

## Meiotic analysis of interspecific hybrids between *Capsicum frutescens* and *Capsicum chinense*

Nádia Fernandes Moreira<sup>1\*</sup>, Telma Nair Santana Pereira<sup>1</sup> and Kellen Coutinho Martins<sup>1</sup>

Crop Breeding and Applied Biotechnology  
17: 159-163, 2017  
Brazilian Society of Plant Breeding.  
Printed in Brazil  
<http://dx.doi.org/10.1590/1984-70332017v17n2n23>

**Abstract:** The aim of this study was to observe the genetic relationship between *C. frutescens* (UENF 1636) and *C. chinense* (UENF 1785) based on the meiotic behavior, on the meiotic index, and on pollen viability of their F<sub>1</sub> hybrids. For meiotic analysis and meiotic index, flower buds were collected and fixed for 24 hours in ethanol: acetic acid (3:1), and then transferred to 70% ethanol, and stored in a freezer. Slides preparation for meiosis, meiotic index and pollen viability was carried out according to the laboratory's protocol. Hybrid meiosis was regular, and 12 pairs of chromosomes were observed in diakinesis, as well as a few anomalies. The MI of 88.16%, as well as the pollen viability of 72.5%, were satisfactory. Given the results, it was confirmed the genetic proximity of these species, which are classified in the *Capsicum annuum* gene complex.

**Key words:** Pepper, chromosomes, cytogenetics.

### INTRODUCTION

The genus *Capsicum* belongs to the Solanaceae family, and is represented by pepper and bell peppers (Eshbaugh 1993). Most of the *Capsicum* species are self-compatible, except for *C. cardenasii*, whose incompatibility is gametophytic, also found in other Solanaceae genera (Pickersgill 1991). According to Barbosa et al. (2011), among the 38 species, which have already been identified, five of them are grown and used by humans: *C. annuum* var. *annuum*; *C. baccatum* var. *baccatum*; *C. chinense*; *C. frutescens*, and *C. pubescens* (Pickersgill 1991, Moscone et al. 2007). Within this genus, species can be distinguished in two groups, according to the basic number of chromosomes: one with  $n = x = 12$  chromosomes, and another with  $n = x = 13$  chromosomes (Moscone et al. 2007).

Wild species are considered as gene repositories, and several varieties of agronomic importance have been improved through interspecific hybridization (Hajjar and Hodgkin 2007). Interspecific crosses may allow the improvement of varieties by the introduction of important alleles, such as those which provide resistance to pathogens (Pickersgill 1997). However, for the success of gene transfer between species, it is necessary that the species are genetically close, i.e., they must present chromosome homology, reducing incongruity problems, and thus making the hybrid viable (Prestes and Goulart 1995). In addition, the knowledge of the direction of the cross is essential, since, in some species, interspecific cross is effective and unique to a given direction (Prestes and Goulart 1995).

**\*Corresponding author:**  
E-mail: [nadia\\_fernandesm@yahoo.com.br](mailto:nadia_fernandesm@yahoo.com.br)

**Received:** 02 March 2015  
**Accepted:** 08 February 2016

<sup>1</sup> Universidade Estadual do Norte Fluminense Darcy Ribeiro, Centro de Ciências e Tecnologias Agropecuárias, Laboratório de Melhoramento Genético Vegetal, Av. Alberto Lamego, 2000, Parque Califórnia, 28.013-602, Campos dos Goytacazes, RJ, Brazil,

In *Capsicum*, species are grouped in three complexes in order to obtain fertile hybrids. The complex *Capsicum annuum* gathers the species *C. annuum*, *C. chinense* and *C. frutescens* and their botanical forms; the complex *Capsicum baccatum* gathers the species *C. baccatum* var. *baccatum* and *C. baccatum* var. *pendulum*; and the complex *Capsicum pubescens* gathers wild species and only one domesticated species, *C. pubescens* (Pickersgill 1991).

Interspecific hybrids can be obtained between the domesticated species of the *Capsicum* genus; however, these hybrids present different degrees of fertility, which may be due to chromosome pairing problems in the hybrid, or due to the presence of different chromosome structural arrangements (Kumar et al. 1987, Lanteri and Pickersgill 1993). According to Shiffriss (1997), the degree of male sterility of interspecific hybrids of *Capsicum* depends on the accessions used in the crosses. Monteiro et al. (2011) observed that the degree of viable interspecific hybrids of *Capsicum* ranged from high, in combinations involving *C. chinense* x *C. frutescens*, to complete male sterility, or hybrid unviability. The authors also observed that pollen viability of the interspecific hybrid depended on the accessions involved in the combination, and this was a genotype-specific viability.

The knowledge of the fertility of the species involved in an interspecific hybridization program is important in order to obtain successful crosses. The fertility of a species, usually measured by the pollen viability, is the result of normal and balanced gametes formation. According to Defani-Scoarize et al. (1996), the success of both intra and interspecific hybridization, in genetic terms, is also determined by a regular meiosis, resulting in the formation of viable gametes.

Considering the importance of evaluating the fertility of interspecific hybrids obtained between *C. frutescens* (UENF 1636) and *C. chinense* (UENF 1785), and of following up with further researches, this study aimed to analyze the meiosis of an interspecific hybrid, and to estimate pollen viability and the meiotic index of this hybrid.

## MATERIAL AND METHODS

In this study, it was used an interspecific hybrid plant obtained between the accessions UENF 1785 (*C. chinense*) and UENF 1636 (*C. frutescens*) (Martins et al. 2015). Hybrid seeds were initially germinated in B.O.D. chamber at 27.5 °C, with photoperiod of 8 hours of light and 16 hours of darkness, and then planted in polystyrene trays with the vegetable substrate Vivatto®, in a greenhouse at the Unit for Research Support (UAP) in the campus of the State University of Northern Rio de Janeiro (UENF). When seedlings presented four to six true leaves, they were transplanted from the tray to 500 mL plastic cups with vegetable substrate Vivatto®, in a greenhouse at the UAP of UENF. About two months after germination, plants were transferred to 5 L plastic pots, using the same type of vegetable substrate, with 15 hybrid plants. Management was employed according to the recommendations for the culture (Filgueira 2000).

During the flowering period, flower buds of different sizes and at different development stages were collected at random in bulk, in fifteen plants of the interspecific hybrid, and fixed in ethanol: acetic acid solution at a ratio of 3:1, for 24 hours. Afterwards, they were transferred to 70% ethanol solution, and kept in the refrigerator until being used. For slides preparation, three anthers of flower buds were macerated (squash) on the slide, in 1% acetic carmine solution, according to the laboratory's protocol. Slides were then observed under optical microscope, in brightfield, and the different stages of meiosis were analyzed. Possible meiotic abnormalities were also recorded, such as laggard chromosomes, early segregation of chromosomes, and problems in the spindle fibers.

To estimate the meiotic index (MI), according to Love (1951), flower buds at anthesis were collected in 70% ethanol solution and kept in the refrigerator. At the time of slides preparation (5 slides), three anthers were macerated in 1% acetic carmine solution and observed under a microscope. For the estimate of the meiotic index, the numbers of the post-meiotic products in the five slides analyzed (500 pollen grains, totaling 1500 post-meiotic products) were counted. The meiotic index is estimated by the ratio of the total normal tetrads by the total post-meiotic products. Tetrad with four nuclei of the same size was considered normal, and any deviation (monad, dyad, triad and polyad) was considered abnormal.

Meiotic Index – MI (Love 1951)

$$MI = \frac{N. \text{ of normal tetrads}}{\text{Total n. of post-meiotic products}} \times 100$$

Pollen viability of the accessions was estimated by the Alexander's triple solution (Alexander 1969). To this end,

flower buds at anthesis were collected in 70% ethanol solution and stored at 4 °C. In the slides preparation, the anthers were macerated in a drop of the Alexander's triple solution, in which viable pollen grains were stained red, and unviable pollen grains were stained green, simultaneously. In order to obtain a random sample of the stained pollen grains, the scanning method was used in the slide (8 slides), and 250 pollen grains/slide were counted. All the slides were observed under optical microscope (Olympus BX60), and the images were captured with the *Image-Pro Plus Software* (version 5.1, Media Cybernetics).

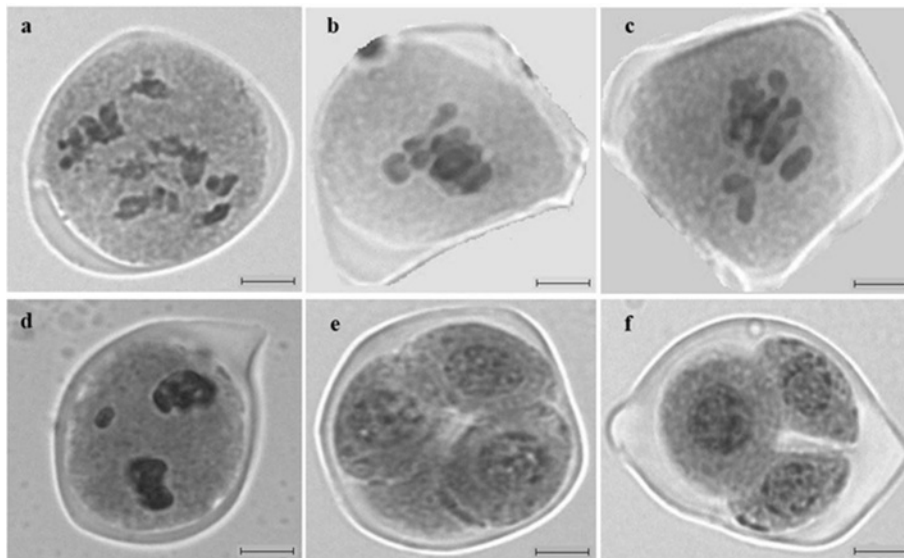
## RESULTS AND DISCUSSION

The meiotic analysis of the hybrids allowed observing 12 chromosomes in bivalent association in diakinesis (Figure 1a), corroborating the literature for diploid parental species, with  $2n=2x=24$  chromosomes (Pozzobon et al. 2006, Moscone et al. 2007). This result is expected, since the parental species, *C. frutescens* and *C. chinense*, differ only by the presence of an annular constriction in the calyx, typical of the species *C. chinense* (IPGRI 1995). For some authors, both species are in fact a single species, depending on the results of morphological analysis (Pickersgill et al. 1979), enzyme analysis (Jensen et al. 1979), and cytogenetic (Egawa and Tanaka 1986). However, Baral and Bosland (2004), when investigating the morphology, crossability, and polymorphism of the DNA of the accessions of these species, concluded that *Capsicum frutescens* and *C. chinense* represent two distinct species.

Meiotic abnormalities were observed in the form of early chromosome segregation (Figure 1b) at the second meiotic division; of laggard chromosomes (Figures 1c and 1d); and of the lack of synchrony. Usually, laggard chromosomes are lost during cell division, causing aneuploid daughter cells (Weinert 1998).

The abnormalities found may result in unbalanced gametes at the end of cell division. According to Pagliarini (2000), the most common meiotic abnormalities observed in several species is the irregular chromosome segregation, characterized by early migration or laggard chromosomes at metaphase I and anaphase I. Considering that the meiotic behavior of a plant is directly related to their degree of fertility, the changes observed during cell division may be reflected in the viability of pollen grains (Defani-Scoarize et al. 1996).

In the hybrid analyzed, it was observed that cytokinesis is not symmetrical, with triad post-meiotic products (Figure 1f). However, there was predominance of normal tetrads (Figure 1e), which is expected at the end of a normal meiotic division. Triads can originate from errors that occurred at the time of the cytoplasm division during meiosis I or II, and are



**Figure 1.** Different stages of meiosis of the hybrid of *Capsicum chinense* and *C. frutescens*: a) Diakinesis; b) Early Segregation; c) Metaphase I, presenting laggard chromosomes; d) Anaphase I, presenting laggard chromosomes; e) Tetrads; f) Triads. Bar.: 10µm

usually related to the formation of unreduced gametes, and may be relevant in the case of a species to which polyploidy is an evolutionary alternative (Pickersgill 1997).

The entire process of cell division is governed by genes that may manifest at any stage of meiotic division. According to Singh (1993), most mutant genes that cause meiosis errors are expressed at the end of the division - tetrad stage and pollen mitosis. Mutant genes that manifest themselves at the tetrad stage are those which affect the chromosomes disjunction, due to errors in the spindle apparatus (Singh 1993).

Love (1951) established that plants with meiotic indices below 90% are meiotic unstable. However, the hybrid under study presented MI of 88.2%, classifying it as an unstable hybrid, but from a confidence interval ( $\alpha=5\%$ ) of 93.63% (upper limit) to 82.77% (lower limit). Considering that the mean MI was 88.2%, it is within the confidence interval.

The formation of the pollen grain follows two cytokinesis patterns, which are determined by the moment when it occurs during meiosis. In simultaneous cytokinesis, no wall is formed after meiosis I (Esau 1977). In this case, microspores are arranged in a tetrahedral tetrad (Hesse et al. 2009). On the other hand, in the successive cytokinesis, a wall is formed after the first and the second division, leading to the formation of several types of tetrads (Schulz and Jensen 1968). As observed in Figure 1e, simultaneous cytokinesi prevails in *Capsicum*, and the arrangement of the tetrad type is tetragonal (Esau 1977).

It was also observed lack of synchronization during cell division, i.e., there were different stages of meiosis in the same flower bud. The literature reports this behavior, which is in accordance with the findings of Picoli et al. (2003), who defined six bud classes, based on the size ratio between calyx and corolla margin shape of the calyx, and on the presence of pigments in the anthers, noting the presence of different stages of meiosis on the same bud class.

Pollen viability was of 72.5%, with predominance of viable pollen grains, resulting from a satisfactory nuclear division for an interspecific hybrid. Thus, it was observed satisfactory relationship between the number of normal tetrads and the viability of pollen grains. Monteiro et al. (2011) evaluated interspecific hybrids obtained between *Capsicum* species and observed that fertility ranged from fertile combinations (*C. chinense* x *C. frutescens*) to male sterility combinations (*C. baccatum* var. *pendulum* x *C. chinense*).

Kumar et al. (1987) mentioned in their work on interspecific hybrids of *Capsicum* the occurrence of irregular meiosis, in which the genome of *C. annuum* differs from *C. chinense* by two translocations and some structural changes, and from *C. baccatum* by two translocations, a single inversion, and some secondary structural changes.

In general, combinations between the species *C. frutescens* x *C. chinense* are considered as possible. According to Pickersgill (1991) and Zijlstra et al. (1991), the species *C. chinense* and *C. frutescens* belong to the same gene complex, which favors and facilitates the cross of these species, obtaining fertile F1 plants. Consequently, the understanding for distinct groups is opposite, i.e., it is more difficult to form fertile interspecific hybrids derived from crosses between species belonging to different gene complexes.

Based on the results, it is concluded that the species *C. frutescens* and *C. chinense* are genetically close, in function of chromosome homology, considering that the interspecific hybrid presented normal meiosis, with 12 bivalent chromosomes, resulting in reduced gametes. The viability of the hybrid was 72.5%, which is considered as satisfactory.

## REFERENCES

- Alexander MP (1969) Differential staining of aborted non aborted pollen. **Stain Technonology** **44**: 117-122.
- Baral JB and Bosland PW (2004) Unraveling the species dilemma in *Capsicum frutescens* and *C. chinense* (Solanaceae): A multiple evidence approach using morphology, molecular analysis and sexual compatibility. **Journal of the American Society for Horticultural Science** **129**: 826-832.
- Barbosa G, Agra MF, Romero MV, Scaldaferrero MA and Moscone EA (2011) New endemic species of *Capsicum* (Solanaceae) from the Brazilian Caatinga: comparison with the re-circumscribed *C. parvifolium*. **Systematic Botany** **36**: 768-781.
- Defani-Scoarize MA, Pagliarini MS and Aguiar CG (1996) Meiotic behavior of inbred lines of maize (*Zea mays* L.) **Nucleus** **39**: 10-18.
- Egawa Y and Tanaka M (1986) Cytogenetical study of the interespecific hybrid between *Capsicum annuum* and *C. baccatum*. **Japanese Journal of Breeding** **36**: 16-21.
- Esau K (1977) **Anatomy of seed plants**. 2<sup>nd</sup> edn, John Wiley & Sons, Madison, 550p.
- Eshbaugh WH (1993) Peppers: history and exploitation of a serendipitous

## Meiotic analysis of interspecific hybrids between *Capsicum frutescens* and *Capsicum chinense*

- new crop discovery. In Janick J and Simon JE (eds) **New crops**. Wiley and Sons, New York, p. 132-139.
- Filgueira FAR (2000) **Novo manual de olericultura: Agrotecnologia moderna na produção e comercialização de hortaliças**. UFV, Viçosa, 402p.
- Hajjar R and Hodgkin T (2007) The use of wild relatives in crop improvement: A survey of developments over the last 20 years. **Euphytica** **156**: 1-13.
- Hesse M, Zetter R, Buchner R, Weber M, Frosch-Radivo A, Halbritter H and Ulrich S (2009) **Pollen terminology. An illustrated handbook**. Springer IV, Vienna, 264p.
- IPGRI (1995) **Descriptors for Capsicum (Capsicum spp)**. IPGRI, Roma, 51p.
- Jensen RJ, McLeod MJ, Eshbaugh WH and Guttman SI (1979) Numerical taxonomic analyses of allozymic variation in *Capsicum* (Solanaceae). **Taxon** **28**: 315-327.
- Kumar OA, Panda RC and Rao KGR (1987) Cytogenetic studies of the F1 hybrids of *Capsicum annuum* with *C. chinense* and *C. baccatum*. **Theoretical and Applied Genetics** **74**: 242-246.
- Lanteri S and Pickersgill B (1993) Chromosomal structural changes in *Capsicum annuum* L. and *Capsicum chinense* Jacq. **Euphytica** **67**: 155-160.
- Love RM (1951) Varietal differences in meiotic chromosomes behavior of Brazilian wheats. **Agronomy Journal** **43**: 72-76.
- Martins KC, Pereira TNS, Souza SAM, Rodrigues R and Amaral Junior AT (2015) Crossability and evaluation of incompatibility barriers in crosses between *Capsicum* species. **Crop Breeding and Applied Biotechnology** **15**: 139-145.
- Moscone EA, Scaldaferrero MA, Grabiele M, Cecchini NM, García YS, Jarret R, Daviña JR, Ducasse DA, Barboza GE and Ehrendorfer F (2007) The evolution of chili peppers (*Capsicum* – solanaceae): a cytogenetic perspective. VI The International Solanaceae Conference. **Acta Horticulturae** **745**: 137-169.
- Monteiro CES, Pereira TNP and Campos KP (2011) Reproductive characterization of interspecific hybrids among *Capsicum* species. **Crop Breeding and Applied Biotechnology** **11**: 241-249.
- Pagliarini MS (2000) Meiotic behavior of economically important plant species: the relationship between fertility and male sterility. **Genetics and Molecular Biology** **23**: 997-1002.
- Pickersgill B (1991) Cytogenetics and evolution of *Capsicum* L. In Tsuchiya T and Gupta PK (eds) **Chromosome engineering in plants: genetics, breeding, evolution**. Elsevier, Amsterdam, p. 139-160.
- Pickersgill B (1997) Genetic resources and breeding of *Capsicum* spp. **Euphytica** **96**: 29-133.
- Pickersgill B, Heiser CB and Mcneill J (1979) Numerical taxonomic studies on variation and domestication in some species of *Capsicum*. In Hawkes JG, Lester RN and Skelding AD (eds) **The biology and taxonomy of the Solanaceae**. Academic, London, p. 679-700.
- Picoli EAT, Carvalho CR, Fári M and Otoni WC (2003) Associação de fases meióticas e estádios dos micrósporos com características morfológicas de botões florais de pimentão. **Ciência & Agrotecnologia** **27**: 708-713.
- Pozzobon MT, Schifino-Wittmann, MT and Bianchetti LB (2006) Chromosome numbers in wild and semidomesticated Brazilian *Capsicum* L. (Solanaceae) species: do  $x=12$  and  $x=13$  represent two evolutionary lines. **Botanical Journal Linnean Society** **151**: 259-269.
- Prestes AM and Goulart LR (1995) Transferência de resistência a doenças de espécies silvestres para espécies cultivadas. **Revisão Anual de Patologia de Plantas** **3**: 315-363.
- Schulz SR and Jensen WA (1968) Capsella embryogenesis: the egg, zygote, and young embryo. **American Journal of Botany** **55**: 807-819.
- Shifriss C (1997) Male sterility in pepper (*Capsicum annuum* L.). **Euphytica** **93**: 83-88.
- Singh, R J (1993) The handling of plant chromosomes. **Plant cytogenetics**. CRC Press, Boca Raton, p. 7-24.
- Weinert T (1998) DNA damage checkpoints update: getting molecular. **Current Opinion in Genetics & Development** **8**: 185-193.
- Zijlstra SC, Purimahua C and Lindout P (1991) Pollen tube growth in interspecific crosses between *Capsicum* species. **Hortscience** **26**: 585-586.