollination. Moreover, not a single mutation was found in the 32-mM series.

If one takes into account such considerations it becomes obvious that the mutation rate is not much higher than the frequency calculated for spontaneous mutations in the control series of the irradiation experiment.

5.3.3 Treatment of inflorescences; effects of EMS on the stylar component of the incompatibility reaction

Fruit and seed set in crosses between EMS-treated pistillate partners and untreated staminate plants are presented in Table 16 and Fig. 18. A clear increase in the number of seeded fruits per 100 pollinations could be observed in the EMS-treated series. The majority of the fruits harvested contained more than 16 seeds. Again seed germination was very low (The exact figure was not established).

The results of the testcrosses are presented in Table 17 and Fig. 18.

Table 16. Fruit and seed yield after crosses between EMS-treated S_2S_3 ♀ (treatment of inflorescences) and untreated S_2S_3 ♂. In brackets number of plants treated.

	Concentration EMS (mM)		
	0 (10)	16 (60)	32 (60)
Pollinations	48	181	146
Seeded fruits per 100 pollinations	8.3	27.6	39.7
Fruits with 1- 4 seeds per 100 pollinations	6.3	12.7	6.8
5-16 seeds per 100 pollinations	2.1	2.8	6.2
> 16 seeds per 100 pollinations	0	12.2	26.7

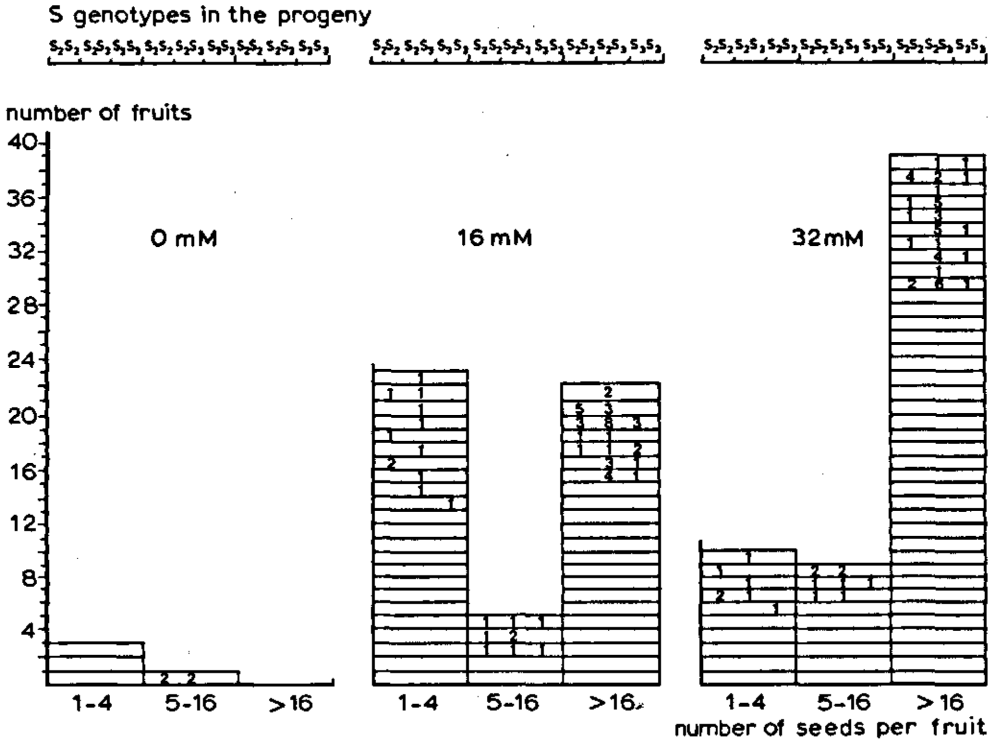


Fig. 18. Distribution of fruit and seed set after crosses between EMS-treated S_2S_3 ♀ (treatment of inflorescences) and untreated S_2S_3 ♂ and S genotypes in the progenies. Plants with erratic behaviour are not included.

Table 17. Classification of S genotypes in the progenies of crosses between EMS-treated S_2S_3 ♀ (treatment of inflorescences) and untreated S_2S_3 ♂. In brackets number of plants analysed.

	Concentration EMS (mM)		
	0 (4)	16 (62)	32 (60)
S_2S_2	2	17	16
S_2S_3	2	33	36
S_3S_3	0	10	7
Erratic behaviour	0	2	1

The majority of the individuals could be classified as self-incompatible S heterozygotes and S homozygotes. However, the observed ratio of 35 S_2S_2 : 71 S_2S_3 : 17 S_3S_3 deviates significantly from the expected 1 : 2 : 1 segregation ($\chi^2_2 = 8.2$; $0.01 < P < 0.02$).

Three plants responded erratically to the testcrosses. An analysis of the advanced progenies of these plants revealed that the unexpected self and/or cross compatibility could be ascribed in two of them to pseudo-compatibility, whereas a partial pollen-part mutation of the S_3 allele was segregating in the progeny of the third plant.

5.3.4 Stage determination (additional experiment)

The incapacity of EMS to induce self-compatibility mutations at the S locus suggested that the pollen used in the experiment had not been harvested from flowers treated at the sensitive stage (PMC). To check this possibility an extensive analysis of pollen stainability after EMS treatment at different bud stages was carried out. The results indicated that the sensitive period did not coincide with the period of 9 - 18 but of 20 - 25 days before anthesis (Fig. 19).

5.4 DISCUSSION

5.4.1 Seed treatment

The fact that all plants raised from EMS-treated seeds were self-incompatible (see Table 12) was unexpected because crosses with the original

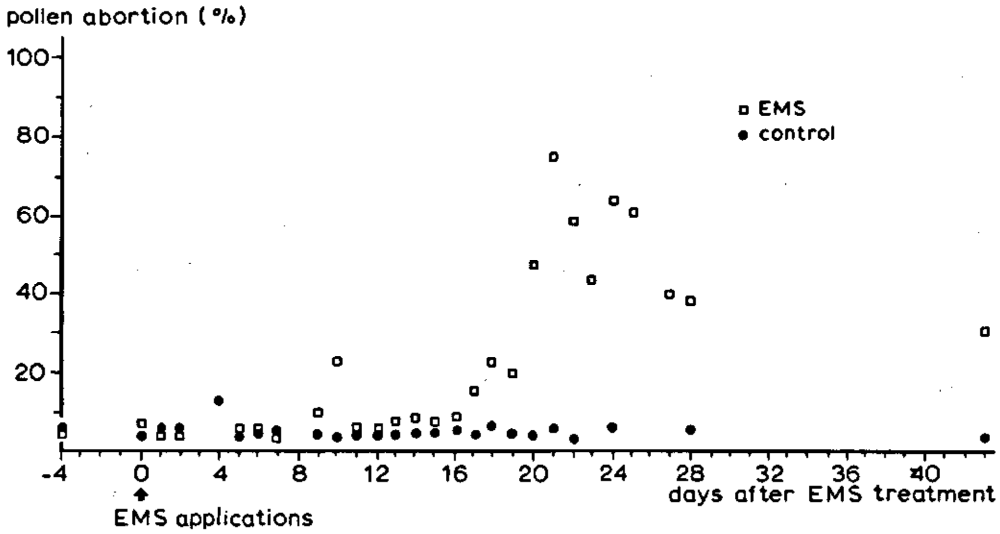


Fig. 19. Relationship between pollen abortion and the number of days to anthesis after EMS treatment of inflorescences.

mother clone as male parent had yielded 26 seeded fruits. This figure may be reduced to 13 because the 26 fruits were harvested from 13 different plants and thus probably resulted from only 13 independent events after 1054 pollinations. Therefore, the chance that 361 self-pollinations do not yield fruits is very small ($0.02 < P < 0.05$). This absence of self-compatibility could also have been due to high levels of EMS-induced pollen abortion. However, this is not likely because it was established in the preliminary experiment that the EMS concentrations used in my study did not induce a high degree of pollen abortion.

The absence of S mutations among M_2 plants is not due to the fact that seeds were treated, because Hoffmann (1969, 1971) obtained self-compatible mutants after nitroso-ethylurethane treatment of *Lycopersicon peruvianum* seeds. As EMS is known to be a very powerful mutagen the absence probably derives from the limited population screened. Hoffmann (1969, 1971) had to screen more than 300 plants to obtain one self-compatible mutant.

The following facts suggest that pseudo-compatibility is involved in my study: (1) compatibility was only observed if plants raised from EMS-treated seeds were used as female (see Table 12), (2) all offspring proved to be self-incompatible (see Table 13), (3) several progenies contained both classes of S homozygotes (see Fig. 16) and (4) only fruits with more than 5 seeds were obtained (see Fig. 16).

However, there is a possibility that stylar-part mutations are induced in the L_1 layer. As was outlined in Section 5.1 the style of such plants allows pollen tube growth and seed set, but the mutation is not transmitted. Since mutations are associated with one S allele, one type of pollen tube is able to penetrate the style. Consequently one class of S homozygotes must be lacking in the progeny. The fact that a number of progenies displayed only one class of S homozygotes (see Fig. 16) supports the view that mutations were induced only in the L_1.

5.4.2 *Treatment of inflorescences*

Because the sensitive period for the induction of pollen abortion did not correspond to day 9 - 18 before anthesis but to approximately three weeks before flower opening, it is concluded that the inefficiency of EMS for inducing S mutations can probably be ascribed to a mistake in the choice of the scoring period. Apparently EMS treatment of inflorescences severely delayed bud development.

It is unlikely that EMS was ineffective because the chemical did not penetrate throughout the anther because high amounts of pollen abortion were found when EMS treatment was applied three weeks before flower opening. However, such observations are not conclusive because one could imagine that EMS does not penetrate PMCs and induces pollen sterility through damages restricted to the tapetum (Singh, 1975). Experiments are now being done to study the effects of EMS at the S locus in pollen grains harvested from flowers which reached anthesis 18 - 25 days after bud treatment.

It remains to explain why significant increases in fruit and seed set were observed after crosses between EMS-treated plants as females and untreated incompatible partners (see Table 16). Here, the effect cannot be attributed to the method of application or to high temperatures (see Section 5.3.2) since the control series (treated with water instead of EMS) responded only slightly. Therefore, EMS had an effect on the pistil of the treated individuals, comparable to those reported after chronic irradiation, heat shocks and acute irradiation (for references and discussion see Section 3.4.1).

The large deficit of S_3S_3 genotypes found in the progenies (see Table 17) was probably not due to a difference in inhibition between S_2 and S_3 pollen tubes, or to preferential fertilization, because a deviation (at the 0.10 level of probability) from the one to one ratio for S homozygous (HOM) and

S heterozygous (HET) individuals was observed (HOM : HET = 52 : 71; $\chi_1^2 = 2.9$; $0.1 > P > 0.05$) and because the number of plants in the S_2S_2 class is approximately half of the number in the S_2S_3 class. Therefore, the deficit in S_3S_3 genotypes is probably the consequence of post-zygotic lethality in S_3S_3 genotypes.

6 Nature of 'self-compatibility' in triploids and aneuploids

6.1 INTRODUCTION

Artificially induced tetraploids of species with a gametophytic self-incompatibility system are usually self-compatible (Crane & Lewis, 1942; Lewis & Modlibowska, 1942; Stout & Chandler, 1942; Lewis, 1943, 1947; Atwood, 1944; Atwood & Brewbaker, 1953; Brewbaker, 1954, 1958; Emery et al., 1960; Pandey, 1968). This phenomenon, competitive interaction, occurs when diploid pollen grains, with two different S alleles, grow in a style with the same two S alleles. Self-incompatibility does not break down in tetraploids derived from S homozygote individuals, because such tetraploids are not able to produce heterogenic di(S)allelic pollen grains.

Theoretically, S heterozygous triploids and the S heterozygous aneuploids which are trisomic for the S locus are able to produce heterogenic di(S)allelic pollen grains and should thus be self-compatible. However, it is known from the literature that male sterility is not uncommon in triploids or that their aneuploid pollen is not functional (Lesley, 1928; Allard, 1960; Emery et al., 1960; Breuer, 1961; Mok et al., 1975).

It depends on the number of additional chromosomes whether aneuploids are sterile or not. It is also possible that no or hardly any functional aneuploid pollen is produced; transmission of extra chromosomes through the pollen is often very limited (Allard, 1960; Tsuchiya, 1964; Khush, 1973; Mohammed and Majumder, 1974).

6.2 MATERIAL AND METHODS

The crosses performed are described together with the results. Material and methods not described here are presented earlier.

6.3 RESULTS

6.3.1 Triploids

An emasculated flower of a tetraploid individual ($S_2S_2S_3S_3$; see Chapter 2) was pollinated, in the bud stage, by the pollen of a diploid plant (S_2S_3). The results of the self-pollinations on 16 resulting (triploid) progeny plants, revealed that none of the triploids set seed after selfing.

Crosses with tester genotypes (S_2S_2, S_3S_3, S_6S_7) yielded erratic results. Generally, no seeds were obtained if the triploid was used as the male parent, but high seed set was obtained when the triploid was pollinated by the pollen of the S_6S_7 clone. The triploid is thus fertile as pistillate parent but produces sterile pollen (male sterile).

6.3.2 Aneuploids

Aneuploid plants were created by crossing a triploid ($S_2S_2S_3$ or $S_2S_3S_3$) and a diploid plant (S_6S_7). The diploid individual was used as staminate parent in the cross (see Section 6.3.1).

The 59 offspring were self-pollinated and crossed with S_2S_2 and S_3S_3 homozygotes. When they were cross-incompatible with both homozygous testers (i.e. had genotype $S_2S_3S_6$ or $S_2S_3S_7$), crosses were performed with the S_6S_6 and S_7S_7 homozygous clones. The homozygotes were always used as staminate

Table 18. Results of testcrosses for S alleles performed on nine aneuploid individuals obtained in the progeny of the cross between a triploid and a diploid plant.

	Number of chromosomes	Self-pollination	S_2(♂)	S_3(♂)	S_6(♂)	S_7(♂)	S genotype
C191 A- 3	25	-	-	-	-	+	$S_2S_3S_6$
C191 A-18	22	-	-	-	+	-	$S_2S_3S_7$
C191 A-19	19 + fr	+	-	-	-	+	$S_2S_3S_6$
C191 A-22	24	-	-	-	+	-	$S_2S_3S_7$
C191 A-26	23	-	-	-	-	±	$S_2S_3S_6$
C191 A-30	23	-	-	-	-	+	$S_2S_3S_6$
C191 A-35	20	-	-	-	+	-	$S_2S_3S_7$
C191 A-41[1]		-	-	-	-	+	$S_2S_3S_6$
C191 A-42	24	-	-	-	±	-	$S_2S_3S_7$

[1] Plant died before chromosome counts could be made.

parent. With this procedure no less than nine aneuploid plants were detected which carried three different S specificities in the style (Table 18). Self-pollinations showed that eight of these plants were self-incompatible. In testcrosses where the aneuploids were used as staminate parent (not entered in Table 18) several crosses yielded a compatible seed set. Therefore, not all pollen of the nine aneuploid plants is sterile.

One of the aneuploids was self-compatible and displayed 19 chromosomes and a centric fragment (see Chapter 8).

6.4 DISCUSSION

Triploids are male sterile because no seed set was obtained in crosses where the triploids were used as staminate parent, whereas good seed set was usually obtained in the reciprocal cross. Male sterility is probably caused by all or nearly all pollen being genomically imbalanced (aneuploid).

Aneuploids are male fertile since they can be used as male parents in crosses. Apparently, they can produce sufficient functional pollen. Since the aneuploids were self-incompatible, it is likely (1) that such functional pollen is euploid (n=x=9) or (2) that, if some aneuploid pollen is also functional, pollen which carries as extra chromosome the S bearing one, is not functional, or (3) that competitive interaction does occur in such pollen grains.

The only aneuploid plant which was self-compatible carried an extra centric fragment. Probably self-compatibility in this plant results from the additional extra centric fragment and not from the extra chromosome. In my theory (presented in Chapter 7) competitive interaction in fragment-carrying pollen grains is excluded, so one must assume a spontaneous pollen-part mutation (see Chapter 2) and self-compatibility must result from complementation by the fragment of the mutant allele.

Furthermore, it is clear that the compatibility behaviour of plants with more than two S specificities cannot be predicted with certainty. This conclusion is particularly important for the identification of the S-bearing chromosome (see Chapter 8; Kessel, 1973; Ramulu et al., 1975) by the competitive interaction test. It means, that the most reliable method for detecting trisomics for the S-bearing chromosome is the analysis of the incompatibility genotypes (which should reject S_a, S_b and S_c pollen grains if the trisomic is $S_aS_bS_c$ in genotype) rather than the search for competitive interaction and compatibility in pollen of plants assumed to be $S_aS_bS_c$ or $S_aS_aS_b$ (see Section 8.1.3).

7 Nature of self-compatibility in pollen-part mutants

7.1 INTRODUCTION

Different theories have been formulated for explaining pollen-part mutations at the S locus of species with a gametophytic monofactorial system of self-incompatibility. These can be summarized as follows.

7.1.1 Fragment mutations

The competitive interaction theory (Brewbaker & Natarajan, 1960) assumes that the additional centric fragment (see Section 4.4.3) carries the S locus and that heterogenic di(S)allelic pollen grains are able to fertilize as a result of competitive interaction (Table 19a). Thus the two different S alleles present in the pollen grains compete for a same substrate to produce the necessary incompatibility substances.

The complementation theory (Pandey, 1965, 1967) originates from the facts that (a), with one exception, all Pandey's pollen-part mutants had a fragment, and (b) from selfing S heterozygous pollen-part mutants not only S heterozygotes, but also S homozygotes arose (see Table 19c). Homozygotes must arise from S_3fr_3 pollen; the compatibility of such pollen cannot be explained by competitive interaction. So such pollen should be denoted by $S_{3pp}fr_3$. To explain why S_{3pp} pollen is not compatible, lethality is assumed. The theory then is that a pollen-part mutant arises by simultaneous mutation of both the pollen-activity part and a closely linked viability unit and that fr_3 restores viability of the pollen (complementation). Implicit is dominance of S_{3pp} over fr_3 or fr_3 does not contain a functional pollen-activity part. Similarly $S_{3pp}fr_2$ pollen is rendered viable by complementation (Table 19b). Pandey (1965, 1967) then explained compatibility by competitive interaction. However, one may also assume that $S_{3pp}fr_2$ is compatible due to dominance of S_{3pp} over fr_2, either in addition to competitive interaction or even in the absence of it.

The restitution theory (de Nettancourt et al., 1975) assumes that the main consequence of the incompatibility reaction is a general cessation of protein synthesis in the pollen tube. The additional fragment, postulated to consist of nucleolar organizer regions from satellited chromosomes, does not lead to competitive interaction or complementation but, simply provides the pollen tube with the necessary machinery for continuation of protein synthesis (Table 19e).

7.1.2 Nonfragment mutations

Two explanations have been given for the origin of pollen-part mutations which are not associated with the presence of an additional centric fragment. Lewis (1961) supposed that the pollen-part mutation results from genetic losses (deletion or inactivation) of the pollen-activity part (see Table 22a). Pandey (1965, 1967) equated the pollen-part mutation to incorporated duplications which, as for the fragment, may lead to competitive interaction (see Table 22b) or complementation (see Table 22c, d).

To discuss these theories further, some analyses of progenies of pollen-part mutants are now presented.

7.2 MATERIAL AND METHODS

The testcrosses are described together with the results.
Material and methods not described here are presented earlier.

7.3 RESULTS AND DISCUSSION

7.3.1 Pollen-part mutants with a fragment

With competitive interaction in heterogenic di(S)allelic pollen grains, the inbred progeny is expected to consist of only S heterozygotes (Table 19a). In the complementation theory the progenies of pollen-part mutants should segregate in a 1 : 0 ratio (mutant $S_2S_{3pp}fr_2$; Table 19b) or a 1 : 1 ratio (mutant $S_2S_{3pp}fr_3$; Table 19c) for S heterozygotes to S homozygotes. My results together with those of Pandey (1965, 1967) show that, in inbred progenies of pollen-part mutants with a fragment, two different types of segregation ratios were found, namely:

Table 19. Theories for self-compatibility in pollen-part mutants with a fragment (fr), pp = pollen-part mutation, fr_2 = centric fragment which carries an S_2 allele, HET = S heterozygote, HOM_2 = S homozygote (S_2).

Cause of self-compatibility	Genotype of mutant	Gametes	Compatible gamete(s)	Offspring	Segregation ratio HOM_2:HET:HOM_3
a Competitive interaction	$S_2S_3fr_2$	S_2 S_3 S_2fr_2 S_3fr_2	S_3fr_2	$S_2S_3fr_2$ $S_3S_3fr_2$ $S_2S_3fr_2fr_2$ $S_3S_3fr_2fr_2$	0 : 1 : 0
b Complementation only	$S_2S_{3pp}fr_2$	S_2 S_{3pp} S_2fr_2 $S_{3pp}fr_2$	$S_{3pp}fr_2$	$S_2S_{3pp}fr_2$ $S_{3pp}S_{3pp}fr_2$ $S_2S_{3pp}fr_2fr_2$ $S_{3pp}S_{3pp}fr_2fr_2$	0 : 1 : 0
c	$S_2S_{3pp}fr_3$	S_2 S_{3pp} S_2fr_3 $S_{3pp}fr_3$	$S_{3pp}fr_3$	$S_2S_{3pp}fr_3$ $S_{3pp}S_{3pp}fr_3$ $S_2S_{3pp}fr_3fr_3$ $S_{3pp}S_{3pp}fr_3fr_3$	0 : 1 : 1
d Complementation and competitive interaction	$S_2S_{3pp}fr_3$	S_2 S_{3pp} S_2fr_3 $S_{3pp}fr_3$	$S_{3pp}fr_3$ S_2fr_3	$S_2S_{3pp}fr_3$ $S_{3pp}S_{3pp}fr_3$ $S_2S_{3pp}fr_3fr_3$ $S_{3pp}S_{3pp}fr_3fr_3$ $S_2S_2fr_3$ $S_2S_2fr_3fr_3$	0 : 3 : 1
e Restitution	S_2S_3fr	S_2 S_3 S_2fr S_3fr	S_2fr S_3fr	S_2S_2fr S_2S_3fr S_2S_2frfr S_2S_3frfr S_3S_3fr S_3S_3frfr	1 : 2 : 1

Table 20. Segregation of S genotypes in inbred progenies of heterozygous pollen-part mutants with a centric fragment (fr) and cytology of inbred plants. pp = pollen-part mutation, fr_2 = centric fragment which carries an S_2 allele, HET = S heterozygote, HOM_2 = S homozygote (S_2), X = X ray induced mutant, Nf = neutron induced mutant, 13(+) = 13 plants analysed and all have a fragment.

Mutant	Number of plants analysed	pp-HET	pp-HOM_2	pp-HOM_3	Cytology	Genotype parent
C1-1(X)	13	10	0	3	13(+)	$S_2S_{3pp}fr_3$
C1231-1(Nf)	13	12	1	0	5(+)	$S_{2pp}S_3fr_2$
C124c-3(Nf)	9	8	0	1	9(+)	$S_2S_{3pp}fr_3$
C124j-1(Nf)	13	12	1	0	13(+)	$S_{2pp}S_3fr_2$

- a complete absence of S homozygotes (Pandey, 1965, 1967), and
- a pronounced deficit of S homozygous plants (see Table 20 and Pandey, 1965, 1967).

The 1 : 0 ratio characterizes the progeny of a mutant where a lethal pollen-part mutant is complemented by a fragment carrying an S allele which is different from the mutated allele ($S_{3pp}fr_2$; Table 19b). According to Pandey (1965, 1967) the pronounced deficit of S homozygotes characterizes the progeny of a mutant where (1) a lethal pollen-part mutation is complemented by a fragment which carries the same S allele as the mutated allele ($S_{3pp}fr_3$) and where (2) competitive interaction between the unmutated allele and the allele on the fragment occurs (S_2fr_3). In other words the S genotype of this mutant is $S_2S_{3pp}fr_3$. Hence, such mutants produce two classes of self-compatible pollen, which are different in genotype (Table 19d; 3 HET : 1 HOM). In addition, a further shortage of S homozygotes may result from disadvantageous certation of $S_{3pp}fr_3$ pollen).

A more simple explanation for the excess of S heterozygotes is to assume that S_2fr_3 pollen is not compatible (no competitive interaction) and that the majority of S homozygotes are (early) embryonic lethals. There is evidence, as will be seen in Section 7.3.2, that this explanation may indeed be true and that there is no need to invoke competitive interaction as an explanation for the self-compatibility in pollen-part mutations.

It now remains to find out to what extent my results and the data obtained by Pandey (1965, 1967, 1970a) are compatible with the restitution theory recently proposed by de Nettancourt et al.(1975). This theory assumes that the fragment does not carry the S locus, but simply overcomes incompatibility by providing the incompatible pollen tube with the necessary material for initiating protein synthesis which is otherwise blocked by the incompatibility reaction.

The fact that the fragment does not carry an S locus (de Nettancourt et al., 1975) is not consistent with the known facts: Pandey's (1967) and Brewbaker & Natarajan's (1960) observations demonstrated that there must be an S allele on the centric fragment associated with certain pollen-part mutants. This demonstration directly stems from their observations that certain plants with a centric fragment in *Nicotiana* and *Petunia* yielded triallelic offspring, when crossed with an unrelated S homozygous plant ($S_1S_2fr_2$ x $S_5S_5 \rightarrow S_1S_5 + S_2S_5 + S_1S_5fr_2 + S_2S_5fr_2$).

A second objection to the restitution theory as an explanation for the self-compatibility in pollen-part mutants with a fragment, is the fact that the fragment is only transmitted to the next generation in association with the mutated allele; this association results in a lack of one class of S homozygotes in the inbred progeny (Table 20). However, on the basis of the restitution theory, the inbred progeny should contain (in addition to S heterozygotes) both classes of S homozygotes, because the fragment is expected to be transmitted to the next generation with either S allele (see Table 19e). Since such a transmission does not occur, the restitution theory is not the most likely explanation for the function of the fragment in radio-induced pollen-part mutants.

It will now be shown by analogy to the pollen-part mutants without a fragment, that complementation is by far the most attractive hypothesis.

7.3.2 Pollen-part mutants without a fragment

Because of the large number of induced pollen-part mutants without a centric fragment (see Section 4.4.3), the segregation patterns which can be observed in the progenies of such mutants can be analysed in detail.

It can be seen from Table 21 that three different types of segregation ratios were found, namely:
- a complete absence of S homozygotes,
- a pronounced deficit of S homozygotes, and
- an equal number of S homozygotes and S heterozygotes.

Table 22 lists the segregation ratios for S heterozygotes and S homozygotes which are expected in the inbred progenies of pollen-part mutants without a

Table 21. Segregation of S genotypes in inbred progenies of heterozygous pollen-part mutants without a centric fragment. pp = pollen-part mutation, (2) = duplication bearing an S_2 allele, HET = S heterozygote, HOM_2 = S homozygote (S_2), X = X ray induced mutant, Nf = neutron induced mutant.

Mutant	Number of plants analysed	pp-HET	pp-HOM_2	pp-HOM_3	Genotype parent
C1-2(X)	22	20	0	2	$S_2S_{3pp(3)}$
C1-4(X)	10	8	0	2	see C1-2
C1-6(X)	9	8	1	0	$S_{2pp(2)}S_3$
C83-1(X)	11	11	0	0	$S_2S_{3pp(2)}$ or $S_{2pp(3)}S_3$
C124m-1(Nf)	14	6	0	8	see C1-2
C123y-1(Nf)	12	12	0	0	see C83-1
C123j-1(Nf)	14	14	0	0	see C83-1

Table 22. Theories for self-compatibility in pollen-part mutants without a fragment. pp = pollen-part mutation, (2) = duplication bearing an S_2 allele, HET = S heterozygote, HOM_3 = S homozygote (S_3).

Cause of self-compatibility	Genotype of mutant	Gametes	Compatible gamete	Offspring	Segregation ratio HET : HOM_3
a Deletion or inactivation	S_2S_{3pp}	S_2 S_{3pp}	S_{3pp}	S_2S_{3pp} $S_{3pp}S_{3pp}$	1 : 1
b Competitive interaction	$S_2S_{3(2)}$	S_2 $S_{3(2)}$	$S_{3(2)}$	$S_2S_{3(2)}$ $S_{3(2)}S_{3(2)}$	1 : 0
c Complementation	$S_2S_{3pp(2)}$	S_2 $S_{3pp(2)}$	$S_{3pp(2)}$	$S_2S_{3pp(2)}$ $S_{3pp(2)}S_{3pp(2)}$	1 : 0
d	$S_2S_{3pp(3)}$	S_2 $S_{3pp(3)}$	$S_{3pp(3)}$	$S_2S_{3pp(3)}$ $S_{3pp(3)}S_{3pp(3)}$	1 : 1

fragment, on the basis of the different assumptions made to explain the nature of such mutations. This table shows that practically all results can be explained by complementation through duplications.

The 1 : 1 ratio characterizes the progeny of a mutant where a lethal pollen-part mutant is complemented by an incorporated segment carrying an S allele similar to the mutated one (Table 22d).

Table 23. Segregation of S genotypes in two outbred progenies of heterozygous pollen-part mutants without a centric fragment. SI = self-incompatible, SC = self-compatible, pp = pollen-part mutation, (2) = duplication bearing an S_2 allele.

Cross performed	S_2S_4(SI)	$S_2S_3S_4$(SC)	S_3S_4(SC)	Genotype parent
C123y-1 x S_4S_4	9	5	0	$S_2S_{3pp(2)}$
C123j-1 x S_4S_4	8	3	2	?

Similarly, the 1 : 0 segregation pattern would be typical of progenies of mutants with a lethal pollen-part mutation which is complemented by an incorporated segment including an S allele different from the mutated one (Table 22c). The latter situation could be demonstrated by testcrosses which showed that two different S specificities were present in the same pollen grain (the cross between the assumed $S_2S_{3pp(2)}$ and S_4S_4 (♂) gave rise to individuals which are $S_2S_3S_4$ in genotype; Table 23). This also excludes "statistical escape" in the progenies.

The third group of progenies had a deficit of S homozygotes. It is difficult to explain this result directly but one could assume that the basic ratio was 1 : 1 and that a large part of the S homozygotes were lethal. The argument is important because there is no alternative interpretation for the deviation from the 1 : 1 ratio which cannot be explained by competitive interaction, such in contrast to the case of the pollen-part mutants associated with a centric fragment. This is obvious because the linked complementing segment in pollen-part mutants without a fragment cannot segregate independently with either the mutated or the unmutated allele, as was the case with the fragment in fragment-carrying mutants.

In conclusion it can be said that competitive interaction is not the most likely explanation for the deficit of S homozygotes in *Nicotiana alata* and is perhaps not involved at all in the manifestation of self-compatibility in pollen-part mutants, i.e. neither in pollen-part mutants without nor in pollen-part mutants with the fragment. Neither Pandey (1967) nor Brewbaker & Natarajan (1960) could have reached such a conclusion because they did not have at their disposal the pollen-part mutants without a fragment, which I induced with X rays and fast neutrons (see Section 4.4.3).

It seems that complementation, which must be at the origin of self-compatibility in homozygous pollen-part mutants with a fragment, may well be the only mechanism by which a pollen-part mutant is self-compatible.

8 Identification of the S-bearing chromosome

8.1 INTRODUCTION

It is puzzling that the numerous analyses that have been made of the self-incompatibility phenomenon in higher plants have never clearly indicated where the S locus is situated in the genome (for a discussion, see de Nettancourt, 1972). Although there is no doubt, from the bulk of data available on the genetic segregation of S alleles, that the S locus is situated on a chromosome, it would be worth while to identify the S-bearing chromosome.

8.1.1 Identification by linked markers

If sufficient genetic markers (and the sites of the corresponding genes within the linkage group) are known, linkage of markers to the S locus automatically reveals the position of the S locus in the genome. However, the procedure is difficult because so far only eight cases of linkage between an S allele and a visible phenotypic trait have been reported in homomorphic species (de Nettancourt, 1972).

- *Nicotiana sanderae*; the S locus is linked to a flower colour locus (Brieger & Mangelsdorf, 1926).
- *Oenothera organensis*; linkage to a gametophytic pollen lethality factor (Emerson, 1941).
- *Nemesia strumosa*; linkage to the bicolour and buff gene (Riley, quoted by de Nettancourt, 1972).
- *Lotus corniculatus*; possible linkage to the gene controlling keel-tip colour (Bubar, quoted by de Nettancourt, 1972).
- *Petunia hybrida*; possible linkage to the undulata gene (Bianchi, 1959; Reimann-Philipp, 1965).
- *Trifolium pratense*; linkage to a flower colour gene (Denward, 1963b).
- *Brassica oleracea*; linkage to (a) female sterility gene(s) (Thompson & Howard, 1959).
- *Lycopersicon chilense*; linkage to the dwarf, the wooly and a necrotic gene

(Martin, 1961).

Except for the last example, where the genes responsible for the dwarf, wooly and necrotic character are known to be situated on chromosome 2, the sites of the marker genes within the genome have not (yet) been determined. It follows that, with the possible exception of tomatoes where the situation was greatly complicated and obscured by interspecific incompatibility and modifiers, no one has yet succeeded to use marker genes for identifying the S-bearing chromosome.

8.1.2 Identification by cytological analysis of pollen-part mutants

If one assumes, as Pandey (1967) and Brewbaker & Natarajan (1960) did, that the additional centric fragment, which characterizes pollen-part mutants, carries an S allele, pachytene analysis of such plants may reveal the segment in the genome which is homologous to the fragment.

Pandey (1967) reported occasional pairing between the fragment and a bivalent at the first metaphase. On the basis of the configuration of the lagging divided fragment at the first meiotic anaphase, Pandey (1967) assumed that a subterminal chromosome without a satellite is the S-bearing chromosome. This conclusion was challenged by Carluccio et al. (1974) who provided evidence that the fragment in certain pollen-part mutants of *Nicotiana alata* originated from a satellited chromosome.

Another interesting approach for identifying the S-bearing chromosome with the help of centric fragments which carry the S locus, is the use of banding techniques which may allow determination of the origin of the fragment. This method is being attempted at the Association EURATOM-ITAL.

8.1.3 Identification by cytological analysis of aneuploids

The chromosome bearing a given gene is often identified with the help of trisomic series and by the analysis of segregation ratios in their inbred or crossed progenies (Hermsen, 1970). If all chromosomes can be distinguished morphologically, then with karyotype analysis, in juxtaposition with genetic data, the chromosomes bearing the various genes characterized by trisomic inheritance can be identified.

With regard to the identification of the S-bearing chromosome the situation is more simple. Karyotype analysis of self-compatible aneuploid plants should, in theory, reveal the S-bearing chromosome, because according to the competitive interaction theory, self-compatible aneuploid plants have three S-bearing chromosomes and self-compatibility results from competitive interaction in heterogenic di(S)allelic pollen grains. However, aneuploids with three different S alleles were self-incompatible (see Section 6.3.2). Therefore, plants with three S-bearing chromosomes are selected in the following way. Since a style does not accept pollen grains with a matched S allele, aneuploid individuals with three different S alleles will reject three different classes of pollen grains and can thus be selected by simple testcrosses with appropriate homozygous tester clones. Karyotype analysis of such triallelic plants then reveals the S-bearing chromosome.

This chapter only describes the results of cytological analysis of aneuploid plants.

8.2 MATERIAL AND METHODS

8.2.1 Production and detection of plants with three different S specificities

To constitute aneuploid individuals a triploid plant, from the progeny of the cross between a tetraploid and a diploid plant (see Section 6.3.1), was crossed with an unrelated diploid plant with the genotype S_6S_7 (see Section 6.3.2). Testcrosses with the homozygous tester stocks (S_2S_2, S_3S_3, S_6S_6 and S_7S_7) as staminate parents were carried out to detect the triallelic offspring $S_2S_3S_6$ and $S_2S_3S_7$ (see Section 6.3.2).

8.2.2 Karyotype analysis of triallelic plants

All individuals which displayed three different S specificities in the style and thus three S-bearing chromosomes were examined cytologically to identify the trisomic chromosomes. The S-bearing chromosome should in all cases be present three times. The idiogram and the arm length ratios for the haploid set of *Nicotiana alata* chromosomes were established by Carluccio et al. (1974) and the idiogram is presented in Fig. 20. It should be noted that the two chromosomes within a group (St_1 and St_2; Sat_1 and Sat_2; M_1 and M_2; M_3 and M_4) cannot reliably be distinguished morphologically from each other.

Material and methods not described here have been given by Carluccio et al. (1974) and in Chapters 2 and 6.

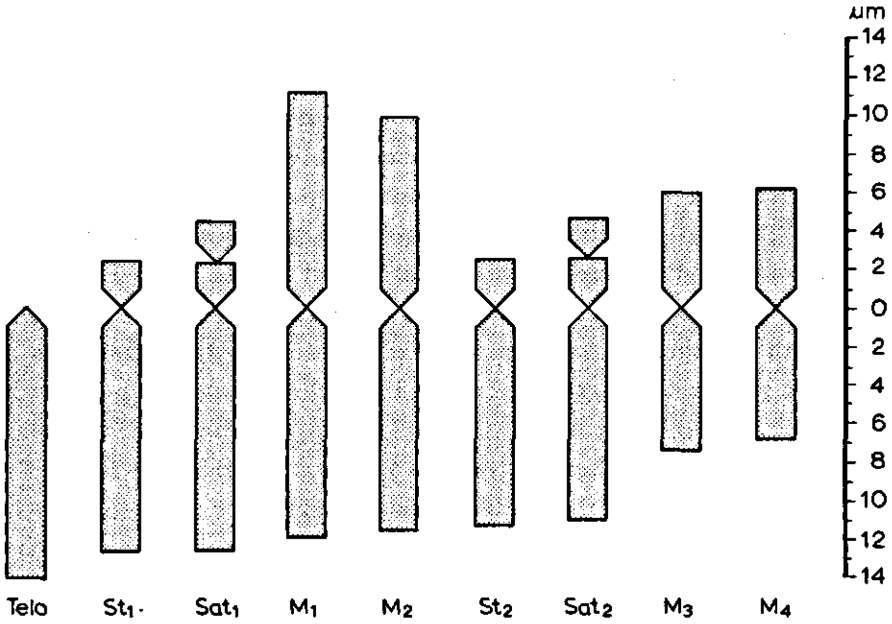


Fig. 20. Idiogram of *Nicotiana alata* (kindly provided by F. Carluccio, Laboratorio per le Applicazioni in Agricoltura, CNEN, Roma, Italia). Telo = telocentric chromosome, St_1 and St_2 = acrocentric chromosomes, Sat_1 and Sat_2 = satellited chromosomes, M_1 - M_4 = metacentric chromosomes.

8.3 RESULTS

Since the behaviour of the triploid individuals and of the aneuploid plants with three different S specificities have already been presented (see Sections 6.3.1 and 6.3.2) and discussed (see Section 6.4), only the results dealing with the karyotype analysis will be outlined here.

Nine plants which displayed three different S alleles in the style were detected among the 59 offspring of the cross between a triploid plant and a diploid individual (see Table 18). Eight of these triallelic plants have been analysed cytologically and the results are presented in Table 24. This table shows that the somatic chromosome number of these aneuploids ranged from 19 to 25.

Theoretically plant C191A-19 should have been the most important plant for the identification of the S-bearing chromosome because it only carries one extra chromosome. However, besides the extra satellited acrocentric chromosome an additional centric fragment was present in the somatic cells (Fig. 21). The origin of this fragment is obscure and since an S allele may

Table 24. Summary of karyotype analyses of eight aneuploid plants which display three different S specificities. Telo = telocentric chromosome, St_1 and St_2 = acrocentric chromosomes, Sat_1 and Sat_2 = satellited chromosomes, M_1-M_4 = metacentric chromosomes.

	Number of chromosomes	Chromosomes which are present three times					S genotype (see Table 18)
		telo	St_1,St_2	Sat_1,Sat_2	M_1,M_2	M_3,M_4	
C191A- 3	25		xx	x	xx	xx	$S_2S_3S_6$
C191A-18	22		xx		x	x	$S_2S_3S_7$
C191A-19	19[1]			x			$S_2S_3S_6$
C191A-22	24	x	x		xx	xx	$S_2S_3S_7$
C191A-26	23		xx		x	xx	$S_2S_3S_6$
C191A-30	23		xx		xx	x	$S_2S_3S_6$
C191A-35	20		xx				$S_2S_3S_7$
C191A-42	24		xx	x	xx	x	$S_2S_3S_7$

[1] This plant carried in addition to the 19 chromosomes an additional centric fragment.

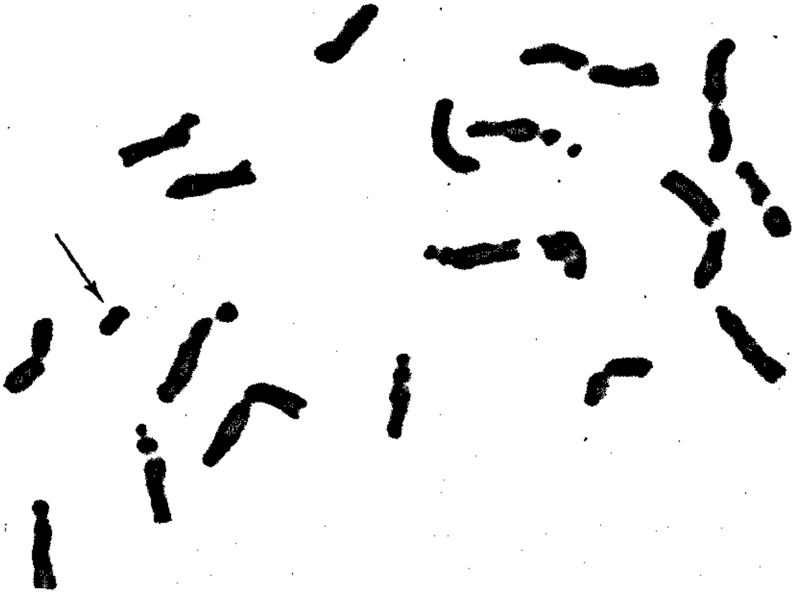


Fig. 21. Karyotype of the self-compatible aneuploid C191A-19, which displays 19 chromosomes and a centric fragment.

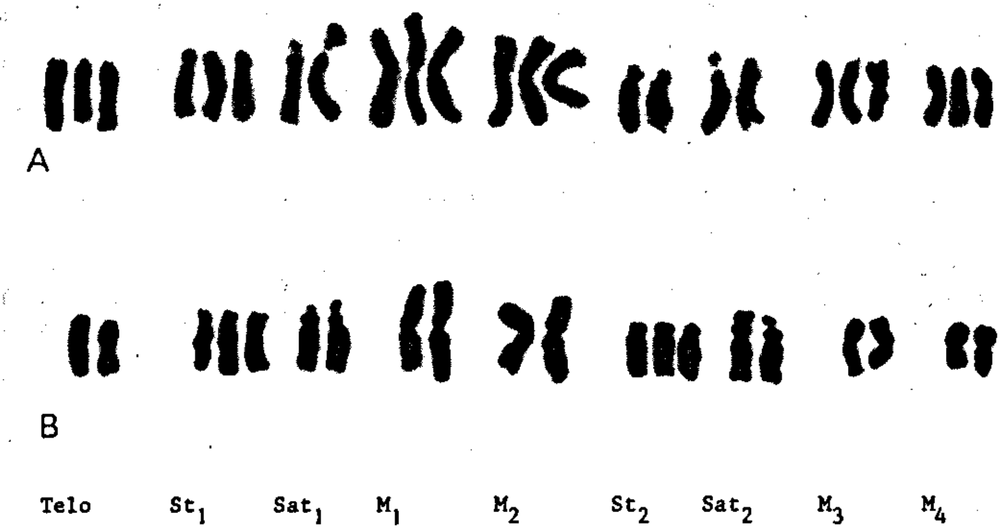


Fig. 22. Idiograms of two aneuploid *Nicotiana alata* plants which are trisomic for the S locus. (A) C191A-22, (B) C191A-35.

be situated on such a fragment the karyotype of this plant could not be used for the identification of the S-bearing chromosome (see Section 6.4). Fig. 22 shows the idiograms of plants C191A-22 and C191A-35.

8.4 DISCUSSION

In Table 24 the results of the karyotype analysis are summarized to take into account the fact that the two chromosomes within a group (St_1 and St_2; Sat_1 and Sat_2; M_1 and M_2; M_3 and M_4) cannot be reliably distinguished morphologically from each other (see Fig. 20). Since in all aneuploids (except C191A-19) the unsatellited acrocentric chromosomes (St_1 and St_2) are the only ones which are always present three times, it can be concluded that one of these two chromosomes is the S-bearing chromosome.

Analysis of the unsatellited acrocentric chromosomes of the aneuploids revealed that in plant C191A-3, C191A-18, C191A-26, C191A-30, C191A-35 and C191A-42, both unsatellited acrocentric chromosomes are present three times and that in plant C191A-22 probably only St_1 is present three times. Therefore, it seems that the longest unsatellited acrocentric chromosome is the S-bearing chromosome in *Nicotiana alata*.

To confirm this conclusion and because the two unsatellited acrocentric chromosomes differ only slightly in their morphology, experiments have been initiated at our Institute, to stain the chromosome of *Nicotiana alata* with Giemsa. The results obtained showed that the Giemsa C-banding technique is very promising (Engels, pers. comm.) and may allow a clear distinction between morphologically identical chromosomes in *N. alata*. Pandey (1967) concluded, on the basis of meiotic configuration and morphology of the centric fragment, that an unsatellited acrocentric chromosome is probably the S-bearing chromosome. My conclusion which is based on the karyotype analysis of tri(S)allelic plants, confirms Pandey's (1967) conclusion.

Summary

Chapter 2 Leaf propagation was very useful for producing large clonal populations of *Nicotiana alata* genotypes. The clones obtained grew more homogeneously than those obtained with stem cuttings. Leaf propagation was a technique for generating self-compatibility mutations which mostly could be ascribed to tetraploidy. Such tetraploid plants could easily be selected because they had thicker stems, broader leaves and larger flowers. The stomata size was larger than that of diploid individuals. In addition to the self-compatible tetraploids five diploid plants were self-compatible. Two of them carried a stylar-part mutation at the S locus, whereas the remaining three self-compatible plants had a pollen-part mutation. Therefore, it seems that the mutability at the pollen-part equals the mutability at the stylar-part of the S locus.

The fact that only one mutant displayed a chimeric structure provides additional evidence that the apex of each adventitious plantlet can be traced back to cells ultimately derived from a single epidermal cell.

Chapter 3 Chronic gamma irradiation did not, at least at the dose rates studied, induce self-compatibility mutations and failed to generate any new incompatibility allele at the S locus. However, chronic gamma irradiation did induce seed set upon selfing in self-incompatible plants. This increase in pseudo-self-compatibility resulted from an effect of irradiation on the pistil and resembled the effect of slight increases in temperature during several days before and after pollination.

Pollen abortion increased with increasing dose rate and amounted to 73.1% at 14.3 rad/h.

Chapter 4 Both fast neutrons and X rays induced large numbers of pollen-part mutations. Fast neutrons were slightly more efficient than X rays for inducing pollen-part mutations. The RBE was greater than one. Neither fast neutrons nor X rays had the capacity to generate new self-incompatibility alleles. Hence, if the S locus is considered as representative for the genes operating in higher plants, it seems that gamma rays, X rays and fast neutrons are probably not effective for reconstructing new functional alleles.

An unexpectedly high fraction of the self-compatibility mutations induced did not display the fragment which usually characterizes such mutations. The ratio of fragment to nonfragment self-compatibility mutations induced by fast neutrons was identical to that of X rays. In other words, there is no difference between the mutation spectra of X rays and fast neutrons. Only very few cases of pseudo-compatibility were probably due to revertible mutations.

Permanent self-compatibility mutations could be used for production of F_1 hybrid seed.

An increase of pollen abortion with increasing dose in both the fast neutron and the X ray series was observed. The RBE value decreased with increasing X ray dose .

Chapter 5 No permanent S mutations were found among the M_1 and M_2 plants raised from EMS-treated seeds.

It is probable that EMS-induced S mutations were not detected after PMC treatment because the choice of the scoring period did not take into account the retardation effects induced by EMS on bud development. Consequently most of the pollen scored for mutations did not originate from buds treated at the proper stage.

EMS had an effect on the pistil of the treated individuals comparable to the ones which have been reported to occur after chronic irradiation, heat shock or acute irradiation.

Chapter 6 Triploids were male sterile because no seed was obtained in crosses where the triploids were used as staminate parent whereas good seed set was usually obtained in the reciprocal cross.

Aneuploids were male fertile since they could be used as male parents. Apparently they produced sufficient functional pollen. Because such functional pollen is euploid or because aneuploid pollen with two S-bearing chromosomes is not functional, the aneuploids with three different S-bearing chromosomes are self-incompatible.

Chapter 7 The segregation ratios in inbred progenies of pollen-part mutants without a centric fragment could only be explained by complementation. A lethal pollen-part mutation is complemented by an incorporated segment which carries essential elements which are deleted at the time a pollen-part mutation is induced. The large excess of S heterozygotes in some progenies must have resulted from lethality of S homozygotes. The same events (complementation and S homozygote lethality) could explain all segregation data in the progenies of pollen-part mutants with a fragment. In this case the fragment complements information outside the S locus which is deleted in the original genome at the moment a pollen-part mutation is induced. Since strong evidence has been obtained that fragments do carry an S allele and that transmission to the next generation of the fragment is S dependent, it appears that the restitution theory cannot be considered as an explanation for the function of the fragment in radio-induced pollen-part mutants. Therefore, complementation is probably the only mechanism by which a pollen-part mutant is self-compatible.

Chapter 8 Evidence has been obtained that the longest unsatellited acrocentric chromosome is the S-bearing chromosome in *Nicotiana alata*.

Samenvatting

Bij bloemplanten zijn een groot aantal mechanismen bekend die zelfbevruchting en daardoor inteeltdepressie verhinderen (o.a. mannelijke steriliteit, protandrie en protogynie). Een veel voorkomend mechanisme (3000 soorten) is zelf-incompatibiliteit. Incompatibiliteit kan gedefinieerd worden als de verhindering van een gametenvereniging binnen een voortplantingssysteem, die niet op defecten van de gameten is terug te voeren. Naast zelf-incompatibiliteit kan kruisings-incompatibiliteit optreden. Indien incompatibiliteit samen gaat met verschillen in bloembouw spreekt men van heteromorfe incompatibiliteit. Treden geen morfologische verschillen op, dan hebben we te maken met homomorfe incompatibiliteit.

Homomorfe incompatibiliteit kan worden ingedeeld in gametofytische en sporofytische incompatibiliteit. *Nicotiana alata* heeft een gametofytisch incompatibiliteitssysteem, dat op één locus berust. Het al of niet plaatsvinden van de incompatibiliteitsreactie is afhankelijk van het genotype van de haploïde pollenkorrel (gametofyt) en het genotype van het diploïde stijlweefsel. Mutatieonderzoek van eerdere auteurs heeft uitgewezen dat in planten met een gametofytisch incompatibiliteitssysteem de S-locus uit drie delen bestaat die onafhankelijk van elkaar kunnen muteren, maar functioneel een eenheid vormen. Deze delen worden respectievelijk specificiteitsdeel, activiteitsdeel voor het pollen en activiteitsdeel voor de stijl genoemd. De transcriptie (?) van het specificiteitsdeel wordt in het pollen en in de stijl gereguleerd door het bijbehorende activiteitsdeel. De incompatibiliteitsreactie vindt plaats indien pollen en stijl hetzelfde specificiteitsdeel (S-allel) hebben.

Uit literatuuronderzoek is gebleken dat mutagene behandeling vaak resulteert in mutaties van het activiteitsdeel (dat wil zeggen de plant wordt zelf-compatibel), terwijl het specificiteitsdeel "ongevoelig" voor mutagene behandeling blijkt te zijn (geen verandering van de S-specificiteit, d.w.z. geen verandering van S_x naar S_y). Mutaties van het activiteitsdeel zijn vaak terug te voeren op chromosoom-aberraties; veranderingen van de specificiteit, die wel optreden in inteeltpopulaties, zijn waarschijnlijk puntmutaties.

De incompatibiliteitslocus biedt in principe de mogelijkheid om mutagene stoffen te onderzoeken voor wat betreft hun vermogen om aberraties en puntmutaties te induceren. Het doel van het onderhavige onderzoek was dan ook om het mutatiespectrum van de S-locus vast te stellen na behandeling met verschillende mutagentia.

Omdat al het experimentele plantmateriaal verkregen is via de bladstekmethode wordt, na de Inleiding, in hoofdstuk 2 aandacht besteed aan mogelijke veranderingen ontstaan door deze wijze van vermenigvuldigen. De methode blijkt zeer goed bruikbaar te zijn om plantmateriaal vegetatief te vermeerderen; de verkregen planten zijn veel homogener dan planten die ontstaan door stengelstekjes te laten wortelen. Ongeveer 7% van de planten blijkt tetraploïd te zijn. Deze tetraploïden zijn eenvoudig te selecteren omdat ze dikkere stengels, bredere bladeren en grotere bloemen hebben. De lengte van de sluitcellen van de huidmondjes is duidelijk groter dan die van de sluitcellen in bladeren van diploïde planten.

Naast zelf-compatibele tetraploïden werden vijf zelf-compatibele diploïde planten gevonden. Drie planten bleken een mutatie in het activiteitsdeel voor het pollen te bezitten. Zulke planten zijn zelf-compatibel en verder alleen compatibel met de oorspronkelijke moederkloon als de mutant als vader wordt gebruikt. De twee andere zelf-compatibele diploïden hadden een mutatie in het activiteitsdeel voor de stijl. In dit geval is de (heterozygote) mutant alleen compatibel als deze wordt bestoven door pollen van de moederplant. De conclusie is getrokken dat de bladstekmethode goed bruikbaar is voor vegetatieve vermenigvuldiging, maar dat men de verkregen planten pas kan gebruiken voor incompatibiliteitsonderzoek nadat tetraploïden (fenotypische selectie) en zelf-compatibele diploïden (zelfbestuiving) zijn verwijderd.

In de hoofdstukken 3, 4 en 5 worden de resultaten vermeld die verkregen zijn na behandeling van planten, pollenmoedercellen en zaad met een bepaald mutagens. Zowel na chronische gammabestraling als na EMS-behandeling treden geen mutaties van de S-locus op. Wel wordt een verhoogde zaadzetting na incompatibele kruisingen verkregen. Worden pollenmoedercellen bestraald met röntgenstralen of snelle neutronen, dan worden vele mutaties in het activiteitsdeel geïnduceerd. Verandering van de specificiteit ($S_x \rightarrow S_y$) is in geen enkel geval gevonden.

In hoofdstuk 6 wordt het kruisingsgedrag van triploïden en aneuploïden beschreven. Triploïden blijken mannelijk steriel te zijn; aneuploïden zijn zowel vrouwelijk als mannelijk fertiel. Aneuploïden met drie verschillende

S-loci zijn zelf-incompatibel.

In hoofdstuk 7 wordt aandacht besteed aan het mechanisme waardoor planten met een gemuteerd pollen-activiteitsdeel zelf-compatibel zijn. Het blijkt dat de complementatietheorie de beste verklaring geeft. Deze theorie houdt in dat een centrisch fragment (of een duplicatie) dat aanwezig is in de celkernen van genoemde mutanten, bepaalde, in het oorspronkelijke genoom gemuteerde, elementen complementeert, waardoor de pollenkorrel met het gemuteerde pollen-activiteitsdeel weer in staat is de incompatibiliteitsbarrière te overwinnen. Complementatie treedt dus zowel op in mutanten met een fragment als in mutanten zonder een fragment. Competitie tussen twee S-allelen in één pollenkorrel speelt geen rol bij de zelf-compatibiliteit van zulke mutanten.

In het laatste hoofdstuk worden experimenten beschreven om het chromosoom te bepalen waarop de locus ligt die verantwoordelijk is voor de incompatibiliteitsreactie. Zeer waarschijnlijk is dit het langste acrocentrische chromosoom dat geen satelliet heeft.

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