Uluslararası Fen Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Science Research 2022, Vol. 6 (4), 185-210
https://doi.org/10.29329/ijiasr.2022.512.2

Original article

Inheritance of Unilateral Incompatibility in the Genus *Capsicum*

Ahmet Naci Onus *

Department of Horticulture, Faculty of Agriculture, Akdeniz University, Antalya, Türkiye

Abstract

Unilateral incompatibility is reported to occur between the species of *Capsicum pubescens* complex (*Capsicum cardenasii Capsicum. eximium* and *Capsicum pubescens*) and other species of genus *Capsicum* when the latter are used as the male parent. The relationship between self-incompatibility and unilateral incompatibility are yet to be resolved even in the most intensively investigated crosses within and between species of *Lycopersicon* and *Solanum*. Unilateral incompatibility in *Capsicum* has been less intensively studied compared to some other genus in family *Solanaceae*. This study was, therefore, conducted to help us to understand the genetic control of unilateral incompatibility in *Capsicum*. Experiment results showed that while the pistil behaviour of F_1 hybrids agrees with the pistil behaviour of F_1 hybrids from unilaterally incompatible crosses in other genera of *Solanaceae*, the pollen behaviour does not agree with data obtained from other genera of *Solanaceae*. No segregation ratio was obtained for unilateral incompatibility in the backcross progenies, this result does not agree with data from other genera in the *Solanaceae* either. Possible reasons for not obtaining segregation for unilateral incompatibility may be the small size of backcross progenies and/or distorted segregation ratios. As a conclusion it seemed more probable that, in *Capsicum*, unilateral incompatibility has arisen as a by-product of genetic divergence between the *C. pubescens* complex and the other chile peppers, not as a product of natural selection.

Keywords: *Capsicum*, Incongruity, Unilateral Incompatibility, Inheritance, F_1 Plants, Backcross Generation.

Received: 30 December 2022 * **Accepted:** 30 December 2022 * **DOI:** https://doi.org/10.29329/ijiasr.2022.512.2

* **Corresponding author:**

Onus, Ahmet Naci is a professor in the Department of Horticulture at Akdeniz University in Antalya, Turkey. His research interests include the Agricultural Sciences, Vegetable Breeding and Breeding, Biotechnology and Genetics. He has lived, worked, and studied in Antalya, Türkiye.
Email: onus@akdeniz.edu.tr

INTRODUCTION

Unilateral incompatibility (UI) occurs when pollen tubes reach and fertilise the ovules in a cross made in one direction, but are inhibited in stigma, style or ovary in the reciprocal cross (Lewis and Crowe, 1958). Unilateral incompatibility is commonly reported between species or groups of species in a genus. But it may also occur within species. UI usually prevents self-incompatible (SI) species from accepting pollen or pollen tubes of self-compatible (SC) species. But there are numerous cases where one-way crossability has been found not only crosses of SC and SI but also between two SC or two SI species. To explain these cases Lewis and Crowe (1958) suggested that the exceptions to SI x SC rule are the self-compatible species and varieties which have recently mutated from SI species. They used the designation Sc to distinguish them from self-compatible species (SC) which obey the rule. Several authors suggested that UI and self-incompatibility have a common basis in the *S* gene complex and UI is a function of *S* alleles (Lewis and Crowe, 1958; Martin, 1963, 1964, 1966; Pandey, 1962).

As stated above in solanaceous plants, SI is controlled by a single multiallelic locus and *S* allele specific pollen rejection occurs as pollen tubes grow through down the transmitting tract. The products of the *S* locus in the style are the S-RNases (Murfett et al., 1996). In some of the recent studies S-RNases have been also implicated in interspecific pollen rejection. In this manner; Kondo et al. (2002) studied the molecular basis of loss of self-incompatibility in genus *Lycopersicon*. In their study, S-RNase and HT-proteins were analysed in seven SC and three SI taxa. No, or low stylar RNase activity was reported in most SC taxa they examined, while high level of RNase activity was present in all SI species. They reported that the *S-RNase* gene was most likely deleted in the SC species of *L. esculentum, L. esculentum* var. *cerasiforme, L. pimpinelliifolium* and *L. cheesmanii* since there was no amplification of *S*-RNase genes from genomic DNA. However the *S-RNase* gene was amplified from the genomes of SC species *L. chmielewski* and *L. hirsutum* f. *glabratum* as these species showed a decreased accumulation of transcripts. S-RNase and interspecific pollen rejection was also studied in other genera, for example *Nicotiana* (Murfett et al., 1996). By using the transgenic, which has been proven to be successful on defining the role of S-RNases in SI mechanism, Murfett et al. (1996) showed that *S*-RNases were indeed involved in UI is some species combinations in the genus *Nicotiana*. The S-RNase mechanism was recently reviewed in detail by Cruz-Garcia *et al*. (2003).

SI was correlated with UI in *Lycopersicon* too in earlier studies, such as Martin (1964). Afterwards mapping studies and QTL analyses provided good evidence that *S* locus plays a major role in UI. For example in genus *Lycopersicon*, Chetelat and DeVerna (1991), working on hybrids involving *Lycopersicon esculentum* (SC), *Lycopersicon pennellii* (Sc) and *S. lycopersicoides* (SI), tested the pollen behaviour of plants from segregating generations on the pistils of *S. lycopersicoides* (SI). They reported that the pollen of plants carrying alleles from *L. pennellii* at three different loci was compatible with style of *S. lycopersicoides*. Three loci were mapped to chromosome 1, 6 and 10. They reported that

the locus on chromosome 1was so close the *S* locus that may be the *S* locus. This finding clearly indicates the involvement of the *S* gene on interspecific incompatibility.

Unilateral incompatibility has been observed in *Capsicum* and it was reported that it occurs between the species in the *Capsicum pubescens* (*C. pubescens*, *Capsicum C. cardenasii* and *Capsicum C. eximium*) complex and all other species (Onus, 1995; Onus and Pickersgill, 2004; Pickersgill, 1991, 1997). In *Lycopersicon* and *Solanum*, self-incompatibility is common among the wild species and self-compatibility seems likely to be derived condition (Pickersgill, 1997). In *Capsicum*, the only species in which self-incompatibility is the norm is *C. cardenasii*. *C. cardenasii* has a typical flower for *Capsicum* and a narrow geographic range. It is, therefore, likely to say that self-incompatibility looks more like a derived than an ancestral condition (Pickersgill, 1997).

Several authors have investigated the genetic control of unilateral incompatibility in the F_1 generation to find out whether there was any association between UI and *S* gene (Martin, 1966; Hardon 1967; Hogenboom 1972, 1973; Pandey, 1962). At the end of all these studies the following points emerged: styles of the F_1 hybrid plants inhibited pollen from their SC parent. This result indicated that stylar rejection of SC pollen behaved as a dominant trait inherited from the Sc/SI parent, pollen of the F_1 hybrid plants was accepted by the pistil of the SC parent. But pollen of the F_1 hybrid plants' was rejected by the pistils of their Sc or SI parents, the results were similar regardless of whether F_1 hybrid plants inherited an active or inactive *S* allele from the wild species.

Several workers have found segregations for behaviour of pistils and pollen in backcross generations of crosses between unilaterally incompatible taxa. For example, Martin (1964) studied F_1 hybrids between two accessions of *Lycopersicon hirsutum* and reported that UI and self-incompatibility were usually associated. Chetelat and DeVerna (1991) also reported that unilateral incompatibility in other *Solanaceae* was at least partially under the control of the *S* gene. Mather (1943) working on self-compatible *Petunia axillaris* x self- incompatible *Petunia. violacea* reported that "the backcross to *P. axillaris* would appear to have introduced so many modifying genes that incompatibility allelomorphs, if present, have largely ceased to be operative".

On the other hand, several other authors argued that self-incompatibility and interspecific incompatibility are two distinct mechanisms and interspecific incompatibility may occur at any stage from pollination to fertilization and the genes which are responsible for interspecific incompatibility may operate at any of these stages and occur at more than one locus (Grun and Aubertin, 1965) and this phenomenon is called as “incongruity” by Hogenboom (1972, 1973).

As can be seen the argument about the relationship between self-incompatibility and unilateral incompatibility are yet to be resolved even in the most intensively investigated crosses within and between species of *Lycopersicon* and *Solanum*. Unilateral incompatibility in *Capsicum* has been less

intensively studied compared to some other genus in family *Solanaceae.* To our knowledge, this is the first comprehensive study on the inheritance of unilateral incompatibility in the genus *Capsicum*. This will help us to understand the genetic control of unilateral incompatibility in the genus. This is of interest for two reasons: 1) To see which of hypotheses concerning unilateral incompatibility best fit data from *Capsicum*, 2) To see how much a practical barrier unilateral incompatibility presents to interspecific gene transfer, whether these barriers continue into F_1 and first backcross (BC_1) generations.

MATERIALS and METHODS

Plant materials

The plant materials used for the experiments were *Capsicum baccatum* SA219 (SC) and Hawkes 6489 (SC), *Capsicum eximium* (Hawkes 3860 (Sc)) a self-compatible accession of *Capsicum cardenasii* (SA268 (Sc)) and F_1 hybrids *Capsicum baccatum* SA219 (SC) x *Capsicum eximium* Hawkes 3860 (Sc) and *Capsicum baccatum* Hawkes 6489 (SC) x *Capsicum cardenasii* SA268 (Sc). The cross between *C. baccatum* (SC) and *C. cardenasii* (Sc) and *C. eximium* (Sc) exhibits unilateral incompatibility when the latter are used as the female (Onus, 1995; Onus and Pickersgill, 2004 a). The F_1 hybrids had been previously synthesized at The University of Reading, UK by Dr. Barbara Pickersgill and Mr. Eri Sofiari, respectively. Four different backcross progenies were produced from 2 unilaterally incompatible interspecific crosses: *C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc) and *C. baccatum* Hawkes6489 (SC) x *C. cardenasii* SA 268 (Sc). The F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc)) was used as female in BCs to both parents. The reciprocal of one of these BCs (*C. baccatum* (SC) x F_1) was also studied. The 4th progeny was *C. baccatum* (SC) x F_1 (*C. baccatum* (SC) x *C. cardenasii* (Sc).

Hand pollinations

All pollinations were made on pot-grown plants on the same day in a greenhouse. Buds which were about to open were emasculated in the morning or early afternoon by removing the corolla and stamens with fine forceps. The stigmas were then pollinated with pollen from flowers whose anthers had dehisced that day. The stigmas were not covered in any way after pollination as it was decided that the pollination by wind and insects was minimal, if there was any, since the plants were planted in protected enclosures and the absence of petals (the emasculation also removes the nectarines) made the pistils unattractive to insects. The number of pistils pollinated per cross varied, depending on the number of flowers available. Flowers of all species under study were selfed to determine the time taken by pollen tubes to reach the ovules. The flowers were harvested at the following time intervals; 4 hours, 8 hours, 24 hours and 48 hours. From the results of these self-pollinations it was decided to harvest the pistils 24 hours after pollination.

Pollen viability

Pollen viability was estimated by staining the pollen grains with 0.1% (w/v) cotton blue in lactophenol (29 g phenol, 1g cotton blue, 25 ml water, 25 ml lactic acid and 25 ml glycerol) for 3-24 hours and observed under a light microscope. After examining a minimum of 200 grains stained and unstained ones were counted, repeated three times and averaged for all plants tested.

Study of pollen tube growth

Pistils were collected 24 hours after pollination and fixed for 3 to 24 hours in 3 parts absolute ethanol: 1 part glacial acetic acid. The fixed pistils were stained by a method modified from Martin (1959). The pistils were rinsed twice with distilled water and hydrolysed in 1M NaOH for 2 hours at room temperature, followed by 15 minutes at 60 ^{o}C. They were then stained, either for 2 hours at room temperature or overnight at 4^{o}C, in a solution of 2 g methyl blue and 20 g K_3PO_4 dissolved in 1 l distilled water. The stained pistils were mounted in drop of stain, squashed gently under a cover slip and examined microscopically under ultra-violet light.

The pistil was divided into 6 regions as follows: 1-stigma; 2-top of the style, just below the stigma; 3-upper half of the style, excluding region 2; 4-lower half of style, excluding stylar base; 5-base of style; 6-ovary. The region reached by the longest pollen tube in any given pistil was recorded, and the results averaged for each cross. The pollen tube growth data were analysed as a completely randomised design and standard error of means were calculated using MSTAT-C software program (MSTAT-C, Michigan State University, Version 1.2).

To estimate the number of pollen tubes, pistils were divided into three different parts: top, which covered the region from stigma to neck, middle, which covered the upper half of the style from the neck to the mid-point and finally bottom and ovary, which covered the region from the lower half of the style to stylar base and ovary. Subjective estimates were made of the number of pollen tubes in each region: many (40-60 pollen tubes), some (20-40 pollen tubes) and few (0-20 pollen tubes).

Self-pollinations

Self-pollinations were made on *C. baccatum* SA219 (SC), *C. baccatum* Hawkes 6489 (SC), *C. eximium* Hawkes 3860 (Sc), *C. cardenasii* SA268 (Sc), and the two interspecific F_1 hybrids (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc), *C. baccatum* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc)). The F_1 hybrids were self pollinated to provide estimates of self fertility for comparison with data from backcross pollinations. Self-pollinations were made between different flowers on the same plant.

Backcross pollinations

Pollinations were made for F_1 hybrid SA219 (SC) x Hawkes 3860 (Sc) and F_1 hybrid Hawkes 6489 (SC) x SA268 (SC). Each pollinated bud was labelled and after 24 hours, stigma and style were carefully removed from the ovary by using a sharp scalpel blade, fixed, stained as described above and examined under u.v. light for pollen tube growth. Ovaries, which were left on the plant, were observed to see whether they set fruit.

Study of morphological and isozyme markers

In order to determine whether any morphological and isozyme markers show similar pattern of segregation with unilateral incompatibility segregation of isozymic and morphological characters were examined. Corolla colour, corolla shape, colour of corolla spots, anther colour, style colour and mature fruit colour were thought to distinguish *Capsicum baccatum* accessions SA219 (SC) and Hawkes 6489 (SC), *Capsicum eximium* Hawkes 3860 (Sc), *Capsicum cardenasii* SA268 (Sc) and to be under simple genetic control or monogenically inherited and were scored in *C. baccatum* accessions SA219 (SC) and Hawkes 6489 (SC), *C. eximium* Hawkes 3860 (Sc), *C. cardenasii* SA268 (Sc), *C. baccatum* F_1 hybrids *C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc) and *C. baccatum* Hawkes6489 (SC) x *C. cardenasii* SA268 (Sc), and backcross generations of *C. baccatum* SA219 (SC) x F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc) and *C. baccatum* Hawkes 6489 (SC) x F_1 (*C. baccatum* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc).

In addition to these morphological markers, selectedisozymes of the enzymes—aconitase, alanine aminopeptidase, esterase, glutamic-oxaloacetic transaminase,glycerate-2-dehydrogenase, malate dehydrogenase, peroxidase, phosphoglucomutase, phosphoglucose isomerases,and shikimate dehydrogenases—were investigated in the plants of *C. baccatum* accessions SA219 (SC) and Hawkes 6489 (SC), *C. eximium* Hawkes 3860, *C. cardenasii* SA268 (Sc), *C. baccatum* F_1 hybrids SA219 (SC) x *C. eximium* Hawkes 3860 (Sc) and *C. baccatum* Hawkes6489 (SC) x *C. cardenasii* SA268 (Sc), and backcross generations of *C. baccatum* SA219 (SC) x F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc) and *C. baccatum* Hawkes 6489 (SC) x F_1 (*C. baccatum* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc).

Forty plants from thebackcross family were used for studies of morphological markers and isozyme segregation. For isozyme analysis, very young leaves were macerated in an extraction medium and the extracts were subjected to horizontal starch gel electrophoresis (Soltis *et al*., 1983). The formulation of 100 ml of stock solution (pH 7.8) for preparing extraction medium was 1.211 g Tris, 1.761 g ascorbic acid, 0.074 g KCl, 0.002 g Na_2 EDTA, and 0.037 g $MgCL_2.6H_2O$. Then 0.20 g polyvinylpyrrolidone-40 and 0.60 g polyvinylpolypyrrolidone were added to 5 ml of stock solution to get an exact extraction medium. Gels were made one night before the extracts were prepared, covered

with cling film, and kept overnight at room temperature. The extract from each sample was absorbed into a small paper wick inserted into horizontal gel. Electrophoresis was carried out at 4 ºC. A constant voltage of 150 V for 20 min was applied to the gel, the power was switched off, and the wicks removed. The power was then restarted and electrophoresis was carried out for some hours, depending on the buffer systems that were developed by Rick *et al*. (1977), Guries and Ledig (1978), and Vallejos (1983). After electrophoresis, the gel was cut into several slices (normally 5 or 6 slices, each 1.5 mm thick) and each slice was stained for a different enzyme system. The slices were in the appropriate staining solution for the enzyme to be visualized in an oven at approximately 37 ºC in the dark (Soltis *et al*., 1983). The staining solution was poured off and the gel was rinsed a couple of times with distilled water. Finally, gel slices were fixed in 50% aqueous glycerol and the number and position of stained bands were recorded. After scoring, stained and fixed gel slices were placed on a light box and photographed with black and white film.The segregation ratios observed for all monogenic characters (morphological and isozymic) were compared against the expected 1:1 ratio using chi-square test (Paddle and Bissell, 1972).

RESULTS and DISCUSSION

Pollen viability

Average pollen viability (stainability) of plants was given in Table 1.

Table 1. Mean pollen stainabilities of species and F_1 hybrids.

Species	Mean Pollen Stainability
C. baccatum Hawkes 6489	91.05
C.baccatum SA219	75.61
C.cardenasii SA268	86.38
C. eximium Hawkes 3860	77.94
F_1 hybrid *C. baccatum* Hawkes 6489 x *C.cardenasii* SA268	12.88
F_1 hybrid *C.baccatum* SA219 x *C. eximium* Hawkes 3860	14.78

C. baccatum Hawkes 6489 (SC): 91.05% (varied between 89.15% and 93.25%), *C. baccatum* SA219 (SC): 75.61% (varied between 71.23% and 84.40%), *C. cardenasii* SA 268 (Sc): 86.38% (varied between 85.29% and 87.79%), *C. eximium* Hawkes 3860 (Sc): 77.94% (varied between 76.42% and 78.49%) F_1 (*C. baccatum* Hawkes 6489 (SC) x *C. cardenasii* SA 268 (Sc): 12.88% (varied between 12.76% and 13.57%), F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc)): 14.78% (varied between 11.48% and 18.98%).

F_1 hybrids had low pollen viabilities since F_1 hybrids are heterozygous for one interchange (Haji Itam, 1988 and Pickersgill, personal communication). In an individual heterozygous for one interchange,

two pairs of chromosomes are usually associated in a ring or a chain at meiosis. The pairing of homologous portions of this group of 4 chromosomes results in an cross shaped configuration at pachytene and this cross shape opens up into a complex of four chromosomes associated mainly at the ends at diakinesis and metaphase 1. The type of orientation and the number of chiasmata formed will affect the conformation of the quadrivalent at metaphase 1 and subsequent separation of chromosomes involved in the interchange. If alternate chromosomes in the quadrivalent are directed towards the same pole (alternate orientation) separation at anaphase 1 usually produces viable gametes. On the other hand if adjacent chromosomes in the quadrivalent are directed towards the same pole (adjacent orientation) separation at anaphase 1 will produce gametes which contain duplications and deficiencies. Many of these gametes will be inviable.

Fruit and seed set

All data about fruit and seed set are presented in Table 2.

Table 2. Fruit and seed set from hand pollinations

Type of pollination	No. of Pistils Pollinated	No. of Fruits Set	% Fruit Set	Total No. of Seed Produced	Mean No. of Seeds per Fruit
Self-pollinations					
C. baccatum Hawkes 6489 (SC)	10	10	100	401	40.10
C. baccatum SA219 (SC)	10	10	100	564	56.40
C. eximium Hawkes 3860 (Sc)	10	9	90	52	5.77
C. cardenasii SA268 (Sc)	10	8	80	25	3.12
Interspecific F_1 hybrids					
C. baccatum SA219 (SC) x *C. eximium* Hawkes 3860 (Sc)	542	45	8	46	1.02
C. baccatum Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc)	40	5	13	8	1.60
Cross pollinations					
C. baccatum SA219 (SC) x *C. eximium* Hawkes 3860 (Sc)	15	8	53	425	53.12
C. eximium Hawkes 3860 (Sc) x F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc))	215	5	2	9	1.80
C. baccatum SA219 (SC) x F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc))	1545	20	3	497	4.55
C. baccatum Hawkes 6489 (SC) x F_1 (Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc))	327	112	34	562	5.08
F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc)) x *C. eximium* Hawkes3860	100	73	73	197	2.69
F_1 (*C. baccatum* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc)) x *C. baccatum* Hawkes 6489 (SC)	40	0	0	0	0.00
F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc)) x *C. baccatum* SA219 (SC)	183	26	14	43	1.65

Self-pollinations

C. baccatum (SA219 (SC) and Hawkes 6489 (SC)), *C. cardenasii* (Sc) and *C. eximium* (Sc) set fruits and seeds readily after experimental self-pollinations. Although much pollination had to be made to obtain F_2 seeds from F_1 plants, since F_1 plants were expected to be heterozygous for one interchange, it, therefore, had very low pollen viability, and probably also low female fertility, it was found that F_1 plants were self compatible.

Cross pollinations

Interspecific crosses between C. baccatum (SC) x C. eximium (Sc)

Pollinations between *C. baccatum* SA219 (SC) x *C. eximium* (Sc) set fruits and seeds. There was a reduction in fruit set, but not any marked reduction in number of the seeds per fruit, in a comparison with self-pollinations of *C. baccatum* accession SA219 (SC).

F_1 hybrid used to pollinate its C. eximium (Sc) parent

This was tested only for the hybrid of *C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc). F_1 pollen set fruits and seeds, when applied to *C. eximium* Hawkes 3860 (Sc) pistils. However, there was a reduction in fruit set, presumably reflecting reduced pollen quality of F_1 plants.

F_1 hybrids used to pollinate their SC parent (C. baccatum)

Pollen of both F_1 hybrid combinations (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc*)* and *C. baccatum* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc) set fruits and seeds with *C. baccatum* (SC).

Much effort was required to obtain fruits and seeds from the cross *C. baccatum* (SC) x F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc)), but the F_1 hybrid *C. baccatum* Hawkes6489 (SC) x *C. cardenasii* SA268 (Sc) set fruit and seeds with *C. baccatum* Hawkes6489 (SC) much more readily (34% fruit set, compared to only 1-2% fruit set when the F_1 *C. baccatum* SA219 (SC) x *C. eximium* Hawkes3860 (Sc) was used). Although this was a considerable difference in the % fruit set, there was not a difference in mean number of seeds per fruit. The general reduction in fruit set presumably reflects the reduced pollen quality of the F_1s pollen.

F_1 hybrid pollinated by its Sc parent

This was tested only for the hybrid *C. baccatum* Hawkes6489 (SC) x *C. eximium* Hawkes3860 (Sc). Pollen of *C. eximium* Hawkes3860 (Sc) set fruit and seeds on the F_1 plants. More fruits per pollination were obtained than when the F_1 was self pollinated, because pollen of *C. eximium* Hawkes 3860 (Sc) was fully fertile. However, there were very few seeds per fruit and this may indicate that the quality of the F_1 female gametes may be as low as that of the male F_1 gametes.

F_1 hybrids pollinated by their SC parent (C. baccatum)

Pollen of *C. baccatum* SA219 and Hawkes6489 (SC) was rejected by the pistils of both F_1 hybrid combinations. When the F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc)) was pollinated by *C. baccatum* accession SA219 (SC) for the first time in summer time, all crosses failed and all pollinated pistils were abscised. During autumn, some of these crosses set fruits and seeds. A similar result was also obtained in following year.

In the other backcross combination, using *C. baccatum* Hawkes6489 (SC) as the male parent and the F_1 hybrid (*C. baccatum* Hawkes6489 (SC) x *C. cardenasii* SA268 (Sc)) as the female parent, no fruits or seeds were obtained, regardless of the pollination time.

Backcross Pollinations and Pollen Tube Growth

All data on pollen tube growth are presented in Table 3, 4 and 5.

Table 3. Pollen tube growth in backcross pollinations. The values are given ± standard error of means (s.e.m.)

Type of pollination	No. of pistils studied	Region reached by the longest pollen tube						Average growth class
		1	2	3	4	5	6	
C. eximium Hawkes 3860 (Sc) x F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc))	10	0	2	5	1	1	1	3.4 ± 0.18
C. baccatum SA219 (SC) x F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc))	10	0	2	4	2	1	1	3.5 ± 0.18
C. baccatum Hawkes 6489 (SC) x F_1 (*C. baccatum* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc))	20	3	2	3	4	4	4	3.8 ± 0.14
F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc)) x *C. eximium* Hawkes 3860 (Sc)	10	0	0	0	0	6	4	5.4 ± 0.09
F_1 (*C. baccatum* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc)) x SA268 (Sc)	10	0	0	0	1	5	4	5.3 ± 0.20
F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc)) x *C. baccatum* SA219 (SC)	10	3	7	0	0	0	0	1.7 ± 0.16
F_1 (*C. baccatum* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc)) x *C. baccatum* Hawkes 6489 (SC)	30	6	12	12	1	0	0	2.0 ± 0.12

Table 4. Behaviour of pistils of BC_1 plants. The values are given ± standard error of means (s.e.m.)

Pistillate parent	**Pollen parent**	**Number of plants tested**	**Av. growth class of pollen tube**	
			Mean of all plants in progeny	**Range of means for individual plants within progeny**
BC_1 [*C. baccatum* SA219 (SC) x F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc))]	*C. baccatum* SA219 (SC)	37	5.1 ± 0.10	5.0-6.0
BC_1 [F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860*(Sc)*) x *C. baccatum* SA219 (SC)]	*C. baccatum* SA219 (SC)	7	5.3 ± 0.15	5.0-6.0
BC_1 [F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc)) x *C. eximium* Hawkes 3860 (Sc)	*C. eximium* Hawkes 3860 (Sc)	37	5.3 ± 0.15	5.0-6.0
BC_1 [F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc)) x *C. eximium* Hawkes 3860 (Sc)]	*C. baccatum* SA219 (SC)	28	1.4 ± 0.16	1.1-2.0
BC_1 [*C. baccatum* Hawkes 6489 (SC) x F_1 (*C. baccatum (SC)* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc))]	*C. baccatum* Hawkes 6489 (SC)	40	5.2 ± 0.06	5.0-6.0
BC_1 [*C. baccatum (SC)* SA219 (SC) x F_1 (*C. baccatum* SA219 (SC) x *C. eximium (Sc)* Hawkes 3860 (Sc))]	Self	30	5.3 ± 0.07	5.0-6.0
BC_1 [*C. baccatum (SC)* Hawkes 6489 (SC) x F_1 (*C. baccatum* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc))]	Self	30	5.3 ± 0.07	5.0-6.0

Table 5. Behaviour of pollen of BC_1 plants. The values are given ± standard error of means (s.e.m.)

Pistillate parent	**Pollen parent**	**Number of plants tested**	**Av. growth class of pollen tube**	
			Means of all plants in progeny	**Range of means for individual plants within progeny**
C. eximium Hawkes 3860 (Sc)	BC_1 [*C. baccatum* SA219 (SC) x F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc))]	37	1.2 ± 0.11	1.1-2.0
C. cardenasii SA268 (Sc)	BC_1 [*C. baccatum (SC)* Hawkes 6489 (SC) x F_1 (*C. baccatum (SC)* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc))]	38	1.3 ± 0.10	1.1-2.0

Behaviour of F_1 hybrids as male parent

In crosses to the Sc parent (C. eximium)

This pollination was tested only for F_1 hybrid *C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc). Pollen tubes from F_1 hybrids grew through the style and reached the ovary. However, only one or two pollen tubes were present in any region of the pistil. No differences were observed in numbers in the upper part of the style versus lower part of the style. The small numbers of the pollen tubes in the style and consequently low fruit set were attributed to the low pollen stainability (hence probably poor viability) of F_1 plants. Segregation is expected in the pollen grains of F_1 plants, since a heterozygous F_1 plant is expected to produce two types of pollen grains. Pollen tubes of F_1 plants were inhibited in region 2, where unilateral incompatibility expresses itself. This may be the expected segregation between the pollen grains produced by the F_1 plants. However, when F_1 plants were used to pollinate *C. baccatum* (SC), there were again two pistils which pollen tubes of F_1 plants were inhibited, although no incompatibility reaction is expected to occur in this type of pollination.

In crosses to the SC parent (C. baccatum)

In both crosses with the F_1 hybrids as male parent and *C. baccatum* accessions Hawkes6489 (SC) and SA219 (SC) as the pistillate parents, pollen tubes grew through the style and reached the ovary.

More pollen grains germinated on the stigma surface and pollen tubes reached the ovary in more pistils when the F_1 hybrid *C. baccatum* Hawkes6489 (SC) x *C. cardenasii* SA268 was used to pollinate *C. baccatum* (SC) Hawkes 6489 (SC) than in corresponding pollinations of *C. baccatum* SA219 (SC) with the F_1 hybrid *C. baccatum* (SC) x *C. eximium* (Sc). This is not related to differences in pollen stainability of two F_1 hybrid combinations, since the length of the longest pollen tube is not the only factor which determines whether a pollinated flower will set a fruit. It may be necessary for a certain minimum number of pollen tubes to reach the ovary. For that reason, it was decided to count number of pollen tubes reaching the ovary. Results showed that very few pollen tubes were able to reach the ovary, when the F_1 hybrids were used as the male parent. Since just a few pollen tubes reached the ovary, there was a drop on fruit set, presumably reflecting the reduced quality of the F_1 pollen as stated earlier.

Behaviour of F_1 hybrids as female parent

In crosses with Sc parent (C. eximium or C. cardenasii)

Pollen grains of *C. eximium* Hawkes 3860 (Sc) and *C. cardenasii* SA268 (Sc) germinated on the stigmas of their respective F_1 hybrids grew in the styles and reached the ovaries (No differences were observed between the two crosses). When F_1 hybrid *C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc) was pollinated with pollen of *C. eximium* Hawkes 3860 (Sc), approximately 50 pollen tubes

were present in the ovary, and this result is an agreement with the results as stated earlier that F_1 hybrid had high fruit set with good quality pollen of *C. C. eximium* Hawkes 3860 (Sc).

In crosses with the SC parent (C. baccatum)

In the summer, both F_1 hybrids behaved like their Sc parent, i.e. *C. baccatum* SA219 and Hawkes6489 (SC) pollen was able to germinate and pollen tubes were able to penetrate the stigma of F_1 hybrid plants, but pollen tubes were inhibited in the upper region of the style. There were many pollen tubes in each stigma and no difference was observed between the two F_1 hybrid combinations.

Behaviour of styles of plants from the BC_1 to *C. baccatum* (SC)

When styles of all backcross plants in the different progenies [*C. baccatum* SA219 (SC) x F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860(Sc)), *C. baccatum* Hawkes6489 (SC) x F_1 (*C. baccatum* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc)) and F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc) x *C. baccatum* SA219 (SC))] were tested with *C. baccatum* SA219 (SC) pollen, the *C. baccatum (SC)* pollen grains germinated, pollen tubes grew through the styles and reached the base of the style or the ovary. The longest pollen tube reached a similar region of the style in all plants. Values of average pollen tube growth class for individual plants in these progenies varied from 5.0 to 6.0.

Although the longest *C. baccatum* (SC) pollen tubes reached the ovary, the relative numbers of pollen tubes in different regions of the pistil varied. The largest numbers of pollen tubes occurred in the top part of the style, and then in the middle part of the style the numbers of pollen tubes was reduced finally in the bottom part of the style and in the ovary there were relatively few pollen tubes present (see Figure 1 and Figure 2).

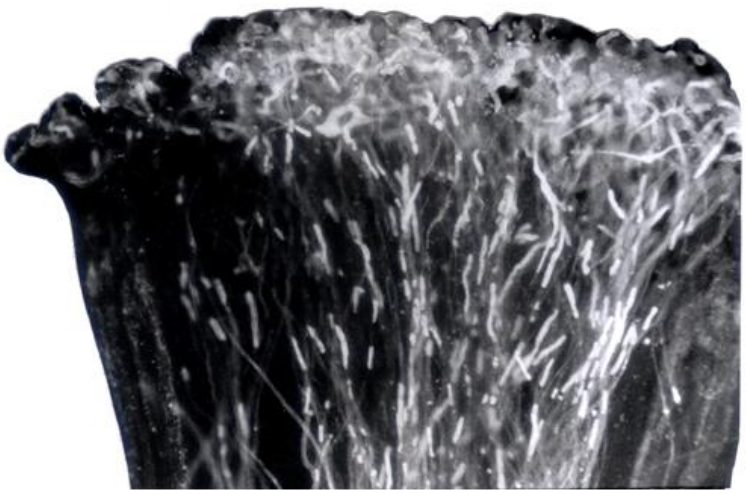

Figure 1. The pollen of *C. baccatum* Hawkes6489 on the pistils of one backcross plants coming from *C. baccatum* SA219 (SC) x F_1 (*C. baccatum* SA219 (SC) x *C. cardenasii* SA268 (Sc)). Please note that the largest numbers of pollen tubes occurring in the top part of the style

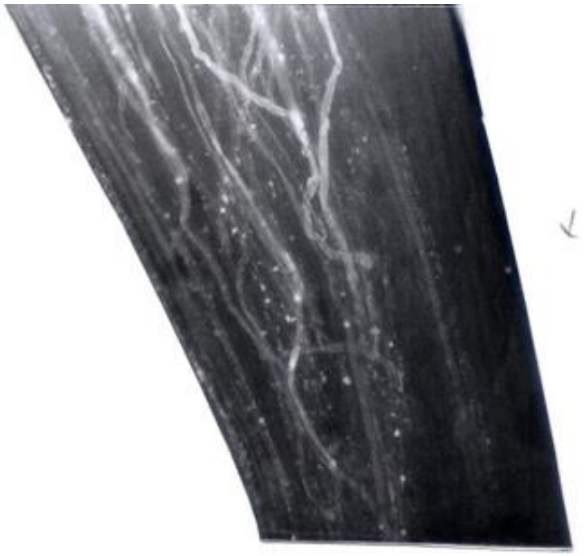

Figure 2. The pollen of *C. baccatum* Hawkes6489 on the pistils of one backcross plants coming from *C. baccatum* SA219 (SC) x F_1 (*C. baccatum* SA219 (SC) x *C. cardenasii* SA268 (Sc)). Please note that the numbers of pollen tubes was reduced in the bottom part of the style

The relative number of pollen tubes in top versus bottom part of the style also varied between backcross plants. In one plant, the top part of the style had more than 50 pollen tubes, while the middle part of the style had less than 25 and at the base of the style there were just 5 or 6 pollen tubes. In another plant the top part of the style had more than 60 pollen tubes, the middle part of the style had 35-40 pollen tubes and 30-35 pollen tubes entered the ovary.

As well as differences within each backcross progeny, there were also differences between the three progenies. For example when backcross plants derived from *C. baccatum* SA219 (SC) x F_1 (*C. baccatum* SA219 (SC) x. *C. eximium* Hawkes 3860 (Sc)) were tested with *C. baccatum* SA219 (SC) pollen, most of the pollen tubes passed to middle part of the style. On the other hand, when backcross plants derived from *C. baccatum* SA219 (SC) x F_1 (*C. baccatum* SA219 (SC) x *C. cardenasii* SA268 (Sc)) were tested with *C. baccatum* SA219 (SC) pollen, most pollen tubes were inhibited in the top part of the style and some of them passed to middle part of the style.

A few plants were obtained from the backcrosses of the F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc)) as female and *C. baccatum* SA219 (SC) as the male parent. When these plants were tested with *C. baccatum* SA219 (SC) pollen, average pollen tube growth was the same as in the reciprocal backcross with F_1 as male parents, but the numbers of pollen tubes differed. Styles of the plants from the backcrosses with the F_1 used as female parent had noticeably fewer *C. baccatum* SA219 (SC) pollen tubes than styles of plants from the two backcrosses in which the different F_1 hybrids were used as male parents.

Behaviour of styles of plants from the BC_1 to *C. eximium* (Sc)

Styles of all BC_1 plants supported growth of *C. eximium* Hawkes 3860 (Sc) pollen. The pollen grains germinated, pollen tubes grew down the style and reached the ovary.

On the other hand when these backcross plants were pollinated by *C. baccatum* SA219 (SC), pollen tubes were always inhibited either in the stigma or in the style (just below the stigma). In some of the pistils, the site of the inhibition was exactly the same as in the original cross between *C. eximium* Hawkes 3860 (Sc) and *C. baccatum* SA219 (SC) and as in the F_1 x *C. baccatum* SA219 (SC); while in some of them inhibition was occurred earlier, in the stigma rather than the style.

Behaviour of pollen from plants from the BC_1 to *C. baccatum* (SC)

Plants of two backcross progenies, derived from the two interspecific F_1 hybrids, were used to pollinate stigmas of their non-recurrent parent (*C. eximium* Hawkes 3860(Sc) or *C. cardenasii* SA268 (Sc) respectively). Some of the pollen grains germinated and some of them did not. In some of the pistils, the germinated pollen tubes penetrated the stigma, then pollen was inhibited either in the stigma or in the style (just below the stigma) as happened in the original cross when *C. baccatum* (SC) pollen was placed on *C. eximium* (Sc) or *C. cardenasii* (Sc) stigmas.

Average growth class of pollen tubes Avaried from 1.1 to 2.0. Some backcross plants with very good pollen stainability produced pollen grains which did not germinate very well on *C. eximium* Hawkes 3860 (Sc) or *C. cardenasii* SA268 (Sc) stigmas, although pollen grains covered the surface of the stigma. There was not any pistil in which backcross pollen reached the base of the style or the ovary.

Self-pollinations of plants of the BC_1 to *C. baccatum* (SC)

When backcross plants, originating from both interspecific crosses, were self pollinated, some pollen tubes reached the ovary in every plant. No self-incompatibility was observed among these backcross plants.

Segregation of isozymes and morphological markers

Results on segregation of isozymes and morphological markers are presented in Table 6 and 7.

Table 6. Segregation of isozyme markers and morphological markers in the backcross progeny of *C. baccatum* Hawkes6489 x F_1 (*C. baccatum* Hawkes6489 (SC) x *C. cardenasii* (Sc))

Gene locus	Heterozygotes	Homozygotes	χ^2 (1 df)
P (fruit persistence)	9	31	11.52***
Acon-2	19	21	0.10
Aap-1	17	23	0.90
Est-5	11	29	8.10***
Got-1	22	18	0.40
Idh-1	13	27	4.90*
Skdh-1	27	13	4.90*
Pgi-1	21	19	0.10
Pgi-2	10	30	10.00***
Pgm-1	20	20	0.00
Pgm-2	9	31	12.00***

Table 7. Segregation of isozyme markers and morphological markers in the backcross progeny of *C. baccatum* Hawkes6489 x F_1 (*C. baccatum* Hawkes6489 (SC) x *C. cardenasii* (Sc))

Gene locus	Heterozygotes	Homozygotes	χ^2 (1 df)
P (fruit persistence)	9	31	11.00***
y (fruit colour)	10	30	9.09***
Acon-2	18	22	0.40
Aap-1	19	21	0.10
Est-5	13	27	4.90*
Got-1	21	19	0.10
Idh-1	12	28	6.40*
Skdh-1	29	11	8.10***
Pgi-1	19	21	0.10
Pgi-2	12	28	6.40*
Pgm-1	18	22	0.40
Pgm-2	10	30	10.00***

Since no segregation was observed for unilateral incompatibility in two families of backcross plants, it could be assumed that the apparent lack of segregation might be due to backcross progenies used in this study not containing enough plants to permit any segregation to be observed and/or distorted segregation ratios. Monogenic segregations of morphological and isozyme markers were, therefore, studied and results of the results related to segregation of these markers and zymotypes of enzyme systems previously published elsewhere (Onus and Pickersgill, 2004 b).

Segregation studies for enzyme systems revealed that 10 single nuclear loci showed analyzable variation, whereas others were invariant. For the backcross combination *C. baccatum* SA 219 (SC) x F_1 (*C. baccatum* SA 219 (SC) x *C. eximium* Hawkes 3860 (Sc)) five loci [*P* (morphological marker for fruit persistence) *Est-5*, *Idh-1*, *Pgi-2*, *Pgm-2*] showed distorted segregation ratios with an excess of homozygotes. For the other backcross combination *C. baccatum* Hawkes 6489 (SC) x F_1 (Hawkes 6489 (SC) x SA 268 (Sc)), six loci *P*, y (morphological markers for fruit persistence and mature fruit colour,

respectively) *Est-5*, *Idh-1*, *Pgi-2*, *Pgm-2*] showed distorted segregation ratios with an excess of homozygotes. In other words both progenies had an excess of individuals carrying alleles inherited from their *C. baccatum* (SC) parents.

Both interspecific F_1 hybrids, *C. baccatum* (SC) x *C. eximium* (Sc) and *C. baccatum* (SC) x *C. cardenasii* (Sc), behaved in a similar way as regards compatibility with their parental species. Firstly, pistils of both F_1 hybrids accepted pollen from their Sc parent (*C. eximium* (Sc) or *C. C. cardenasii* (Sc) but rejected pollen from their SC parent (*C. baccatum*). Secondly, some pollen of both F_1 hybrid combinations was able to grow down through the styles of both SC and Sc parents. Pistil behaviour of both the F_1 hybrid combinations in *Capsicum* is in agreement with pistil behaviour of other F_1 hybrids from unilaterally incompatible crosses in different genera of *Solanaceae*. But on the other hand, pollen behaviour of both the F_1 hybrids in *Capsicum* is not in agreement with data obtained from other genera of *Solanaceae*. It is important to remind the reader that when it is said that data obtained in this study is or is not an agreement with other genera in the family *Solanaceae*, this means when species used in this present study and those used in other studies are comparable, e.g. when there is no active *S* allele. There may be a possible explanation of why pollen of F_1 hybrids of *Capsicum* and *Lycopersicon* behave differently. As assumed above when either one gene or two genes is involved in penetration capacity, respectively 50% and 25% of the pollen is expected to be compatible with *C. eximium* (Sc) pistil. But if multiple loci are involved in penetration capacity, a pollen grain has to carry alleles from the Sc parent at each of these loci to grow all the way down to ovary. So it can be possible that the number of penetration loci required to overcome the stylar barriers may be different for *Capsicum* and *Lycopersicon*. In other words, in *Capsicum* the number of the loci required to overcome the stylar barriers in Sc species may be less than the number of the loci required in *Lycopersicon*.

As an alternative explanation, there may be different mechanisms controlling unilateral incompatibility between *Capsicum* and *Lycopersicon*. For example Martin (1964, 1966) questioned the genetic control of unilateral incompatibility in *Lycopersicon*. In a study involving SC *L. esculentum* and a Sc accession from *L. hirsutum* (comparable species to those involved in this study of *Capsicum*), he reported that unilateral incompatibility in *Lycopersicon* is a function of the self-incompatibility which may remain as a relic even when self-incompatibility is inactive and can be reactivated by the influence of some other gene(s). Presence of these other gene(s) with the *S* gene in the pistil prevents growth of any pollen carrying SC allele or Sc allele. Pandey (1962) also postulated second gene acting together with *S* gene, to explain some of his results obtained for *Solanum*. So in *Lycopersicon* presence of *S* gene and its interaction with other gene(s) may prevent Sc species accepting any pollen from their F_1 hybrids. In this case, one can assume that either there is no relic *S* gene present in *Capsicum* (although it does not seem likely for *C. cardenasii* (Sc)) or there is no other gene(s) to activate the *S* gene. Thus unilateral incompatibility in

Capsicum and *Lycopersicon* may be controlled by two different mechanisms so that some pollen of F_1 hybrids of *Capsicum* can grow in the pistils of their Sc parent.

Since F_1 hybrids will be heterozygous for those dominant allele(s), stylar behaviour is expected to segregate in the backcrosses to *C. baccatum* (SC). Some plants are expected to accept pollen grains from *C. baccatum* (SC) and some plants are expected to reject *C. baccatum* (SC) pollen.

If one considers the longest pollen tube in each individual backcross plant, it is possible to say that styles of all plants from backcrosses of both F_1 hybrids to *C. baccatum* (SC) accepted pollen grains from *C. baccatum* (SC). This does not fit to expectations and does not agree with data from other genera in family *Solanaceae*. For example, Martin (1964) backcrossed the F_1 hybrid *Lycopersicon esculentum* (SC) x *L. hirsutum* (SI) and found segregation among the backcross plants for cross incompatibility with *L. esculentum*. Most of the backcross plants accepted pollen from *L. esculentum*, but one quarter of the backcross progeny rejected *L. esculentum* pollen.

On the other hand, if one considers the relative number of the pollen tubes in different parts of the style of backcross plants of both F_1 hybrids to *C. baccatum* (SC), in some of the pistils, most of the *C. baccatum* (SC) pollen stopped growing in upper part of the style and just few of them reached to stylar base or the ovary. This occurrence may be considered as "leaky" incompatibility in which a few pollen tubes get through and reach the ovary but most of them inhibited. In a similar way, Grun and Aubertin (1965) reported that in *Solanum* 2 genes control interspecific incompatibility, one of them is operating in upper part of the style and the other one is operating in the bottom part of the style and in the ovary. And also, in the backcross progenies of F_1 hybrid of *Lycopersicon esculentum* x *L. peruvianum* Hogenboom (1972) reported three of the interaction patterns with *L. esculentum* pollen: 1) uniform stop of pollen tube growth after penetration of about one third of the style; 2) thinning bundle (25-30 tubes at the stylar base); 3) little or no inhibition (more than 25-30 pollen tubes at the stylar base).

In this study of *Capsicum*, no backcross plant was found to show absolute inhibition of pollen of *C. baccatum* (SC) after penetration one third of the style. But in two of the backcross plants "thinning bundle" with some pollen tubes at the stylar base was observed and none of the pollen tubes entered the ovary. So this may be considered as some sort of inhibition pattern. In the pistils of the rest of the backcross plants, little or no inhibition of pollen tubes was observed as some of the pollen tubes entered the ovary. This information can be used to argue that there may be different barriers in different parts of the pistil in *Capsicum* too, as reported for *Solanum* and *Lycopersicon.*

Styles of all plants of the backcross plants to *C. baccatum* (SC) accepted pollen grains from their Sc (*C. cardenasii*, *C. eximium*) parents. These results are in agreement with expectations and with results obtained from *Lycopersicon* (Martin, 1966). Styles of all plants of the backcross to Sc parent *C. eximium*

rejected pollen grains from SC parent (*C. baccatum*). This result fits expectations and agrees with data from *Lycopersicon* (Martin, 1966).

Styles of all plants of the backcross to Sc parent *C. eximium* (Sc) rejected pollen grains from SC parent (*C. baccatum*). This result fits expectations and agrees with data from *Lycopersicon* (Martin, 1966).

F_1 pollen was able to grow down the styles of the SC and Sc parents (*C. baccatum* (SC*)*, and *C. eximium* (Sc) respectively), which means F_1 pollen behaved like that of the Sc parent. So F_1 pollen should segregate into different classes, which should behave in different ways on Sc styles (though not on SC styles).

Pollen of plants from backcrosses of *C. baccatum* (SC) with both F_1 hybrids was inhibited in the styles of Sc parents (*C. cardenasii* and *C. eximium*). This result does not fit expectations, since some of the pollen grains of backcross plants, derived from the BC_1 to *C. baccatum* (SC), are expected to be able to grow in the styles of their Sc parent (*C. eximium*, *C. cardenasii*).

It was expected segregation for behaviour of the pistil and behaviour of the pollen of backcross plants. The segregation ratio could have suggested how many genes are involved in each case. If unilateral incompatibility in *Capsicum* was due to the *S* gene acting alone, then 1:1 segregation for unilateral incompatibility should be obtained in the backcross progenies of *C. baccatum* (SC) x F_1 (*C. baccatum* (SC) x *C. eximium* (Sc)), and *C. baccatum* (SC) x F_1 (*C. baccatum* (SC) x *C. C. cardenasii* (Sc)). Since no segregation occurred, some other gene(s) may be involved in the unilateral incompatibility mechanism.

In this manner, Hogenboom (1972, 1973, 1975) hypothesised that unilateral incompatibility affects many different processes which operate between pollination and fertilization, so is controlled by many genes in many different linkage groups. He explained his theories with different models. In his simplest model, the barrier capacities (b) of related species (for example *C. baccatum* (SC), *C. eximium* (Sc), *C. cardenasii* (Sc) for this present study) differ in alleles of only one gene. The barriers associated with allele, for example A, can be overcome by the dominant allele of the corresponding penetration gene (p). For the sake of discussion we tested different models put forward by Hogenboom. In all models tested it looked that model assuming two parents differ by three complementary genes controlling barrier capacity, A_1, A_2, A_3. These genes are unlinked and presence of all three genes is necessary for the barrier to operate. One penetration gene A can overcome this barrier. In this case the genotypes of the parents may be as down stated:

C. baccatum (SC)		x	*C. cardenasii* (Sc)	
$b:a_1a_1a_2a_2a_3a_3$	p:aa		$b:A_1A_1A_2A_2A_3A_3$	p:AA

According to this model, in the BC_1 generations sixteen genotypes will be obtained. Fourteen will accept pollen from parent p:aa, (*C. baccatum* (SC) pollen for present study) while two will reject.

Although the idea that barrier capacities of the species *C. baccatum* (SC), *C. eximium* (Sc) and *C. cardenasii* (Sc) are controlled by three genes, A_1, A_2, A_3, with one corresponding penetration gene A, is the best possible model to explain the results of this study, although it is highly speculative, one question is still unanswered. According to this model, two-sixteenths of the backcross progeny should have had genotypes which do not accept pollen from *C. baccatum* (SC). No such plants were found. So it is necessary to explain what may be the possible reasons not to get this specific genotype. These reasons may include size of the BC_1 progenies used in this study (less than 40 plants) and/or distorted segregation ratios. As stated earlier, to assess these two possibilities, segregation of other single gene characters (isozymic and morphological) were examined. Segregation for morphological and isozyme markers suggested that in backcrosses to *C. baccatum* (SC), more *C. baccatum* alleles are transferred to the next generation than the expected Mendelian share. This may be one reason for lack of segregation for unilateral incompatibility. But how can this happen?

Firstly, pollen competition is a likely reason for distorted segregation ratios and may explain lack of segregation for unilateral incompatibility. Pollen tube competition is common in flowering plants and has been demonstrated in cases of artificial interspecific pollination. Variation in pollen germination and pollen tube growth expressed, and selection for a variety of characters is possible, in the stylar environment (Grant, 1975). When pollens are pollinated with mixtures of self and alien pollen, alien pollen of then grows more slowly and is less effective in fertilization than the self pollen (Grant, 1975). In this study, growth rates of F_1 pollen tubes varied. After 24 hours, some of the F_1 pollen tubes had reached the ovary and some were in the middle part of the style. Since there were no burst pollen tubes, slow growth of the pollen was not attributed incompatibility gene(s). Some plants were produced from backcrossing F_1 (*C. baccatum* (SC) x *C. eximium* (Sc)) as female parent with *C. baccatum* (SC). In this backcross, no pollen tube competition is expected and yet the very few plants in this backcross family also showed no segregation for unilateral incompatibility. Pollen tube competition therefore cannot be the only reason for not having segregation for unilateral incompatibility.

Another factor might be non-random loss/non-functioning of some zygotes. Marshall and Folsom (1991) reported several ways in which selective elimination of zygotes may occur. For example, they reported that the fitness of maternal plants may be increased by abortion and accumulation of additional resources for particular embryos to which maternal plants are more closely related. So in this study, backcrosses to *C. baccatum* (SC) would be favoured.

Distorted ratios for marker genes can be due to selective elimination of heterozygous genotypes, not only after fertilization and during seed development, but also during seed germination and early seedling stages. In the backcross progenies studied here, empty seeds (16-22%) (Onus, 1995) indicate either failure for fertilization or elimination of zygotes after fertilization, during embryogenesis. If these empty seeds

carried predominantly the embryos which were heterozygotes for the genes showing distorted ratios and which also carried the genes responsible for the unilateral incompatibility, this may explain the unbalanced ratios and lack of segregation for unilateral incompatibility which were observed. Rick (1969) working on an F_1 hybrid between *Lycopersicon pennellii* and *Lycopersicon esculentum* reported that even a 5% loss of embryos can affect the genetic ratio.

This model, where there is complementary action of three barrier genes A_1, A_2, A_3, is better than simple models involving fewer genes in explaining findings from these hybrids in *Capsicum*. This thought can be supported with the results of Chetelat and DeVerna (1991). They reported that the expression of unilateral incompatibility in pollen is controlled by three major loci on chromosome 1, 6 and 10. Since *Lycopersicon* and *Capsicum* are both in the family *Solanaceae* and high genomic homology was reported (Tanksley *et al.* 1988) between them, unilateral incompatibility in *Capsicum* and *Lycopersicon* may be controlled by similar number of genes.

The number of the pollen tubes of *C. baccatum* (SC) in different parts of the style of backcross plants *(C. baccatum* (SC) x F_1s) showed variation. In the backcross plants of *C. baccatum* Hawkes 6489 (SC) x F_1 (*C. baccatum* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc)) there were relatively more pollen tubes in the top part of the style, fewer pollen tubes in the middle part of the style and finally at the bottom part of the style very few pollen tubes entered the ovary. On the other hand, in plants from the backcross of *C. baccatum* SA219 (SC) x F_1 (*C. baccatum*) SA219 (SC) x *C. eximium* Hawkes 3860 (Sc)) there was no obvious reduction in the number of pollen tubes between the top part of the style and the middle part of the style, although there was a clear reduction in the number of the pollen tubes entering the ovary. In other words styles of the backcross plants derived from the F_1 hybrid with *C. eximium* (Sc) did not inhibit *C. baccatum* (SC) pollen tubes in the upper parts of the pistil, whereas in pistils of backcross plants derived from the F_1 hybrid with *C. cardenasii*, many *C. C. baccatum* (SC) pollen tubes were inhibited either in the stigma or in the style.

These differences in relative numbers of pollen tubes in different parts of the pistil in these two different backcrosses to *C. baccatum* (SC) indicated that barrier genes from *C. eximium* (Sc) and *C. cardenasii* are of different strengths or are expressed differently. So how can this happen? *C. eximium* (Sc) has been self-compatible for a long time, while *C. cardenasii* SA268 has only recently evolved self-compatibility (during the last 20-25 years of cultivation). So one may say that *S* gene may affect the UI mechanism in genus *Capsicum*. If it is the case *C. eximium* Hawkes 3860 (Sc) and specifically *C. cardenasii* SA268 (Sc) may still contain a hidden or inactive *S* gene possessing a relic of its incompatibility functions. A similar result was also reported in *Lycopersicon* by Martin (1966). Martin (1966) reported self-incompatibility among plants derived from backcrossing the hybrid between *Lycopersicon esculentum* (SC) and *L. hirsutum* (Sc) after backcrosses to *L. esculentum* (SC). In Martin's study unilateral incompatibility

data in this type of backcross plants suggested that one or two more dominant genes from *Lycopersicon hirsutum* are also operating. For this reason, "*L. hirsutum* may contain a hidden or inactive *S* gene.

But for this present study it should be highlighted that there was no SI plants in all backcross plants coming from *C. baccatum* SA219 (SC) x F_1 (*C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc) and *C. baccatum* Hawkes 6489 (SC) x F_1 (*C. baccatum* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc) combinations as pollen stainabilities (viabilities) of backcross plants varied between 30% and 90%, in other words some of them were partially sterile but not self-incompatible (Onus, 1995). This is because F_1 hybrids *C. baccatum* SA219 (SC) x *C. eximium* Hawkes 3860 (Sc) and *C. baccatum* Hawkes 6489 (SC) x *C. cardenasii* SA268 (Sc) are heterozygous for one interchange (Haji Itam, 1988) and that can also be reason why both F_1 hybrid combinations had low pollen viability (stainability). Naturally when an F_1 heterozygous for one interchange and for various other genetic loci is backcrossed to one parent, the resulting backcross progeny will consist of some plants which are heterozygous for one interchange and partially sterile and other plants which are homozygotes and fully fertile.

Chetelat and DeVerna (1991) reported that although pollen grains needed 3 loci from *Lycopersicon pennellii* to overcome the incompatibility barriers in the styles of diploid hybrids which had one *L. esculentum* genome and one *Solanum lycopersicoides* genome, only 2 loci were necessary to overcome the barriers in the styles of sesquidiploid hybrids which had two *L. esculentum* genomes and one *S. lycopersicoides* genome. These results indicate a dosage effect, in which the strength of UI in the style, associated with the presence of gene(s) from *S. lycopersicoides*, can be diluted by presence of an additional *L. esculentum* genome. In this study in *Capsicum*, backcrosses to *C. baccatum* (SC) could have also diluted the gene(s) controlling the strength of unilateral incompatibility in the style so that these barrier genes cannot operate any more and this could be the reason why there was no segregation for unilateral incompatibility.

Conclusion

In *Capsicum*, unlike *Lycopersicon, Solanum* and *Nicotiana*, most wild species are self-compatible. Self-incompatibility is characteristic only of *C. cardenasii*, which appears to be uniformly self-incompatible throughout its limited range in Bolivia. This species is closely related to, and may be sympatric with, *C. eximium* (Sc). On the other hand *C. baccatum* (SC) is domesticated with its conspecific wild relative and the closely related *C. praetermissum*. *C. cardenasii* (Sc) is geographically isolated from all the wild species with which it is unilaterally incompatible. The self-compatible species with which it is most likely to introgress are wild *C. eximium* (Sc) and domesticated *C. pubescens* with both of which it is bilaterally compatible. The geographic distributions of the wild species of *Capsicum* make it difficult to picture unilateral incompatibility originating as a device to prevent introgression of self-compatibility into a self-incompatible taxon, as suggested for other taxa and for unilateral incompatibility in general. It therefore seems to more probable that, in *Capsicum*, unilateral incompatibility has arisen as a by-product of genetic divergence between the *C. pubescens* complex (*C. pubescens*, *C. cardenasii* (Sc) and *C. eximium* (Sc)) and the other species, not as a product of natural selection. But it should be pointed out that experiments results of this paper presented no clear evidence to rule out the influence of *S* locus in UI in genus *Capsicum*. Further studies such as S-RNAse-like genes in Capsicum *would* be helpful to determine the cause of unilateral incompatibility in the genus.

Acknowledgement

Special thanks to Dr. Barbara Pickersgill at The University of Reading, UK, for her knowledge and guidance throughout the research. This paper was supported by Scientific Research Projects Administration Unit of Akdeniz University.

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