Acta Agron. (2017) 66 (4) p 612-617 ISSN 0120-2812 | e-ISSN 2323-0118 *https://doi.org/10.15446/acag.v66n4.59162*

Cytogenetic evaluation of chili (*Capsicum spp*., Solanaceae) genotypes cultivated in Valle del Cauca, Colombia

Evaluación citogenética de genotipos de ají (*Capsicum* spp., Solanaceae) cultivados en el Valle del Cauca, Colombia

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Rec.: 19.09.2016 Accep.: 04.04.2017

Abstract

The genus *Capsicum* comprises a large group of hot and sweet peppers, with different morphological varieties and degree of fruits pungency. Due to its active principles is widely used in cooking and in traditional medicine, being sold in large worldwide. In order to provide cytogenetic information which supporting plant breeding programs, this study aimed to analyze the meiotic behavior, chromosome number and pollen viability of the three species of greater economic importance of *Capsicum* (*C. annuum*, *C. frutescens* and *C. chinense*). Therefore, fruits and flower buds were collected from the Experimental Center of the Universidad Nacional de Colombia, campus Palmira- CEUNP to the corresponding mitotic, meiosis and pollen viability analysis. Plants presented different meiotic abnormalities, where all can disrupt the process of meiosis cell division, creating unbalanced chromosome numbers and decreasing pollen fertility. Chromosome number variation, checking 2n=2x=24, 2n=2x=26, 2n=4x=48 to 2n=6x=72 chromosomes, also occurred. A low degree of non-viable pollen grains was observed for *C. frutescens* genotype, so these accession should not considered as a parental for breeding programs.

Key words: Chromosome number, meiotic behavior, meiotic index, plant genetic resources conservation, ploidy level, pollen viability.

Resumen

El género *Capsicum* comprende un gran grupo de ajís picantes y dulces con diferentes variedades morfológicas y el grado de pungencia de los frutos. Debido a sus principios activos, es utilizado ampliamente en la cocina y en la medicina tradicional, siendo comercializado ampliamente en todo el mundo. Con el fin de aportar una información citogenética apropiada que apoye los programas de mejoramiento genético vegetal, este estudio tuvo como objetivo analizar el comportamiento meiótico, el número cromosómico y la viabilidad de polen de las tres especies de *Capsicum* (*C. annuum, C. frutescens* y *C. chinense*) de mayor importancia económica. Frutos y botones florales fueron colectados en el Centro Experimental de la Universidad Nacional de Colombia sede Palmira - CEUNP para los correspondientes análisis mitótico, meiótico y polínico. Las plantas presentaron diferentes anormalidades meióticas, afectando la estabilidad, conllevando al desbalance cromosómico y reduciendo la fertilidad del polen. También se encontró una variación en el número de cromosomas, desde 2n=2x=24, 2n=2x=26, 2n=4x=48 hasta 2n=6x=72. Se observó un bajo grado de granos de polen viables para *C. frutescens,* de tal manera que tal accesión no puede ser considerada como parental para programas de mejoramiento genético.

Palabras clave: Comportamiento meiótico, conservación de recursos fitogenéticos, índice meiótico, nivel de ploidía, número cromosómico, viabilidad de polen.

Introduction

The genus *Capsicum* belongs to the Solanaceae family, which is represented by approximately 3000 species, distributed in 150 genera (Barth & Duarte, 2008). *Capsicum* comprises a large group of hot and sweet peppers with different morphological varieties and degree of fruits pungency. In fact, due to their active ingredients are widely used and marketed worldwide (Büttow, Barbieri, Neitzke, Heiden & Carvalho, 2010; Neitzke, Barbieri, Vasconcelos, Fischer, Vilella & Castro, 2014).

The taxonomy of this genus is still confused, there is controversy as to the number of species, ranging from 25 (Büttow *et al.*, 2010), 30 (Moscone, Scaldaferro, Grabiele, Cecchini, Garcia, Jarret, Daviña, Ducasse, Barboza & Ehrendorfer, 2007) and 31 (Martins, Pereira, Souza & Costa, 2010) described species, being classified as domesticated (five species), semi-domesticated and wild (Souza, Martins & Pereira, 2011).

The origin center of this genus is the Tropical America (Pickersgill, 1997). Spanish and Portuguese people were the first to discover this kind in the Americas, one of the products most commonly used in cooking and traditional medicine for these people (Ribeiro, Lopes, Carvalho, Henz & Reifschneider, 2008). In cooking, as constituents of salads and condiments; in traditional medicine, in the fight and prevention of diseases (Signorini, Renesto, Machado, Bespalhok & Monteiro, 2013), some countries like Peru and Mexico use peppers in large-scale, because of the color characteristics and assets of the fruit principles (Teodoro, Garcia & Corona, 2007).

According to Rufino & Penteado (2006), Asia is the continent that more grows peppers, with approximately 89% of the total cultivated. India, Korea, Thailand, China, Vietnam, Sri Lanka and Indonesia are the most prominent production countries. Production of the United States and Mexico is around 7%, followed by countries in Europe, Africa and Middle East, with 4% of the total cultivated peppers. In Brazil, the production of peppers is growing, being an important diversity center, and harboring domesticated, semi-domesticated and wild species, with emphasis on the cultivation of domesticated species *C. annuum*, with greater economic importance in this country (Rufino & Penteado, 2006; Neitzke *et al.*, 2014).

Among the widely consumed species and commercially cultivated are *C. annuum* (pepper, sweet pepper); *C. baccatum* (finger-to-girl in-hat-friar); *C. chinense* (pepper-of-smell, pepper-goat, murici); *C. frutescens* (chilli pepper) and *C. pubescens* (hairy pepper, rocoto), which is a no cultivated species into Brazil (Carvalho, Maciel, Beckman & Poltronieri, 2014).

The basic chromosome number usually found in domesticated species, *C. annuum*, *C. frutescens*, *C. chinense* and *C. baccatum* is x=12 (2n=24) (Moscone *et al.*, 2007), but in some wild species as *C. buforum*, *C. campylopodium* and *C. cornutum*, a number of x=13 (2n=26) was found (Moscone *et al.*, 2007; Pozzobon & Wittmann, 2006; Teodoro, Garcia & Corona, 2007).

Given these concerns, this study aimed to analyze cytogenetically *C. annuum*, *C. frutescens* and *C. chinense* genotypes from Valle del Cauca, Colombia. In addition, chromosome number, meiotic behavior, meiotic index and pollen viability were studied, in order to detect chromosome abnormalities related to the ploidy level and pollen fertility. Since, to amplify cytogenetic information for this group of plants, which have allowed a basis for plant breeding programs.

Materials and methods

We collected flower buds, flowers and fruits of plants of three species of *Capsicum*: *C. annuum* (Cayenne), *C. frutescens* (Tabasco) and *C. chinense* (Habanero) grown at the Experimental Center at the Universidad Nacional de Colombia, campus Palmira - CEUNP, in the municipality of Candelaria, Valle del Cauca, Colombia.

The plant materials collected were stored in plastic bags and transferred to the cytogenetics laboratory of the Universidad Nacional de Colombia, campus Palmira, for the meiosis analysis in flower buds, and mitosis in root meristems. Then, floral buds were fixed in Carnoy I solution (3:1 ethanol:glacial acetic acid), for a maximum time of 3 to 4 hours, transferred to fresh solution and then preserved under refrigeration until the moment of preparation of the slides. To prepare the slides, technique of squash were employed; the anthers were macerated into a drop of Carnoy I solution and stained by acetic carmine 2%.

Chiasmata frequency were analyzed at 10 cells in diakinesis of each species. Spindle patterns were evaluated at meiosis II (metaphase, anaphase and telophase, respectively). The meiotic index (MI), analyzing a minimum of 100 tetrads of microspores. Pollen viability was counted in 400 grains for each genotype.

For the root meristem analysis, the seeds were germinated in Petri dishes, with moistened filter paper, at 25°C. The onset of germination varied from 5 to 10 days to *C. annuum* and *C. frutescens*, and 10 to 20 days to *C. chinense*. Roots were subjected to a pretreatment by using 8-hydroxyquinoline (8-HQ) solution followed by

cooling for 2 hours at 14°C. Thereafter, the roots were washed with distilled water three times for 5 minutes to eliminate 8-HQ. Then, this material was fixed in 3:1 Carnoy I solution and stored under refrigeration for slide mounting. For slides preparation, the rootlets were washed in distilled water and hydrolyzed 1N HCl for 20 minutes at room temperature to the degradation of the cell wall. After hydrolysis, were washed again in distilled water. The rootlets were cut vertically, macerated, stained with orceine 2% and viewed under an optical microscope with objective at 10, 40 and 100x.

To determine the morphological floral structure patterns of the *Capsicum* 10 flowers were preliminarily evaluated, registering the variables as floral radius, length of peduncle, number and color of the petals, and number of stamens, respectively.

The quantitative and morphological traits were studied according to the standard procedure of recording data in chili crop and were analyzed by using the GLM procedure from Statistical Analysis Systems-SAS ®. To identify significant difference among treatments and statistical significance for all comparisons was made at $p<0.05$. Tukey's multiple range test was used to compare the mean values of treatments.

Results

Both mitosis and meiosis analysis for *C. annuum, C. frutescens* and *C. chinense,* confirmed chromosome number 2n=2x=24 (Figures 1 a, b, c), In the genotypes of *C. annuum* and *C. frutescens*, there was a numerical variation of 2n=26 chromosomes (Figure 1d), suggesting that resulting from a process of aneuploidy or an upward disploidy, causing an increasing in chromosome number.

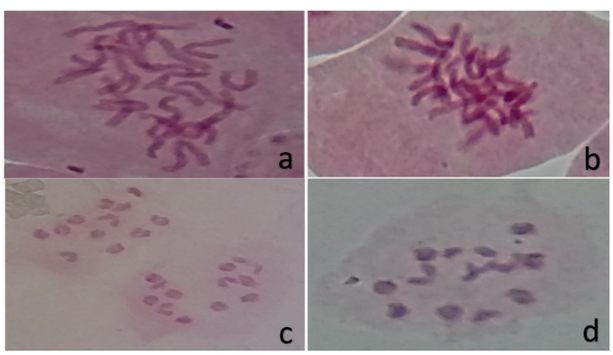

Figure 1. Mitotic cells in prometaphase (a) and metaphase (b) in *C. annuum*, with 2n=24; meiosis (diakinesis), *C. frutescens* with 2n=2x=24 (c) and 2n=2x=26 (d).

Some chromosomal abnormalities as additional nucleoli (Figure 2a-b), micronuclei (Figure 2c) and nuclear reconstitution at telophase II (Figure 2d) possibly leading to triad; irregular segregation in both metaphase and anaphase II (Figures 2e-f), and asyncronic chromosome division (Figure 2i) during meiosis II were observed in *C. annuum* and *C. frutescens*.

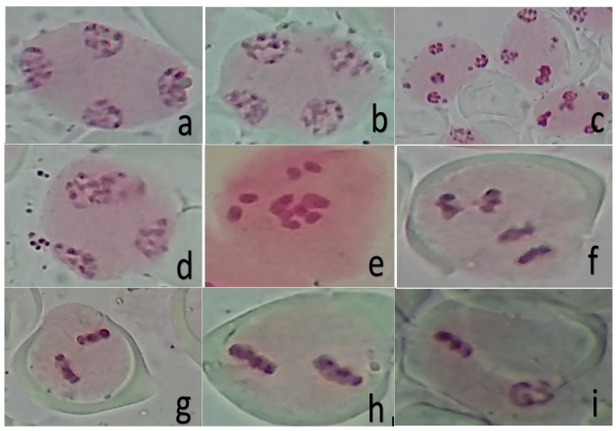

Figure 2. Spindles patterns and chromosomal abnormalities during meiosis II in *C. annuum* (a-e) and *C. frutescens* (**f-i**). **a**) parallel spindles in telophase II (TII), with additional nucleoli; **b**) perpendicular spindles in TII, with additional nucleoli; **c**) TII with micronuclei; **d**) nuclear restitution in TII, which possibly giving rise to an unreduced gamete; **e**) early ascension of chromosomes at metaphase II (MII); **f**) perpendicular spindle at anaphase II, with lagging chromosome; **g**) convergent spindle at MII; **h**) parallel spindle at MII; **i**) asynchronous division (prophase-metaphase) in parallel spindle.

C. frutescens presented polyploid cells with 2n=4x=48 to 2n=6x=72, which could be the result of defective cell wall formation during pre-meiotic mitosis or processes of endomitosis (Figures 3a, 3b).

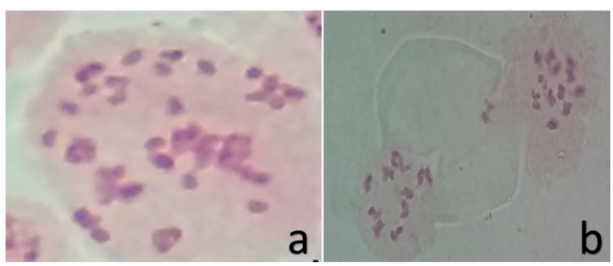

Figure 3. Poliploidy in *C. frutescens;* **a**. 2n=6x=72; **b**. 2n=4x=48

The meiotic index (MI) of the three evaluated species of *Capsicum* can be seen in Table 1 and 2. *C. annuum,* showed 98.7% and *C. frutescens* 100%, respectively.

Table 1. Spindle patterns at meiosis II, tetrads of microspores and meiotic index in *C. annuum.*

Spindle patterns	N°	%	Tetrad patterns	N°	%	MI%
Parallel	98	87.5	Isobilateral	48	61.5	
Linear	-	-	Linear	1	1.28	
Perpendicular	2	1.7	Decussate	2	2.5	
Convergent	12	10.71	Tetrahedral	26	33.3	
-		-	Triads	1	2.5	
TOTAL	112	100	-	78	100	98.7

Table 2. Spindle patterns at meiosis II, tetrads of microspores and meiotic index in *C. frutescens.*

Spindle patterns	N°	%	Tetrad patterns	N°	%	MI%
Parallel	52	52.5	Isobilateral	18	85.7	
Linear	-	-	Linear	-	-	
Perpendicular	1	1.0	Decussate	-	-	
Convergent	46	46.4	Tetrahedral	3	14.2	
-	-	-	Triads	-	-	
Total	99	100	-	21	100	100

It was found a pollen fertility equal to 94.6% in *C. annuum*, while *C. frutescens* showed 19.8% and *C. chinense,* 97.0% (Table 3, Figure 4). The genotypes of *C. annum* and *C. chinense*, because they have a high pollen fertility rate, are recommended as parental in breeding programs. *C. frutescens* genotype can be considered as a male sterile.

Table 3. Pollen fertility of *C. annuum*, *C. frutescens* and *C. chinense*.

Genotype	Pollen fertility			
	Normal		Abnormal	
	N°	%	N°	%
C. annuum	388	94.6	22	5.4
C. chinense	354	97	11	3
C. frutescens	66	19.8	267	80.2

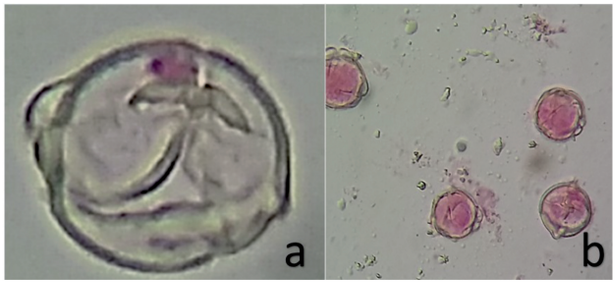

Figure 4. Pollen grains in *C. annuum;* **a**. no viable; **b**. viable

The genotype of *C. frutescens* showed a high degree of no viable pollen, as to be regarded a male-sterile, since pollen is the final product of male meiosis not recommended as a parental. This large number of no viable grains shows a meiotic instability of this species, or a post-meiotic event which leads to partial or complete sterility.

The number of floral structures of the species of *C. annuum* and *C. frutescens* varied between 5 and 7, while in *C. chinense* this variation was higher, 4 to 7 (Tables 4-6; Figure 5).

Table 4. Floral structure in *C. annuum.*

Floral diameter mm	Floral peduncle mm	No. of petals	Color of petals	No. of stamens
56.8	55.7	7	Violet	6
60.0	57.6	7	White	7
59.7	62.0	6	White	6
56.5	56.5	5	White	5
58.0	55.5	7	Violet	7
55.5	53.4	7	White	7
53.5	53.9	6	Violet	6
55.1	54.4	6	White	6
57.5	55.5	5	White	5
55.1	57.8	5	White	5

Table 5. Floral structure in *C. frutescens.*

Floral diameter mm	Floral peduncle mm	No. of petals	Color of petals	No. of stamens
39.4	58.2	7	White	7
54.8	69.2	6	White	5
55.0	64.1	5	White	5
54.8	58.6	7	White	7
55.3	63.2	7	White	6
53.6	61.0	6	White	7
53.8	59.2	6	White	6
58.5	59.8	6	White	6
54.2	63.5	5	White	5
52.8	58.6	5	White	5

Table 6. Floral structure in *C. chinense*.

Floral diameter mm	Floral peduncle mm	No. of petals	Color of petals	No. of stamens
51.9	58.5	7	White	7
58.8	57.4	6	White	5
54.5	57.1	5	White	5
54.9	52.4	4	White	4
52.5	50.9	6	White	6
54.7	57.9	6	White	6
52.8	66.4	6	White	6
52.7	58.4	6	White	6
52.5	56.7	5	White	5
54.1	66.0	7	White	7
52.4	52.5	4	White	4

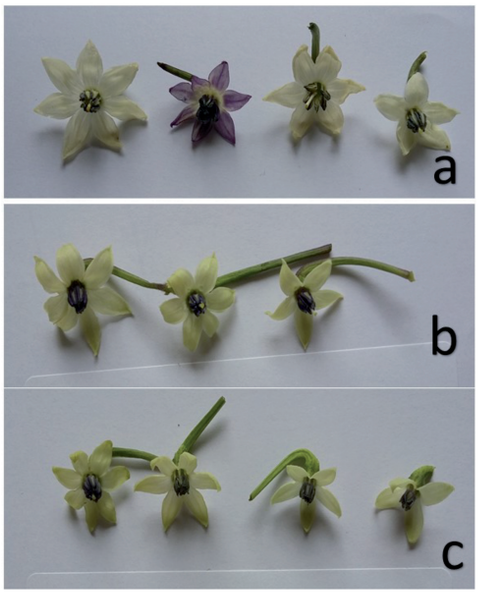

Figure 5. Floral structures in *Capsicum*. **a.** *C. annuum* , numbers ranging from 5 to 7; **b**. *C. frutescens*, numbers between 5 and 7; **c**. *C. chinense* with a greater variation, between 4 and 7.

Discussion

Both mitosis and meiosis analysis for *C. annuum, C. frutescens* and *C. chinense,* confirmed chromosome number 2n=2x=24 (Figures 1 a, b, c), similarly to reported by Shopova (1966); Souza *et al.* (2011); Pozzobon *et al.* (2015).

In addition, Moscone *et al.* (2007), reported the same chromosome number for the three species. Pozzobon & Wittmann (2006), and Teodoro, Garcia & Corona (2007), reported the same chromosome number for some wild species, among them *C. buforum*, *C. campylopodium*, *C. cornutum, C. villosum* var. *villosum*, *C. schottianum* and *C. pereirae*.

Following Moscone *et al.* (2007), the chromosome basic number of *Capsicum* would be x=12, where x=13 would have arisen with the evolution of the genus. According to Pozzobon & Wittmann (2006), the genus has two lines of separate evolution, one of the wild species with x=13 chromosomes, and the ancestral number of domesticated, x=12, emerged after the loss of a pair of chromosomes.

These abnormalities were also reported by Caetano (2003), in maize and Caetano, Sandoval, Posada, Caetano-Nunes & Lagos (2008), in *Vasconcellea*. By other hand, Martins, Pereira, Souza & Costa (2010), reported an early chromosome ascension and laggards, which may be lost during cell division, resulting in aneuploid cells.

Pickersgill (1977), reported a chromosome number of 2n=4x=48 for *C. annuum*, indicating the occurrence of polyploidy, or a natural tetraploid.

This ploidy level phenomenon, could facilitate the emergence of self-fertilization of these plants the self-incompatibility as a result of the same process, affecting pollen fertility and reducing the likelihood of genetic variability (Bateman, 1952).

According to Love (1951), meiotic index larger than 90% indicate elite materials to be used as parental in plant breeding programs. Martins *et al.* (2010), and Pozzobon, Bianchetti, Santos, Carvalho, Reifschneider & Ribeiro (2015), observed pollen viability higher than 90% for the species *C. chinense* and *C. annuum*, *C. frutescens.* Conversely, Pozzobon, Souza, Carvalho & Reifschneider (2011), found a large number of unviable pollen grains, suggesting that due to the high temperature and physical factors, can influence the low viability of this species.

Moscone *et al.* (2007), suggest that changes may occur in chromosome number and morphology of the genus *Capsicum*, making necessary cytogenetic studies for chromosomal determination of this group, which has distinct genetic variation.

Conclusion

The occurrence of differences in ploidy level, including polyploidy of *Capsicum annuum, C. chinense* and *C. frutescens,* is believed to be an outcome of morphological traits changes seen in the flowers, with the number of floral structures.

Acknowledgments

We would like to thank to GIRFIN - Grupo de Investigación en Recursos Fitogenéticos Neotropicales, Universidad Nacional de Colombia Sede Palmira, Colombia.

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