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## Meiotic behavior and fertility of *Capsicum* interspecific hybrids

Sara I de Oliveira <sup>1</sup>; Elba H Ribeiro <sup>1</sup>; Nádia F Moreira <sup>1</sup>; Larissa S Vianna <sup>1</sup>; Telma NS Pereira <sup>1</sup>

<sup>1</sup>Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes-RJ, Brasil; [telmasp@uenf.br](mailto:telmasp@uenf.br); [sarasilvbio2@gmail.com](mailto:sarasilvbio2@gmail.com); [elba\\_hr@hotmail.com](mailto:elba_hr@hotmail.com); [nadia.fernandesm@gmail.com](mailto:nadia.fernandesm@gmail.com); [larissasouzav@hotmail.com](mailto:larissasouzav@hotmail.com)

### ABSTRACT

This study was performed to evaluate the meiotic behavior and fertility of four hybrids obtained from the crossing of *Capsicum baccatum* and its botanical forms with *Capsicum chinense* and *Capsicum frutescens*. We aimed to identify the causes of the low fertility in interspecific hybrids involving species from different gene pools of *Capsicum*. Hybrid flower buds were fixed in fixative solution and the slides were prepared using 1%-acetic carmine solution. Meiosis was observed in all phases and the main meiotic abnormality observed was the complete or partial lack of chromosome pairing, indicating that the species are either genetically distant or that some asynaptic gene was present in the hybrids. Meiotic index (MI), the frequency of unreduced gametes (type 2n), and pollen viability were estimated. MI and pollen viability of the hybrids were low. We concluded that due to the complete or partial lack of chromosome pairing, the species used in the hybrid combinations are genetically distant. We also noticed that the low-fertility hybrids are a consequence of the lack of chromosome homology between the two genomes involved. The hybrids were considered partially sterile since their pollen viability percentage was lower than 50%.

**Keywords:** *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens*, meiosis, meiotic abnormality, chromosome pairing.

### RESUMO

#### Comportamento meiótico e fertilidade de híbridos interespecíficos de *Capsicum*

Esta pesquisa teve o objetivo de estudar o comportamento meiótico e a fertilidade de quatro híbridos, obtidos do cruzamento entre acessos das espécies *Capsicum baccatum* e suas formas botânicas com *Capsicum chinense* e *Capsicum frutescens*, afim de identificar as possíveis causas da baixa fertilidade observada em híbridos interespecíficos envolvendo espécies pertencentes a diferentes complexos gênicos de *Capsicum*. Para tal, botões florais dos híbridos foram fixados em solução fixadora e as lâminas foram preparadas utilizando carmim acético a 1%. A meiose foi observada e a principal anormalidade registrada foi a falta total ou parcial de pareamento dos cromossomos indicando que as espécies ou são distantes geneticamente ou algum gene assínaptico se manifestou no híbrido. Foram estimados o índice meiótico (IM), a frequência de gametas não reduzidos (tipo 2n), e a viabilidade polínica. O IM e a viabilidade polínica dos híbridos foram baixos. Conclui-se que, devido à falta total ou parcial de pareamento dos cromossomos, as espécies utilizadas nas combinações híbridas, são distantes geneticamente, e que a baixa fertilidade dos híbridos é consequência dessa falta de homologia entre os cromossomos dos dois genomas envolvidos. Os híbridos podem ser considerados parcialmente estéreis pois a sua viabilidade polínica foi inferior a 50%.

**Palavras-chave:** *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens*, meiose, irregularidade meiótica, pareamento de cromossomos.

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Despite having several species, the genus *Capsicum*, green peppers and chili consist of only five domesticated species: *Capsicum annuum*, *Capsicum chinense*, *Capsicum frutescens*, *Capsicum baccatum* and *Capsicum pubescens* (Moscone *et al.*, 2007). Domesticated *Capsicum* species are, preferably, autogamous with hermaphrodite flowers, showing  $2n = 2x = 24$  chromosomes, but some wild species, showing  $2n = 2x = 26$  chromosomes (Pozzobon *et al.*, 2006).

The species of *Capsicum* genus are grouped into three gene pools, according to their crossability and

fertility of the obtained hybrids. *C. annuum* complex consists of *C. annuum*, *C. chinense*, *C. frutescens*, *C. chacoense* and *C. galapagoense*; *C. baccatum* complex consists of *C. baccatum* var. *baccatum*, *C. baccatum* var. *pendulum*, and *C. praetermissum* and *C. pubescens* complex shows some wild species. *C. pubescens* is the only cultivated species, though (Pickersgill, 1991; Zijlstra *et al.*, 1991).

Interspecific hybrids are obtained if the crossing involves species of the same complex (Pickersgill, 1991). According to literature, sometimes hybrids are obtained between *Capsicum* species

which belong to different gene pools; however, these hybrids are sterile or show very low pollen fertility (Egawa & Tanaka, 1986; Yoon *et al.*, 2006; Manzur *et al.*, 2015) which prevents or hinders gene transfer. This hybrid infertility is explained by the low pollen viability of the obtained combinations, which is due to the genetic distance between the species participating in crossing. The closer the species genetically involved in obtaining the hybrid, the greater the chance to obtain a viable or fertile hybrid (Singh, 2016).

Evaluating meiosis and fertility of a specific obtained hybrid is,

among several analyses, a way to study chromosomal homology between two species (Singh, 2016), mainly concerning the genomic homology. Cytogenetic analysis is one of the tools used to analyze chromosomal or genomic homology, through meiosis analysis and consequent hybrid fertility. If no chromosomal homology between parents is noticed, chromosome pairing in interspecific hybrids will be impaired and the gene transfer between species will not occur, as no pairing between chromosomes will be verified, no crossing will occur (Brambatti *et al.*, 2016).

The aim of this study was to evaluate the meiotic behavior and fertility of four hybrids obtained between *C. baccatum* var. *pendulum* and *C. chinense*; *C. baccatum* var. *pendulum* and *C. frutescens*; *C. baccatum* var. *baccatum* and *C. chinense*; and *C. baccatum* var. *baccatum* and *C. frutescens* in order to clarify the reasons for the low fertility of interspecific *Capsicum* hybrids reported in literature.

## MATERIAL AND METHODS

### Plant material

In this study, we used four interspecific hybrids obtained from the crossing between the following species: *C. baccatum* var. *pendulum* and *C. chinense* (UENF 1496 x UENF 1785); *C. baccatum* var. *pendulum* and *C. frutescens* (UENF 1624 x UENF 1636); *C. baccatum* var. *baccatum* and *C. chinense* (UENF 1495 x UENF 1785) and *C. baccatum* var. *baccatum* and *C. frutescens* (UENF 1495 x UENF 1636) obtained in breeding program at Universidade Estadual do Norte Fluminense (Oliveira, 2019).

Hybrid seeds and parents were sown in styrofoam trays, filled with commercial vegetal substrate Vivatto®. After one month, the seedlings were transferred into 300-mL plastic cups containing the same vegetal substrate. The material was kept in this container for one month, then we transferred the plantlets into 5-liter pots and kept them in a greenhouse. The experimental

design was completely randomized, with three replicates. Each replicate consisted of four plots (hybrid combination), each combination was represented by five pots with one plant per pot, totalizing five plants. Cultural practices were performed according to the necessity of the plant and according to the recommendations proposed by Filgueira (2008).

### Meiotic analysis

In order to evaluate the meiotic behavior of the hybrids, flower buds, in different development stages, were collected and fixed in an ethanol solution: acetic acid, at a 3:1 ratio, for 24 hours. After, the buds were transferred to the 70% ethanol solution and kept in the refrigerator. The microscope slides were prepared according to the squash method: three anthers of each bud were squashed in drops of 1% acetic carmine. After removing the debris, the material was covered with a coverslip and the slides were observed under an optical microscope (Olympus BX60). The analysis of prophase I was prioritized in order to observe the pairing of chromosomes in hybrids and the images were captured with the aid of the Software CellSens Standard 1.8 (Olympus).

In order to estimate the meiotic index (IM) (Love, 1951) and to estimate the frequency of unreduced gametes (Yan *et al.*, 1997), three anthers were macerated in drops of 1% acetic carmine, and five slides per hybrid were prepared. We counted 400 post-meiotic products/slides, totalizing 2000 post-meiotic products, for each estimate.

Hybrid fertility was estimated via pollen viability test. Floral buds at anthesis were collected, kept in 70% ethanol solution, in a freezer at 4°C. For preparing the slides, the authors macerated the anthers in a drop of triple Alexander solution (Alexander, 1969). For each hybrid, five slides were prepared. In each slide, 400 pollen grains were counted, totalizing 2000 pollen grains. Purple-colored pollen grains were considered viable and green-colored pollen grains were considered non-viable. All slides were observed under an optical microscope

(Olympus BX60), images were captured using CellSens Standard 1.8 (Olympus). Pollen viability data were submitted to analysis of variance using the Genes software (Cruz, 2013).

## RESULTS AND DISCUSSION

The authors observed that, in hybrid meiosis, the vast majority of cells analyzed in Prophase I presented unpaired chromosomes or part paired chromosomes and part univalent chromosomes (Figure 1). Hybrids UENF 1496 x UENF 1785 and UENF 1624 x UENF 1636 (Figures 1A and 1B), whose pollen receptor accession was *C. baccatum* var. *pendulum* (UENF 1496 and UENF 1624), showed complete lack of chromosome pairing, indicating a lack of homology among the genomes of this species, belonging to *C. baccatum* complex, with pollen donor species (*C. chinense* and *C. frutescens*), both belonging to *C. annum* complex. Another reason for the lack of pairing can be due to the presence of a synaptic gene in the genome of the parental species of the hybrid. Synaptic genes are genes which affect the normal chromosome pairing (Wani & Bhat, 2017). They can be asynaptic genes which can cause a complete lack of chromosome pairing during Prophase I, or desynaptic genes which cause a pairing at the beginning of Prophase I, failing to maintain this association in the subsequent stages and, thus, separate prematurely (Wani & Bhat, 2017). If no pairing is verified, no crossing-over between non-sister chromatids occurs and, consequently, a failure in the introgression of genes is verified (Singh, 2016).

On the other hand, the authors also observed that hybrids whose female parent was the accession UENF 1495 (*C. baccatum* var. *baccatum*) showed some paired chromosomes, being possible to verify some bivalent and univalent ones (Figure 1C and 1D). Some cytogenetic studies on hybrids of *C. baccatum* x *C. frutescens* reported that, in both F1 and F2, the hybrids presented irregular meiosis, with an association of eight bivalent and one

quadrivalent chromosomes (Rao *et al.*, 1992). One study indicated the possibility of using *C. chinense* as a bridge species in crossings involving *C. baccatum* species, aiming at posterior crossing of the hybrid with *C. annuum*. There was success in using it as a bridge species, so the crossing is indicated (*C. baccatum* x *C. chinense*) x (*C. annuum*). Nevertheless, *C. frutescens* was not considered an effective bridge species when the goal was to transfer *C. frutescens* genes to *C. annuum* (Manzur *et al.*, 2015). Thus, this combination *C. baccatum* x *C. chinense* can be a good alternative to transfer the *C. baccatum* genes, since *C. chinense* species shows average crossability with the cultivated form (Martins *et al.*, 2015).

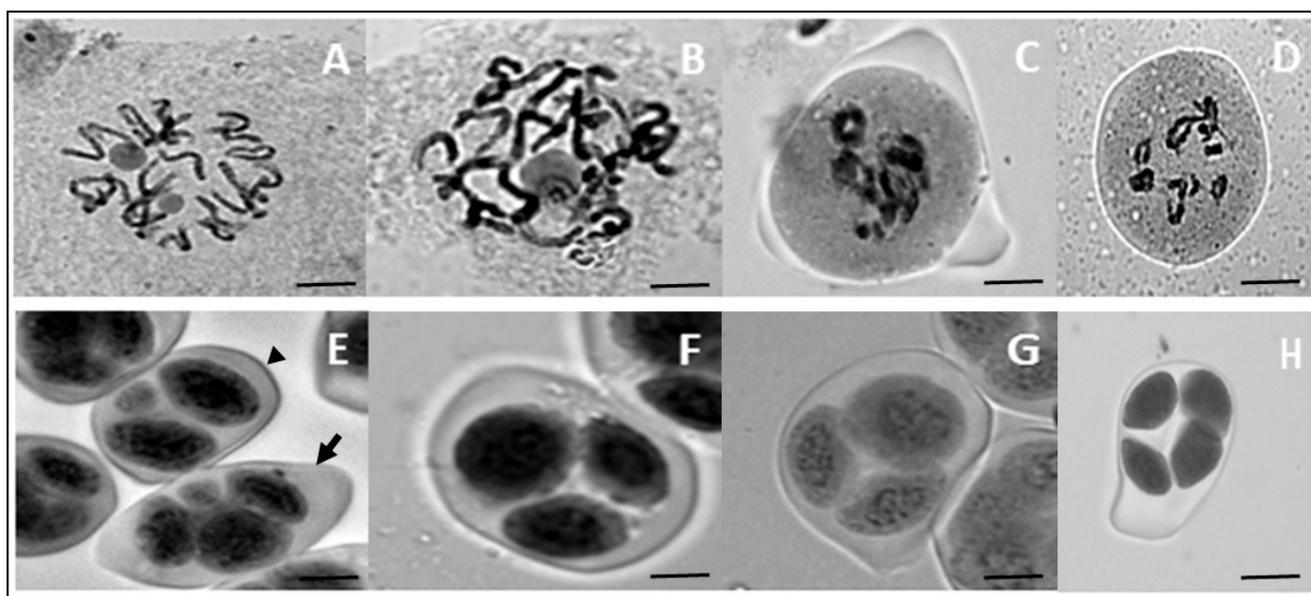
Many irregularities have been observed, such as lagging chromosomes, as a consequence of lack of pairing observed in hybrids. The presence of lagging chromosomes can generate unbalanced or aneuploid gametes, due to the possibility of being trapped in the cytoplasm, not following the set of chromosomes which follow cell division normally, and in the end, be eliminated in the form of micronuclei (Figure 1E) (Kodoru & Rao, 1981; Pozzobon *et al.*, 2011).

In this study, the meiotic index, a complementary data in the meiotic

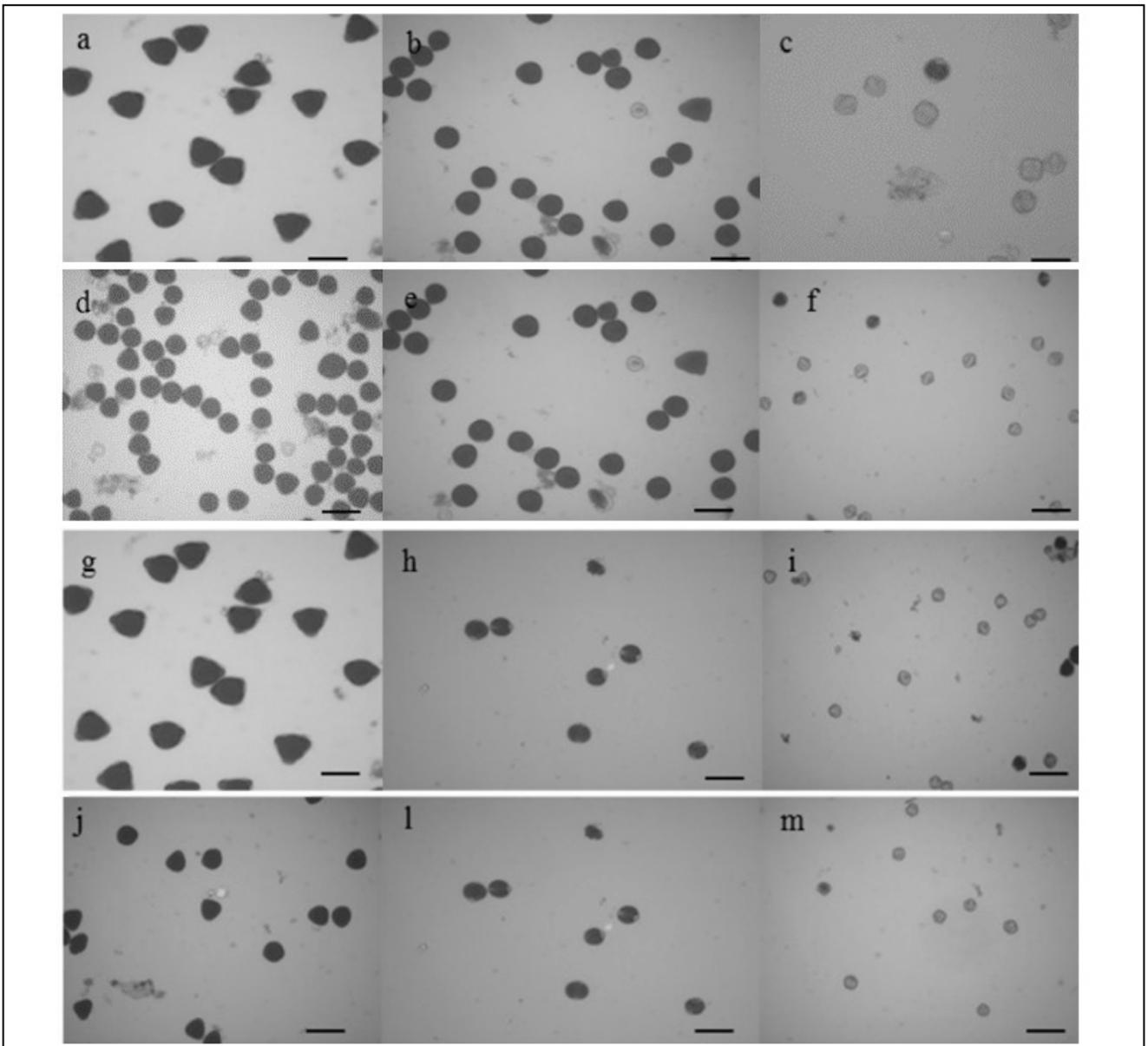
analysis and an indicator of regularity (Love, 1951), was estimated for the four hybrid combinations. The hybrids which showed *C. chinense* as male parent (UENF 1496 x UENF 1785 and UENF 1495 x UENF 1785) showed meiotic indexes of 58.50% and 55.25%, respectively. Whereas the hybrids which showed *C. frutescens* as male parent (UENF 1495 x UENF 1636 and UENF 1624 x UENF 1636) showed meiotic indexes of 60.4% and 64.3%, respectively. Genotypes with meiotic indexes inferior to 90% showed low meiotic stability, which can cause reproductive problems when used in crossings, as they are considered cytologically unstable. Thus, we can state that the hybrids analyzed in this study presented low meiotic stability (Love, 1951).

The authors observed some dyads (Figure 1E), triads (Figure 1F and 1G) which generated as a consequence unreduced gametes in a frequency which ranged from 6.05% in UENF 1496 x UENF 1785 to 13.2% in UENF 1495 x UENF 1636. Unreduced gametes, type 2n, resulted from an abnormal meiotic process, in which no reduction in chromosome number is verified. This failure in reduction may occur due to the restitution in the first division (RFD) when in meiosis I, chromosomes do

not move to the poles during anaphase I and, instead of two cells with haploid number of chromosomes in telophase I, the formation of a cell with diploid number is noticed. In this case, meiosis II occurs normally, resulting in a dyad instead of triad, though (which was already expected). This division (RFD) occurs when the plants in which pairing between homologues or homeologues (interspecific hybrids) is completely absent as in synaptic mutants or distant hybrids and in plants whose cytokinesis is of the successive type as is the case of most dicots (Schifino-Wittmann & Dall'Agnol, 2001; Ramanna & Jacobsen, 2003). The other way to form 2n gametes is in meiosis II, by the restitution in the second division (RSD), in which a cytokinesis failure and diploid restitution nucleus (with formation of dyads or triads) can be verified (Ramanna & Jacobsen, 2003). Unreduced gametes occur spontaneously in virtually all natural plant populations but, in general, in very low percentages, around 1% or less (Schifino-Wittmann & Dall'Agnol, 2001). According to literature, frequency of unreduced male gametes in hybrids was 50 times higher (27.52%) than in non-hybrids (0.56%) (Ramsey & Schemske, 1998). However, in this study, the frequency of triads (Figures 1F and 1G) was average, which



**Figure 1.** A-D) Chromosomal pairing in Prophase I in interspecific hybrids; A and B= lack of chromosome pairing; C and D= partial chromosome A) UENF 1496 x UENF 1785; B) UENF 1624 x UENF 1636; C) UENF 1495 x UENF 1636; and D) UENF 1495 x UENF 1785. E-F) Pos-meiotic products. E= dyad (arrow head) and triads (arrow) with micronuclei; F and G= triads; H= tetrads. Bar= 20µm. Campos dos Goytacazes, UENF, 2019.



**Figure 2.** Viable (a, b, d, e, g, h, j, and l) and non-viable (c, f, i, and m) pollen grains of parents and interspecific hybrids a= UENF 1495, b= UENF 1636, c= UENF 1495 x UENF 1636, d= UENF 1624, e= UENF 1636, f= UENF 1624 x UENF 1636, g= UENF 1495, h= UENF 1785, i= UENF 1495 x UENF 1785, j= UENF 1496, l= UENF 1785, m= UENF 1496 x UENF 1785. Bar=20 $\mu$ m. Campos dos Goytacazes, UENF, 2019.

generated some viable pollen grains.

Hybrid fertility was tested using pollen viability (Figure 2). Variance analysis did not verify any significant differences between the pollen viability values of hybrids. In hybrid UENF 1496 x UENF 1785 the value was 34.85% and in hybrid UENF 1495 x UENF 1785 was 27.05%, inferior to the values obtained for the accession, male parent UENF 1785, whose estimate was 92.85%. In hybrids UENF 1495 x UENF 1636, pollen viability was 20.95% and for UENF 1624 x UENF 1636 was 17.95%, estimates inferior

when compared with male parent, which showed viability of 69% for UENF 1636 (*C. frutescens*). The low viability obtained in hybrids was relatively expected due to unbalanced gametes resulting from meiotic irregularities observed in this study, showing a partial sterility. The hybrid fertility between *Capsicum* species has been studied and low pollen viability has been estimated for combinations like *C. baccatum* var. *pendulum* x *C. chinense* (25%) and for *C. baccatum* var. *pendulum* x *C. frutescens* (46%) (Monteiro *et al.*, 2011). Using the estimates of pollen viability, the authors

could infer the hybrid fertility, which in this study was partial, considering that the viability was lower than 50%, due to lack of homology between the chromosomes of species involved in the crossing, probably in relation to the genetic distance between them (Pickersgill, 1997).

Despite the partial fertility found in the obtained hybrids, these hybrids can be used as pollen receptors, that is, female parents in backcross breeding programs (Yoon *et al.*, 2006), or they can be used as bridge species in breeding programs to improve the cultivated

species (Manzur *et al.*, 2015). Thus, further studies would be welcomed to help out breeders establish breeding programs on the cultivated form, using interspecific hybridization as a viable methodology.

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