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A. NACI ONUŞ

BARBARA PICKERSGILL

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Monogenic Segregations in Backcross Progenies of *Capsicum baccatum* x Two Interspecific F₁ Hybrids and Some Possible Explanations for Distorted Segregation Ratios in *Capsicum*

Ahmet Naci ONUS

Akdeniz University, Department of Horticulture, Faculty of Agriculture, Antalya 07059 - TURKEY

Barbara PICKERSGILL

The University of Reading, Department of Agricultural Botany, School of Plant Sciences, Whiteknights, Reading RG6 6AS, UK

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Abstract : Monogenic segregations of certain morphological and isozymic characters were studied in backcross progenies of *Capsicum baccatum* L. (*Solanaceae*) x two interspecific F₁ hybrids and distorted segregation ratios were noted. It was observed that isozyme markers *Est-5*, *Idh-1*, *Pgm-2*, and *Pgi-2*, and morphological marker gene *P* for fruit persistence showed distorted segregation ratios in the first backcross generation of both *C. baccatum* Hawkes 6489 (P.G.Smith) x F₁ (*C. baccatum* Hawkes 6489 x *Capsicum cardenasii* Heiser Smith) and *C. baccatum* SA219 (P.G.Smith) x F₁ (*C. baccatum* SA219 x *Capsicum eximium* A.T.Hunz). Both progenies and an excess of individuals carrying alleles inherited from *C. baccatum*. A further gene, *y*, controlling fruit colour and segregating in the backcross of *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii*), also showed a distorted segregation ratio with an excess of homozygous individuals. However, in backcross families from interspecific crosses, *Skdh-1* showed a distorted segregation ratio with an excess of heterozygous individuals.

Key Words: Morphological characters, isozymic characters, monogenic segregation, distorted segregation ratios

***Capsicum baccatum* ile Türler Arası İki F₁ Hibridin Geriye Melezlenmesi Sonucu Elde Edilen Generasyonlarda Tek Gen Açılımları ve Mendel Açılımı Göstermeyen Bazı Karakterlerin Sapma Nedenlerinin Açıklanması**

Özet : *Capsicum baccatum* L. (*Solanaceae*) ile türler arası iki F₁ hibridin geriye melezlenmesi sonucu elde edilen generasyonlarda tek gen açılımı gösteren morfolojik ve izoenzimik karakterler üzerinde çalışılmış ve izoenzimik karakterlerden *Est-5*, *Idh-1*, *Pgm-2*, *Pgi-2* ile morfolojik karakterlerden meyvenin kalyx'den ayırımı kontrol eden *P* geninin her iki geriye melezleme sonucu elde edilen generasyonlarda, *C. baccatum* L. Hawkes 6489 (P.G.Smith) x F₁ (*C. baccatum* Hawkes 6489 x *Capsicum cardenasii* Heiser Smith) and *C. baccatum* SA219 (P.G.Smith) x F₁ (*C. baccatum* SA219 x *Capsicum eximium* A.T.Hunz), Mendel açılımına sahip olmadıkları, 1:1 oranından sapma gösterdikleri belirlenmiştir. Her iki generasyon homozigot yapıda daha fazla bireye sahip olmuştur. Meyve rengini kontrol eden *y* geni ise sadece *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii*) generasyonunda varyasyon göstermiş ve daha fazla homozigot yapıda bireylere sahip olarak 1:1 oranından sapma göstermiştir. Diğer taraftan *Skdh-1* geni, her iki geriye melezleme generasyonunda daha fazla heterozigot bireye sahip olarak Mendel açılımından sapma göstermiştir.

Anahtar Sözcükler: Morfolojik karakterler, izoenzimik karakterler, tek gen açılımı, Mendel açılımından sapma

Introduction

There are two different hypotheses concerning the genetic control of unilateral incompatibility (UI). Pandey's hypothesis (1) assumes that UI reflects the primary specificity of the S gene, and so genetic control is associated with a single locus in a single linkage group.

According to Hogenboom (2), on the other hand, UI is a consequence of evolutionary divergence affecting many different stages of the processes which occur between pollination and fertilisation, and so genetic control is governed by many different genes in many different linkage groups. Both researchers reached these

conclusions by studying segregation of UI in backcross generations

Monogenic segregations have been investigated in the family *Solanaceae* as well as in other plant families and distorted segregations have been found by several researchers. For example, Stephens (3) found skewed segregations in some backcross generations of crosses between two different cotton species (*Gossypium* L./*Malvaceae*). He concluded that "selective elimination in both male and female gametes rather than zygotic selection is mainly responsible for the skewed ratios".

Rick (4) working on a tomato species (*Lycopersicon* Mill. (*Solanaceae*)) also suggested some kind of selective elimination of male gametes as a possible explanation for distorted backcross segregation ratios. According to Rick (4), this could occur at pollen germination or during pollen tube growth stages.

Zamir et al. (5) studied isozyme segregation in backcross plants from a cross between a cultivated tomato and one of its wild relatives. The backcross plants showed a significant excess of wild species alleles at *Pgi-1*, *Est-4* and *Adh-2*. According to their data, these backcross populations were not subject to a strong differential selection during embryogenesis and seed germination, so it is likely that factors occurring before fertilisation caused the distorted ratios.

Vallejos and Tanksley (6) also studied the same interspecific backcross between a cultivated tomato and one of its wild relatives and detected significant distortions in segregations at six enzyme coding loci. Four loci (*Got-3*, *Aps-2*, *Got-4*, *Prx-4*) showed an excess of cultivated species alleles, while two (*Est-1* and *Aps-2*) showed an excess of wild species alleles. They reported that selection at the germination/seedling stage might account for some of the skewness. In other words, it is probable that factors occurring after fertilisation caused the skewed ratios.

Tanksley and Loaiza-Figueroa (7) studied gametophytic self-incompatibility and segregation of isozyme characters in crosses among individuals within two different populations of *Lycopersicon peruvianum* Mill. (*Solanaceae*). They reported that *Idh-1* and *Prx-1* showed skewed segregation ratios with particular female parents when a single pollen parent was used. They concluded that linkage between *Idh-1*, *Prx-1* and the self-incompatibility gene caused the distorted segregation ratio.

Zamir and Tadmor (8) reviewed distorted segregation ratios in interspecific crosses in *Lycopersicon* and *Capsicum* L. (*Solanaceae*) and reported that these segregation ratios occurred as a result of linkage between the markers and gene (s) that operate in pre-zygotic and post-zygotic phases of reproduction.

In this study, the genetic control of unilateral incompatibility in *Capsicum* was tested and no segregation was observed in plants of two backcross families, *C. baccatum* L. Hawkes 6489 x F₁ (*C. baccatum* x *C. cardenasii*) (Heiser Smith) and *C. baccatum* SA219 x F₁ (*C. baccatum* x *C. eximium*) (A.T.Hunz). At the end of this study, it was suggested that the apparent lack of segregation might be due to distorted segregation ratios. The segregation of certain morphological and isozymic characters was also studied to see whether any known monogenic traits show the same pattern of segregation observed for unilateral incompatibility in *Capsicum*.

Materials and methods

Plant materials

C. baccatum L. accessions SA219 (P.G.Smith) and Hawkes 6489 (P.G.Smith); *Capsicum cardenasii* Heiser Smith accession SA268 (P.G.Smith) and *Capsicum eximium* A.T./Hunz Hawkes 3860 (J.G.Hawkes); two interspecific F₁ hybrids, *C. baccatum* Hawkes 6489 x *C. cardenasii* and *C. baccatum* SA219 x *C. eximium*; and two backcross families, *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii* SA268 and *C. baccatum* SA219 x F₁ (*C. baccatum* SA219 x *C. eximium* Hawkes 3860). Forty randomly chosen plants from each backcross family were used in our studies. All plants were raised by culturing the seeds in a special medium as described below.

Fully mature dry seeds were imbibed in distilled water for up to 24 hours. The following operations were carried out in a laminar air-flow cabinet. All the seeds were kept in 2% hypochlorite solution for 5-10 minutes to ensure surface sterilisation and then they were rinsed three times for five minutes and kept in the last wash. The testa of each seed was removed under a dissecting microscope by a no. 11 scalpel blade.

Each endosperm-embryo complex was put on the surface of a culture medium consisting of 5 ml Murashige and Skoog's medium (9), modified by excluding edamin,

kinetin and thiamine HCl, with an agar concentration of 0.7% (w/V), pH 5.8.

The culture vials were placed in a constant temperature room (25°C) until the seedlings had developed one or two true leaves and some lateral roots. The seedlings were then removed from the vials, rinsed in tap water, and then transplanted into vermiculite to encourage full development of the root system. The transplanted seedlings were kept in a growth room under a transparent cover to reduce transpiration while they became established. After 7-10 days, seedlings were moved into compost in the greenhouse.

Pollen studies

Pollen from the hybrids *C. baccatum* Hawkes 6489 x *C. cardenasii* and *C. baccatum* SA219 x *C. eximium* were used to pollinate the pistils of *C. baccatum* accessions SA219 and Hawkes 6489.

To test pollen viability, pollen was collected from flowers whose anthers had dehisced on the day of collection. One flower per plant of each F₁ hybrid combination and three plants per hybrid were examined. Pollen grains were stained with 0.1% (w/V) cotton blue in lactophenol (29 g phenol, 1 g cotton blue, 25 ml water, 25 ml lactic acid and 25 ml glycerol) for 3-24 hours and observed under a light microscope. Stained (considered viable) and unstained (considered inviable) pollen grains were counted in a sample of at least 200 pollen grains per flower.

To visualise the pollen tubes, pollinated pistils were collected and fixed from 3 to 24 hours in a solution made up of three parts absolute ethanol and one part glacial acetic acid. After fixation the pistils were treated according to a method modified from Martin (10). Pistils were rinsed twice with distilled water and hydrolysed in 1M NaOH for 2 hours at room temperature, then 15 min at 60°C. To remove excess NaOH, pistils were rinsed twice with distilled water. They were then stained in a solution of 2 g methyl blue and 20 g K₃PO₄ dissolved in 1 litre distilled water. The pistils were kept in the staining solution either for 2 hours at room temperature or overnight at 4°C. The stained pistils were then mounted on the slides in one drop of stain under a cover slip and gently squashed and examined under fluorescence microscope.

Morphological markers and isozyme analysis

Characters thought to distinguish *C. baccatum* accessions SA219 and Hawkes 6489, *C. cardenasii* SA268 and *C. eximium* Hawkes 3860 and to be under simple genetic control or monogenically inherited, which were corolla colour, corolla shape, colour of corolla spots, anther colour, style colour, and mature fruit colour were scored in *C. baccatum* accessions SA219 and Hawkes 6489; *C. cardenasii* SA268, *C. eximium* Hawkes 3860; two F₁ hybrids, SA219 x Hawkes 3860 and Hawkes 6489 x SA268; and all plants in the backcross families *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* x *C. cardenasii*) and *C. baccatum* SA219 x F₁ (*C. baccatum* x *C. eximium*).

In addition to these morphological markers, selected isozymes 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 and Hawkes 6489; *C. cardenasii* SA268, *C. eximium* Hawkes 3860; two F₁ hybrids, SA219 x Hawkes 3860 and Hawkes 6489 x SA268; and backcross families *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* x *C. cardenasii*) and *C. baccatum* SA219 x F₁ (*C. baccatum* x *C. eximium*). Forty randomly chosen plants from each backcross family were used for studies of morphological markers and isozyme segregation.

For isozyme analysis, very young leaves were macerated in extraction medium based on the procedure given by McLeod et al. (11) and the extracts were subjected to horizontal starch gel electrophoresis.

The segregation ratios observed for all monogenic characters (morphological and isozymic) were compared against the expected 1:1 ratio using the chi-square test (12).

Results

Morphological characters

Results with morphological characters are given in Table 1.

Table 1. Data on morphological characters observed in the species, F₁ hybrid combinations, and backcross plants. If the characters segregate quite clearly in the backcross, the number of plants in each of the phenotypic classes is given.

Morphological character	C.baccatum		C.eximium	C.cardenasii	F ₁ ■	F ₁ ■■	BC ■	BC ■■
	SA219	Hawkes 6489	Hawkes 3860	SA268				
Corolla colour	White	White	Purple	Purple	Purple	Purple	33 white, 13 purple	36 white 14 purple
Fruit persistence	Non-Deciduous (nd)	Non-Deciduous	Deciduous (d)	Deciduous	Deciduous	Deciduous	10 d,32 nd	11 d,33 (nd)
Corolla shape	Rotate	Rotate	Rotate	Campanulate	Rotate	Rotate	All plants rotate	All plants rotate
Anther colour	Brownish yellow	Brownish yellow	Brownish yellow	Purple	Brownish yellow (by)	Bluish(b)	Brownish yellow	38 by,2b
Style colour	Cream	Cream	Cream	Purple	White	Purple	White	Purple to white
Corolla spotting	Brownish yellow	Brownish yellow	Diffuse green	Diffuse green	Brownish yellow	Brownish yellow	Brownish yellow	Brownish yellow
Mature fruit colour	Red	Yellow	Red	Red	Red	Red	Red	12 red,32 yellow

F₁ (■) SA219 x Hawkes 3860, F₁ (■■) Hawkes 6489 x SA268
 BC (■) SA219 x F₁ (SA219 x Hawkes 3860), BC (■■) Hawkes 6489 x F₁ (Hawkes 6489 x SA268)

Corolla colour

Both accessions of *C. baccatum* have a white corolla colour, and *C. cardenasii* and *C. eximium* have a purple corolla colour. Both F₁ hybrids SA219 x Hawkes 3860 and Hawkes 6489 x SA268 had purple corollas, although these were slightly lighter than those of the *C. cardenasii* and *C. eximium* parents. Both backcross families segregated into plants with purple flowers and those with white flowers. Segregation ratios for both of the backcross families deviate significantly from 1:1 (see Table 1) but fit a ratio of 3 white:1 purple.

Colour of spots

While both accessions of *C. baccatum* have brownish yellow corolla spotting, *C. cardenasii* and *C. eximium* have diffuse green corolla spotting. F₁ plants of SA 219 x Hawkes 3860 and Hawkes 6489 x SA268 had brownish yellow corolla spots similar to their *C. baccatum* parent as well. Thus, it is quite likely that this character is determined by dominant allele(s) of the *C. baccatum* parent.

Corolla shape

Both accessions of *C. baccatum* and *C. eximium* have rotate corollas, and *C. cardenasii* has campanulate

corollas. This character, therefore, can be scored only in the *C. baccatum* x *C. cardenasii* cross. F₁ plants of this cross had rotated corollas. In the backcross *C. baccatum* x F₁ (*C. baccatum* x *C. cardenasii*) all plants had rotated corollas, indicating that the rotated corolla was probably dominant over the campanulate corolla. Thus, it is not possible to determine the genetic control of this character.

Anther colour

Both accessions of *C. baccatum* and *C. eximium* have brownish yellow anther colour and *C. cardenasii* has purple anther colour. Anther colour, thus, also can be scored only in the *C. baccatum* x *C. cardenasii* cross. The F₁ hybrid between *C. baccatum* and *C. cardenasii* had bluish (b) colour. On the other hand, in the backcross family, 38 plants had brownish (by) yellow anthers, while two plants had purple (bluish) anthers (see Table 1). Thus, this character did not fit the expected pattern of segregation that it would be controlled by a single gene. It might be determined by more than one gene.

Style colour

Both accessions of *C. baccatum* and *C. eximium* have white style colour and *C. cardenasii* has a purple style

colour. Therefore, style colour can only be scored in the cross between *C. baccatum* and *C. cardenasii*. The F₁ hybrid of this cross had purple styles, although the intensity of the pigment yielding a purple colour was less than in the *C. cardenasii* parent. In the backcross plants, style colour varied from dark purple to light purple or white. It appears that additional gene(s) modify the shade of the purple colour in different parts of the style. Since it was not possible to divide the backcross plants into two groups, it appears that this character might be controlled by two complementary genes. Further research is needed for a more definite decision.

Fruit colour

C. baccatum accessions SA219 and Hawkes 6489 have red and yellow fruits respectively, *C. cardenasii* and *C. eximium* have red fruits. Fruit colour can only be scored in the cross between *C. baccatum* and *C. cardenasii*. F₁ hybrids of this cross had red fruit. Backcross plants segregated into two classes: plants with red fruits and with yellow fruits. While 12 plants had red fruit colour, 32 had yellow fruit colour. The segregation ratio obtained was similar to the 2-gene control mechanism such that either fruit colour was controlled by a single gene (*y* gene) and the ratio obtained here was a skewed 1-gene ratio, or this character was controlled by 2 genes in the interspecific cross studied (see Table 1 and Table 3).

Fruit Persistence

Both *C. baccatum* accessions have non-deciduous fruits, while *C. eximium* and *C. cardenasii* have deciduous fruits. Both F₁ hybrid combinations had deciduous fruits,

indicating that deciduous was dominant over non-deciduous. The *C. baccatum* SA219 x F₁ (*C. baccatum* SA219 x *C. eximium* Hawkes 3860) backcross family had 10 deciduous and 32 non-deciduous plants, while the *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii* SA268) backcross family had 11 deciduous and 33 non-deciduous plants. The ratios obtained for both backcross families were again similar to the ratio expected when two unlinked genes were segregating. Therefore, again this character was controlled by a single gene, and the ratio obtained in this present study was a skewed 1-gene ratio.

Isozyme banding patterns

Zymograms of the enzymes given in this study have been published previously elsewhere (13)

Aconitase (ACON)

Two zones of activity were observed. ACON-1 exhibited a single band, whose position was the same in all species and accessions used. In the second region ACON-2, *C. baccatum* carried a slow band, while *C. cardenasii* and *C. eximium* had the same fast band. Backcross generation *C. baccatum* SA 219 x F₁ (*C. baccatum* SA 219 x *C. eximium* Hawkes 3860) had 19 heterozygous plants and 21 homozygous plants, and backcross generation *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii* SA 268) had 19 heterozygous and 21 homozygous plants. The segregation ratio obtained for these alleles was an acceptable fit to the 1:1 ratio in each family (see Tables 2 and 3)

Gene locus	Heterozygotes	Homozygotes	χ^2
P (Fruit persistence)	10	32	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*
Pgi-1	20	20	0.00
Pgi-2	9	31	12.00***
Pgm-1	21	19	0.10
Pgm-2	10	30	10.00***
Skdh-1	27	13	4.90***

Table 2. Segregation of isozyme markers and morphological markers in the backcross progeny of *C. baccatum* SA 219 x F₁ (*C. baccatum* SA 219 x *C. eximium* Hawkes 3860).

*, ***, significance levels p 0.05 and 0.01 respectively
Numbers indicate the total number of plants.

Gene locus	Heterozygotes	Homozygotes	χ ²
P (Fruit persistence)	11	33	11.0***
y (Fruit colour)	12	32	9.09***
Acon-2	18	22	0.40
Aap-1	19	21	0.10
Est-5	19	21	0.10
Got-1	21	19	0.10
Idh-1	12	28	6.40*
Pgi-1	18	22	0.40
Pgi-2	10	30	10.00***
Pgm-1	19	21	0.10
Pgm-2	12	28	6.40*
Skdh-1	29	11	8.10***

Table 3. Segregation of isozyme markers and morphological markers in the backcross progeny of *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii* SA 268).

*, *** significance levels p 0.05 and 0.01 respectively
Numbers indicate the total number of plants.

Alanine aminopeptidase (AAP)

A single zone of activity was found and within this zone differences in band positions were observed. While *C. baccatum* accessions displayed a slow band, *C. cardenasii* and *C. eximium* had the same fast band. Backcross generation. *C. baccatum* SA 219 x F₁ (*C. baccatum* SA 219 x *C. eximium* Hawkes 3860) had 17 heterozygous plants and 23 homozygous plants, and backcross generation. *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii* SA 268) had 19 heterozygous and 21 homozygous plants. The segregation ratio obtained for these alleles was an acceptable fit to the 1:1 ratio in each family (see Table 2 and 3).

Esterase (EST)

Region 1 for this enzyme system gave a single band. The position of this band was invariable for all species used in this study.

Regions 2,3 and 4 did not stain well and bands were poorly resolved. Therefore, these regions were not studied any further. Region 5 had bands in two different positions. Both accessions of *C. baccatum* had a fast band, while *C. eximium* and *C. cardenasii* showed a slow band. Backcross generation *C. baccatum* SA 219 x F₁ (*C. baccatum* SA 219 x *C. eximium* Hawkes 3860) had 11 heterozygous plants and 29 homozygous plants and backcross generation *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii* SA 268) had 13 heterozygous and 27 homozygous plants. The segregation ratio obtained for these alleles was a skewed

ratio and both backcross families contained significantly more homozygotes than expected (see Tables 2 and 3).

Glutamic-oxaloacetic transaminase (GOT)

Two zones of activity were found. In region, 1 both accessions of *C. baccatum* had a fast band, while *C. eximium* and *C. cardenasii* showed a slow band. Backcross generation *C. baccatum* SA 219 x F₁ (*C. baccatum* SA 219 x *C. eximium* Hawkes 3860) had 22 heterozygotes plants and 18 homozygotes plants and backcross generation *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii* SA 268) had 21 heterozygous and 19 homozygous plant. The segregation ratio obtained for these alleles was an acceptable fit to 1:1 ratio in each family (see Table 2 and 3).

Glycerate-2-dehydrogenases (G-2DH)

Single zone of activity with a single band was obtained and the position of this band did not vary in any of the species (data not shown).

Isocitrate dehydrogenases (IDH)

One zone of activity with a single band was obtained and the position of this band varied. Both accessions of *C. baccatum* had a fast band, while *C. eximium* and *C. cardenasii* showed a slow band. Backcross generation *C. baccatum* SA 219 x F₁ (*C. baccatum* SA 219 x *C. eximium* Hawkes 3860) had 22 heterozygous plants and 18 homozygous plants and backcross generation *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii* SA 268) had 21 heterozygous and 19 homozygous plants. The segregation ratio obtained for

these alleles was a skewed ratio and both backcross families contained significantly more homozygotes than expected (see Tables 2 and 3).

Peroxidase (RPX)

Two zones of activity were found. One moved cathodally and the other moved anodally. Both zones contained a single band, whose position did not vary.

Phosphoglucosmutase (PGM)

Two zones of activity were found. PGM-1 had a single band, whose position varied. Both accessions of *C. baccatum* had a fast band, while *C. eximium* and *C. cardenasii* showed a slow band. Backcross generation *C. baccatum* SA 219 x F₁ (*C. baccatum* SA 219 x *C. eximium* Hawkes 3860) had 21 heterozygous plants and 19 homozygous plants and backcross generation *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii* SA 268) had 21 heterozygous and 19 homozygous plants. The segregation ratio obtained for these alleles was an acceptable fit to the 1:1 ratio in each family (see Tables 2 and 3).

PGM-2 also showed bands in different positions. While *C. baccatum* accessions displayed a slow band, *C. cardenasii* and *C. eximium* had the same fast band. Backcross generation *C. baccatum* SA 219 x F₁ (*C. baccatum* SA 219 x *C. eximium* Hawkes 3860) had 10 heterozygous plants and 30 homozygous plants and backcross generation *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii* SA 268) had 12 heterozygous and 28 homozygous plants. The segregation ratio obtained for these alleles was a skewed ratio and both backcross families contained significantly more homozygotes than expected (see Tables 2 and 3).

Phosphoglucose isomerase (PGI)

Two zones of activity were found. PGI-1 exhibited a single band whose position varied. Both accessions of *C. baccatum* had a slow band, while *C. eximium* and *C. cardenasii* had the same fast band. Backcross generation *C. baccatum* SA 219 x F₁ (*C. baccatum* SA219 x *C. eximium* Hawkes 3860) had 20 heterozygous plants and 20 homozygous plants and backcross generation *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii* SA 268) had 18 heterozygous and 22 homozygous plants. The segregation ratio obtained for these alleles was an acceptable fit to the 1:1 ratio in each family (see Tables 2 and 3).

PGI-2 also gave a single band and the position of this band varied. Both accessions of *C. baccatum* had a slow band and *C. eximium* and *C. cardenasii* both had the same fast band. Backcross generation *C. baccatum* SA 219 x F₁ (*C. baccatum* SA 219 x *C. eximium* Hawkes 3860) had 9 heterozygous plants and 31 homozygous plants and backcross generation *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii* SA 268) had 10 heterozygous and 30 homozygous plants. The segregation ratio obtained for these alleles was a skewed ratio and both backcross families contained significantly more homozygotes than expected (see Tables 2 and 3).

Shikimate dehydrogenase (SKDH)

Two zones of activity were found. SKDH-1 exhibited a single band, whose position varied. Both accessions of *C. baccatum* had a slow band and *C. eximium* and *C. cardenasii* both had the same fast band. Backcross generation *C. baccatum* SA219 x F₁ (*C. baccatum* SA219 x *C. eximium* Hawkes 3860) had 27 heterozygous plants and 13 homozygous plants and backcross generation *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii* SA268) had 29 heterozygous and 11 homozygous plants. The segregation ratio obtained for these alleles was a skewed ratio and both backcross families contained significantly more heterozygotes than expected (see Tables 2 and 3).

Culturing the seeds

In the cross *C. baccatum* SA219 x F₁ (*C. baccatum* SA 219 x *C. eximium* Hawkes 3860), 20 fruits having 97 seeds were obtained. Nineteen seeds were empty, and thus 78 seeds were cultured. The number of inviable seeds (no growth in the culture) and the number of embryos dying in the culture were 24 and 5, respectively. The number of seedlings transferred to soil was 48, of which 45 survived.

In the cross *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* Hawkes 6489 x *C. cardenasii* SA 268), 112 fruits having 562 seeds were obtained. Ninety-two seeds were empty. A total of 100 seeds were cultured. The number of inviable seeds (no growth in the culture) and the number of embryos dying in the culture were 41 and 7, respectively. The number of seedlings transferred to soil was 52, of which 51 survived.

Pollen tube growth

In both crosses with F₁ hybrids as the male parent and *C. baccatum* accessions Hawkes 6489 and SA 219 as the

pistillate parents, pollen tubes grew through the style and reached the ovary. However, the growth rates of F₁ pollen tubes varied. After 24 hours, while some of the F₁ pollen tubes had reached the ovary, some were in the middle of the style. There were no burst pollen tubes.

Pollen viability

While the mean pollen viability (stainability) of F₁ hybrid *C. baccatum* SA 219 x *C. eximium* Hawkes 3860 was 12.88%, it was 14.78% for F₁ hybrid *C. baccatum* Hawkes 6489 x *C. cardenasii* SA 268.

Discussion

Isozyme markers *Est-5*, *Idh-1*, *Pgm-2* and morphological marker gene P for fruit persistence showed distorted segregation ratios in the first backcross generation of both *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* x *C. cardenasii*) and *C. baccatum* SA 219 x F₁ (*C. baccatum* x *C. eximium*). Both progenies had an excess of individuals carrying alleles inherited from *C. baccatum*. A further gene, y, controlling fruit colour and segregating in the backcross of *C. baccatum* Hawkes 6489 x F₁ (*C. baccatum* x *C. cardenasii*), showed a distorted segregation ratio in favour of homozygous individuals. However, in backcross families from both interspecific crosses, *Skdh-1* showed distorted segregation ratios with an excess of heterozygous individuals.

This results indicated that skewed segregation ratios can be found in *Capsicum* as well as in other genera of the family *Solanaceae* as reviewed in the introduction section of the present study.

Factors which might explain distorted ratios or the lack of segregation in the backcross families were as follows:

- a) pollen competition
- b) non-random loss/non-functioning of some zygotes
- c) non-random loss/non functioning of some gametes
- d) Pollen tube competition

Pollen tube competition, a likely reason for distorted segregation ratios, is common in flowering plants and has been demonstrated in the case of artificial interspecific pollination. Variation in pollen germination and pollen tube growth is observed, and selection for a variety of characters is possible in a stylar environment (14). When flowers are pollinated with mixtures of self and alien

pollen, alien pollen often grows more slowly and is less effective in fertilisation than the self pollen (14 and references therein).

In this study it was observed that the growth rates of F₁ pollen tubes varied. After 24 hours, some pollen tubes reached the ovary while some remained in the middle of the style. Since there were no burst pollen tubes, the slow growth of the pollen was not attributed to incompatibility gene (s). Thus, pollen tube competition is one of the likely reasons for the distorted segregation ratios observed in this study. Another factor might be the non-random loss/non functioning of some zygotes. Marshall and Folsom (15) suggested several possibilities as to how the selective elimination of zygotes occurs. One possibility was that the fitness of the maternal plants might be increased by the abortion of some seeds and the accumulation of additional resources for the particular embryo to which maternal plants were more closely related. Perhaps, in this study, backcrosses to *C. baccatum* were favoured.

The distorted ratios for marker genes may be due to the selective elimination of heterozygous genotypes, not only after fertilisation or during seed development, but also during the seed germination and early seedling stages. In the backcross progenies studied, here empty seeds indicated either elimination of zygotes after fertilisation or during embryogenesis, or failure of fertilisation. In the backcross families studied here, the percentage of empty seeds ranged from 16.22%. If these empty seeds carried predominantly the embryos which were heterozygous for the genes showing distorted ratios, this may explain the unbalanced ratios. Rick (4), working on an F₁ hybrid obtained between two tomato species, reported that even a 5% loss of embryos could affect the genetic ratio.

Fully developed backcross seeds were germinated in the culture to achieve the maximum survival rate. Approximately 39% of the seeds were lost in the backcross generation of *C. baccatum* x F₁ (*C. baccatum* x *C. eximium*), while 45% of the seeds were lost in the initial stage of germination in the backcross germination of *C. baccatum* x F₁ (*C. baccatum* x *C. cardenasii*). These figures are very different from those given for empty seeds the (percentage of empty seeds ranged from 16% to 22%) However, ratios were distorted within similar ranges in the two backcross generations. This result may

indicate that factors occurring during the pre-fertilisation and post-fertilisation stages play more important roles than do factors occurring during the seed germination stage.

Another factor causing distorted segregation ratios for marker genes and lack of segregation for unilateral incompatibility may be the non-random loss/non-random functioning of some gametes. Pollen viabilities (stainabilities) of both F₁ hybrids were approximately 15%, so losses of male gametes were high. Both F₁ hybrid combinations were reported to be heterozygous for one interchange (16). In an individual heterozygous for an interchange, two pairs of chromosomes are usually associated in a ring or chain at meiosis. The pairing of homologous portions of this group of four chromosomes results in a cross-shaped configuration at pachytene and this cross shape opens up into four complex chromosomes associated mainly at the ends of diakinesis and metaphase I (17). The type of orientation and the number of chiasmata formed will affect the conformation of the quadrivalent at metaphase I 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 I usually produces viable gametes. If adjacent chromosomes in the quadrivalent are directed towards the same pole (adjacent orientation), separation at anaphase I will produce gametes which contain

duplications and deficiencies. If alternate and adjacent orientations occur with equal frequency in the pollen mother cells, plants heterozygous for one interchange will produce up to 50% viable and 50% inviable pollen. This may explain the values for pollen stainability obtained in the two F₁ hybrids. However, interchanges should restrict the recovery of the recombinant gametes, not result in the selective loss of gametes from one parent. Therefore, this is not likely to be one of the reasons for the distorted segregation ratios obtained in this present study.

In conclusion, selection favouring the *C. baccatum* genotype occurring in different phases of the plants life cycle for the present study, pollen tube competition, and non-random loss/non-functioning of some zygotes) is one of the most likely reasons for the segregation distortions (and lack of segregation for unilateral incompatibility) observed in relation to marker genes. On the other hand, one should take into consideration the fact that isozymes and morphological markers were used to survey the *Capsicum* chromosomes for the gene(s) responsible for unilateral incompatibility. One should also always keep in mind the fact that isozymes and morphological markers mark a limited part of the *Capsicum* genome. Other major genes involved in unilateral incompatibility might therefore have gone undetected because of a lack of markers on certain chromosomes.

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