

A STUDY ON CROSSABILITY RELATIONSHIPS BETWEEN SOME OF THE WHITE-AND PURPLE-FLOWERED *CAPSICUM* SPECIES

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Abstract

The main objective of this study is to reveal the crossability relations between some of the white-and purple-flowered *Capsicum* species by making pollination and studying pollen tube growth of crosses conducted between the white-and purple flowered species. Results showed that while there was a strong inhibition of pollen tubes, when a purple-flowered species, excluding *C. tovarii*, was the female and no F₁ hybrid was obtained. On the other hand, in reciprocal crosses pistils of the white-flowered species showed no inhibition of pollen tubes of purple-flowered species, including *C. tovarii*, and in most of the crosses seeds were obtained.

Keywords: *Capsicum*, white-flowered species, purple-flowered species, hybridisation

Capsicum Cinsi İçerisinde Bazı Beyaz ve Mor Çiçekli Türler Arasında Melezlenebilme İlişkileri Üzerine Bir Araştırma

Özet

Bu çalışmanın ana amacı bazı beyaz ve mor çiçekli *Capsicum* türleri arasında melezlemeler yaparak ve polen tüpü gelişimini inceleyerek bu iki grup *Capsicum* türleri arasında melezlenebilme olanaklarını ortaya koymaktır. Araştırma sonuçlarına göre; *C. tovarii* hariç geriye kalan mor çiçekli türler melezlemede dişi form olarak kullanıldıkları zaman beyaz çiçekli türlerin polenleri inhibe edilip ve hiçbir tohum elde edilemezken, resiprokal melezlemelerde beyaz çiçekli türlerin pistilleri mor çiçekli türlerin, *C. tovarii* dahil, polenlerini inhibe etmemiş ve melezlemelerin çoğunluğundan tohum elde edilmiştir.

Anahtar Kelimeler: *Capsicum*, Beyaz Çiçekli Türler, Mor Çiçekli Türler, Melezleme

I. Introduction

Several authors studying relationships in the genus *Capsicum* have informally classed a number of species into two groups (Ballard *et al.*, 1970; Pickersgill, 1971). This informal classification is based mainly corolla colour, one group having white corollas and the other purple. The former group includes four of the domesticated species as *C. annuum* L., *C. baccatum* L., *C. frutescens* L., *C. chinense* Jacq. Although these species are called domesticated species, there are wild accessions within most of the so-called domesticated species. There are also some species in the white-flowered group that have not been domesticated and these are; *C. chacoense* A.T.Hunz, *C. galapagoense* A.T.Hunz, *C. praetermissum* A.T.Hunz.

The purple-flowered group includes domesticated *C. pubescens* R&P., which is

unknown in the wild, and three wild species; *C. eximium* A.T. Hunz, *C. cardenasii* Heiser and Smith, *C. tovarii* Eshbaugh, Smith and Nickrent.

Apart from above stated species, more than 20 wild species of *Capsicum* have been found, mostly restricted to South America. These species have not yet been completely evaluated, but they have been reported to contain many useful characters, especially disease resistance. For example *C. chacoense* carry resistance to bacterial leaf spot and this resistance has been incorporated into sweet pepper (Cook, 1982).

From the plant breeder's point of view, it is necessary to know the "crossability" relationships among the *Capsicum* species. But limits to wide hybridisation are not yet completely known, partly because many species have not yet been studied intensively.

Limited number of studies on the crossability of species within the same group showed that they can be usually intercrossed easily, whereas crosses between species from different groups are much more difficult. For example Smith and Heiser (1957) were not able to get any seeds from their cross between *C. pubescens* and *C. annuum*.

Zijlstra *et al.* (1991) showed that pollen tubes of the wild purple-flowered species can penetrate the egg cells of *C. annuum* but not vice versa. They showed that pollen tubes of *C. annuum* were inhibited either on the stigma or in the style of the purple-flowered species.

Bermawie (1990) showed an interesting result in her crossing programme between the purple-flowered species *C. tovarii* and the white-flowered species *C. chacoense*. Pollen tubes of *C. tovarii* reached the ovary of *C. chacoense* and vice versa. Fruits were set from the cross between *C. chacoense* x *C. tovarii*, but not from the reciprocal cross. It was thought that failure of fruit set in the latter might be due to late-acting incompatibility.

As can be seen from the above stated examples, in *Capsicum* interspecific crosses usually fail in one direction only, i.e. incompatibilities are usually unilateral. However, it must be stated, in most interspecific crosses it is not known whether the pollen tubes are inhibited and if so where they are inhibited.

The main aim of this study was the clarify the crossability relationships between two different *Capsicum* groups by studying certain pre-post fertilisation barriers which occur between particular species in the groups. More knowledge about pre-post fertilisation barriers in interspecific crosses in *Capsicum* can be helpful for plant breeders to develop different methods to overcome these breeding barriers to achieve different breeding purposes and it can be also useful for the researchers trying to clarify the evolutionary ties between different species in the genus *Capsicum*.

2. Materials and Methods

2.1 Plant materials

The species and accessions which have been used in this study were as follows:

The white-flowered species:

C. annuum var. *glabrisculum* (wild BP225), *C. annuum* var. *annuum* (domesticated C70-7a), *C. baccatum* var. *baccatum* (wild SA250), *C. baccatum* var. *pendulum* (domesticated SA219), *C. frutescens* (wild 6240), *C. frutescens* (domesticated SA36), *C. chinense* (wild BP605), *C. chinense* (domesticated C248), *C. praetermissum* (wild C343), *C. chacoense* (wild BP281).

The purple-flowered species:

C. pubescens (domesticated BP43), *C. cardenasii* (wild SA268), *C. eximium* (wild Hawkes3860), *C. tovarii* (wild BP382).

These accessions were chosen to represent both wild and domesticated forms within the species which contained both. Only one accession of the wild species *C. praetermissum*, *C. eximium*, *C. cardenasii* and *C. tovarii* was used. Accessions were germinated by sowing the seeds in soil.

2.2. Method for pollination

Most of the pollinations were made in the morning or early afternoon between 10 am and 1 pm according to Koopai's method (Koopai, 1976).

While 10 pistils were pollinated for pollen tube growth studies, 15 pistils per accession were pollinated to observe the fruit set.

2.3 Procedures for visualising pollen tubes

Pollinated pistils were collected and fixed from 3 to 24 hours in a solution made up of 3 parts of absolute ethanol and one part

of the glacial acetic acid. After fixation the pistils were treated according to Martin (1959)'s method.

2.4. Scoring of pollen tubes

Pistils were divided into six regions according to a method modified from James (1975) as follows: Region 1; stigma surface, Region 2; just below the stigma, Region 3; middle of the style, Region 4; bottom half of the style, Region 5; stylar base, Region 6; ovary region, in contact with ovules.

The region reached by the longest pollen tube in any given pistil was recorded and growth classes were then calculated (James, 1975).

3. Results

3.1. Fruit setting results when purple-flowered species used to pollinate white-flowered species

Results are given in Table 1.

3.2. Fruit setting results when white-flowered species used to pollinate purple-flowered species

Results are given in Table 2.

3.3. Pollen tube growth observations in crosses white-flowered species used to pollinate purple-flowered species

Pollen tubes of the white-flowered species were arrested either in the stigma or in the style just below the stigma of the purple flowered species except *C. tovarii*. Some pollen tubes showed non-directional growth in the stigma. The tips of the inhibited pollen tubes showed a heavy callose deposit accompanied by an opening.

On the other hand, in crosses between the white-flowered species and *C. tovarii*, pollen grains of the white-flowered species germinated, pollen tubes grew down through the style, and pollen tubes reached to the

stylar base or to the ovary in 24 hours.

3.4. Pollen tube growth observations in crosses purple-flowered species used to pollinate white-flowered species

In all the crosses between purple-flowered species, including *C. tovarii*, male parent and the white-flowered species as the female parent, pollen grains germinated and pollen tubes penetrated the stigma, grew straight and down through the style, callose plugs were formed at regular intervals and reached the stylar base or ovary.

4. Discussion

Results showed that there was strong inhibition of pollen tubes when a purple-flowered species was the female parent, excluding *C. tovarii*, and a white-flowered species was the male. This is why no F₁ hybrid was obtained in crosses with white-flowered species used to pollinate purple-flowered species. In contrast, in reciprocal crosses, pistils of the white-flowered species showed no inhibition of pollen grains of purple-flowered species, including *C. tovarii* and in some of the crosses seeds were obtained.

As it was stated in crosses purple-flowered species used to pollinate the white-flowered species, pollen tubes grew straight and callose plugs were formed at regular intervals. Williams *et al.* (1982) considered such phenomena as characteristics of compatible pollinations.

On the other hand, in crosses white-flowered used to pollinate purple-flowered species, the tips of the inhibited pollen tubes showed a heavy callose deposit which was sometimes accompanied by an opening at the tips of the pollen tubes. This phenomenon is a typical incompatible reaction, i.e., a heavy deposit of callose at the tip of the pollen tube and the disappearance of the tube apex (Netlancourt *et al.*, 1974). White-flowered species and the purple-flowered species are

Table 1. Fruit and seed setting results when purple-flowered species used to pollinate white-flowered species.

Cross	Total fruits	Total seeds	Av.tube growth class
<i>annuum</i> BP225 x <i>C. cardenasii</i> SA268	4	57	5.9
<i>C. annum</i> C70-7a x <i>C. cardenasii</i> SA268	-	-	5.2
<i>annuum</i> BP225 x <i>C. eximium</i> H3860	4	84	5.8
<i>C. annum</i> C70-7a x <i>C. eximium</i> H3860	-	-	5.2
<i>C. annum</i> BP225 x <i>C. pubescens</i> BP43	1	10	5.3
<i>C. annum</i> C70-7a x <i>C. pubescens</i> BP43	-	-	5.2
<i>C. annum</i> BP225 x <i>C. tovarii</i> BP382	3	21	5.9
<i>C. annum</i> C70-7a x <i>C. tovarii</i> BP382	-	-	5.7
<i>C. baccatum</i> SA250 x <i>C. cardenasii</i> SA268	4	24	5.8
<i>C. baccatum</i> SA219 x <i>C. cardenasii</i> SA268	8	172	5.8
<i>C. baccatum</i> SA250 x <i>C. eximium</i> H3860	2	1	5.7
<i>C. baccatum</i> SA219 x <i>C. eximium</i> H3860	8	53	5.6
<i>C. baccatum</i> SA250 x <i>C. pubescens</i> BP43	-	-	5.1
<i>C. baccatum</i> SA219 x <i>C. pubescens</i> BP43	-	-	5.1
<i>baccatum</i> SA250 x <i>C. tovarii</i> BP382	1	22	5.8
<i>C. baccatum</i> SA219 x <i>C. tovarii</i> BP382	2	24	5.6
<i>C. frutescens</i> 6240 x <i>C. cardenasii</i> SA268	5	26	5.8
<i>frutescens</i> SA36 x <i>C. cardenasii</i> SA268	4	16	5.6
<i>C. frutescens</i> 6240 x <i>C. eximium</i> H3860	12	7	5.8
<i>frutescens</i> SA36 x <i>C. eximium</i> H3860	1	5	5.6
<i>C. frutescens</i> 6240 x <i>C. pubescens</i> BP43	3	11	5.4
<i>C. frutescens</i> SA36 x <i>C. pubescens</i> BP43	2	6	5.2
<i>C. frutescens</i> 6240 x <i>C. tovarii</i> BP382	2	8	5.8
<i>C. frutescens</i> SA36 x <i>C. tovarii</i> BP382	1	5	5.8
<i>praetermissum</i> C343 x <i>C. cardenasii</i> SA268	3	23	5.8
<i>C. praetermissum</i> C343 x <i>C. eximium</i> H3860	2	15	5.8
<i>praetermissum</i> C343 x <i>C. pubescens</i> BP43	2	16	5.1
<i>C. praetermissum</i> C343 x <i>C. tovarii</i> BP382	1	6	6.0
<i>C. chacoense</i> BP281 x <i>C. cardenasii</i> SA268	2	17	5.9
<i>C. chacoense</i> BP281 x <i>C. eximium</i> H3860	2	8	5.6
<i>chacoense</i> BP281 x <i>C. pubescens</i> BP43	1	12	5.4
<i>C. chacoense</i> BP281 x <i>C. tovarii</i> BP382	1	12	5.3

growth inhibition either in the stigma or in the style close to the stigma (Onus, 1995).

Present results showed that UI occurs in *Capsicum* when the white-flowered species are the male parent and the purple-flowered species, excluding *C. tovarii*, are the female parent. No differences were observed between wild and domesticated forms of the species in terms of unilateral incompatibility. Present results are therefore agree with and extend those of previous researchers.

According to these results, some purple-flowered species such as *C. praetermissum* and *C. tovarii* belong in the white-flowered group on the basis of their crossing behaviour. So, this result may

support the thought that *C. praetermissum* is close to *C. baccatum* as suggested by Hunziker (1971).

In the case of *C. tovarii*, Mc Leod *et al.* (1983) reported that *C. tovarii* is isozymically different from the rest of the purple-flowered species and Bermawie (1990) reported that the pollen of *C. tovarii* grew in the style of the *C. chacoense*, reached the ovary and vice versa. So findings of this study support the findings of Mc Leod *et al.* (1983) and Bermawie (1990) that *C. tovarii* is different from the rest of the purple-flowered group species. *C. tovarii* and *C. chacoense* which are isozymically distinct from one another and from all other

Table 2. Fruit setting results when white-flowered species used to pollinate purple-flowered species.

Cross	Total Fruits	Total seeds	Av.tube growth class
<i>C. cardenasii</i> SA268 x <i>C. annuum</i> BP225	-	-	1.2
<i>C. cardenasii</i> SA268 x <i>C. annuum</i> C70-7a	-	-	1.1
<i>C. eximium</i> H3860 x <i>C. annuum</i> BP225	-	-	1.4
<i>C. eximium</i> H3860 x <i>C. annuum</i> C70-7a	-	-	1.1
<i>C. pubescens</i> BP43 x <i>C. annuum</i> BP 225	-	-	1.2
<i>C. pubescens</i> BP43 x <i>C. annuum</i> C70-7a	-	-	1.1
<i>C. tovarii</i> BP382 x <i>C. annuum</i> BP225	-	-	5.9
<i>C. tovarii</i> BP382 x <i>C. annuum</i> C70-7a	-	-	5.6
<i>C. cardenasii</i> SA268 x <i>C. baccatum</i> SA250	-	-	1.2
<i>C. cardenasii</i> SA268 x <i>C. baccatum</i> SA219	-	-	1.2
<i>C. eximium</i> H3860 x <i>C. baccatum</i> SA250	-	-	1.7
<i>C. eximium</i> H3860 x <i>C. baccatum</i> SA219	-	-	1.7
<i>C. pubescens</i> BP43 x <i>C. baccatum</i> SA250	-	-	1.2
<i>C. pubescens</i> BP43 x <i>C. baccatum</i> SA219	-	-	1.2
<i>C. tovarii</i> BP382 x <i>C. baccatum</i> SA250	-	-	5.9
<i>C. tovarii</i> BP382 x <i>C. chacoense</i> BP281	-	-	5.6
<i>C. tovarii</i> BP382 x <i>C. baccatum</i> SA219	-	-	5.7
<i>C. cardenasii</i> SA268 x <i>C. frutescens</i> 6240	-	-	1.2
<i>C. cardenasii</i> SA268 x <i>C. frutescens</i> SA36	-	-	1.2
<i>C. eximium</i> H3860 x <i>C. frutescens</i> 6240	-	-	1.4
<i>C. pubescens</i> BP43 x <i>C. frutescens</i> 6240	-	-	1.4
<i>C. pubescens</i> BP43 x <i>C. frutescens</i> SA36	-	-	1.2
<i>C. tovarii</i> BP382 x <i>C. frutescens</i> 6240	-	-	5.5
<i>C. tovarii</i> BP382 x <i>C. frutescens</i> SA36	-	-	5.6
<i>C. cardenasii</i> SA268 x <i>C. chinense</i> BP605	-	-	1.1
<i>C. cardenasii</i> SA268 x <i>C. chinense</i> C334	-	-	1.2
<i>C. eximium</i> H3860 x <i>C. chinense</i> BP605	-	-	1.4
<i>C. eximium</i> H3860 x <i>C. chinense</i> C334	-	-	1.2
<i>C. pubescens</i> BP43 x <i>C. chinense</i> BP605	-	-	1.1
<i>C. pubescens</i> BP43 x <i>C. chinense</i> C334	-	-	1.2
<i>C. tovarii</i> BP382 x <i>C. chinense</i> BP605	-	-	5.6
<i>C. tovarii</i> BP382 x <i>C. chinense</i> C334	-	-	5.6
<i>C. cardenasii</i> SA268 x <i>C. praetermissum</i> C343	-	-	1.2
<i>C. eximium</i> H3860 x <i>C. praetermissum</i> C343	-	-	1.2
<i>C. pubescens</i> BP43 x <i>C. praetermissum</i> C343	-	-	1.2
<i>C. tovarii</i> BP382 x <i>C. praetermissum</i> C343	-	-	5.7
<i>C. cardenasii</i> SA268 x <i>C. chacoense</i> BP281	-	-	1.3
<i>C. eximium</i> H3860 x <i>C. chacoense</i> BP281	-	-	1.2
<i>C. pubescens</i> BP43 x <i>C. chacoense</i> BP281	-	-	1.1

species of *Capsicum* (Mc Leod *et al.*, 1983) belong in the same crossability group as far as UI is concerned.

As can be seen from the results, although all cross between the white-flowered species and *C. tovarii* pollen grains germinated, grew down through the style and reached to the stylar base or to the ovary, no seed was obtained when white-flowered species used to pollinate *C. tovarii*. Failure

of fruit set in this case may be due to late-acting incompatibility.

The crossing results showed that domesticated species were harder to cross than wild species. This finding is in agreement with Pickersgill (Pickersgill, 1971; 1977) who found that wild accessions of various species were easier to cross with each other than the domesticated accessions in this group were. For example Pickersgill

(1977) reported that it was possible to obtain a hybrid between two domesticated white-flowered species but it was either have a very low percentage of stainable pollen (viable pollen) (e.g. domesticated *C. chinense* x *C. annuum* var. *annuum*) or was completely sterile (e.g. domesticated *C. chinense* x *C. baccatum* var. *baccatum*). The reason for the decrease in pollen viability of interspecific hybrids of the cultivated types as compared with wild types is probably due to independent evolution under domestication causing increased cytogenetic divergence and hence decreased infertility.

Furthermore as cultivated peppers are predominantly self-pollinating, the cultivated species may not able to tolerate the increased heterozygosity this would cause in an interspecific F_1 . This would explain why the cultivated *C. pubescens* was so unsuccessful as a pollen donor when crossed to the cultivated white-flowered species yet managed to effect fertilisation some of the wild white-flowered species. It may also be the reason why the cultivated *C. annuum* var. *annuum* did not set seed when pollinated by any of the purple-flowered species.

5. Conclusion

It has proved possible to cross white and purple-flowered species, which have been considered distinct major groups on different aspects. It, thus, seems likely that genes from purple-flowered species could ultimately be transferred to white-flowered species. This should give impetus to the screening and evaluation of species other than *C. annuum* for useful characters and conserving material of all species of *Capsicum* should be collected and conserved in gene bank to be used by plant breeders if or when they need them.

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