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RESEARCH ARTICLE

Chromosomes behavior in three species of *Capsicum* genus

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Abstract

The genus *Capsicum* consists of 35 species out of which five are widely domesticated and cultivated in the tropical and subtropical regions of the world. The research was undertaken at the Faculty of Life Sciences, Department of Plant Science, Modibbo Adama University, Yola. Pollen were collected by crushing with tweezers to release pollen grains. Harvesting of the pollen was done between hours of 9:30 am and 10:00 am. The excised anthers were immersed in 0.02 % Colchicine for 4 hours. The pre-fixed flower buds were fixed directly into Carnoy's Fluid ("Glacial acetic acid and absolute ethanol" in a 3:1 ratio) solution for 24 hours. The anthers were then washed in distilled water at room temperature, transferred into 1N hydrochloric acid (HC l) at 60º C in a water bath for 5-8 minutes. The hydrolyzed flower buds were rinsed in water and one anthers at a time was squashed in aceto-orcein stain. Photomicro-graphs of suitable cells were taken for illustration. The result revealed that all the three species revealed a diploid chromosome count of n = 12 = 24. Also, it is observed that diplonema, zygotene, leptotene, pachytene and diakinesis at prophase I. Non-oriented chromosomes at metaphase II. One lagging chromosomes as well as chromatid separation at diakinesis and scattered chromosomes at anaphase I were observed in all the three species. Pollen viability in *C. annuum* L, *C. chinenses* Jacqs. *C. frutescens* L. were 71.3 %, 68.4 % and 84.2 % , respectively. The pollen viability was scored according to staining level (pollen with bold red color as viable and colorless as nonviable). Consequently, viable and non-viable pollen grains of variable sizes were ascertained. This study helped in unraveling the different evolutionary trends in the *Capsicum* genus.

Key words: Meiotic, chromosomes, behavior, capsicum, pollen

Introduction

The genus *Capsicum* belong to *Solanaceae* family (Bosland and Votava, 2000). Approximately, the genus Capsicum consists of 35 species out of which five are widely domesticated and cultivated in the tropical and subtropical regions of the world. These are *Capsicum annum* L., *Capsicum chinenses* Jacqs., *Capsicum frutescens* L., *Capsicum* pubescens R. and Capsicum baccatum L. (Garcia *et al.*, 2016).

Capsicum species are immensely valued not only because of their economic importance but also for their rich nutritional value (Xiao-min *et al.,* 2016). Meiosis is the process whereby the genetic content of cells is reduced from the somatic to the gametic content, in order to maintain a fixed volume of genetic material upon fusion of gametes (Barlow and Hult´en, 2015).

Meiosis is one of the most dynamic processes for a plant genome (Prusicki *et al.*, 2019). To achieve a reductional division, the meiotic cell goes through one round of DNA replication followed by two cell divisions (Mercier *et al.*, 2015). Meiosis, a complicated event of high evolutionary fidelity and genetically programmed cellular processes, ensures gametic viability and is regarded as one of the most sensitive stages in the life cycle of seed plants (Tantry *et al.,* 2021).

Cytological studies on the meiosis of Capsicum have been chiefly concerned with the processes of the divisions and the chromosome numbers characteristic of the different species, forms and the behavior of meiotic chromosomes has not been thoroughly studied in the *Capsicum* genus (Olatunji and Afolayan, 2019). These potential genetic resources have not been properly used for Capsicum breeding or evolutionary studies mainly due to the bottleneck of lack of basic information as chromosome numbers and meiotic behavior. Over the years, identification and classification of the cultivated *Capsicum* species are based mainly on morphological, chemical and anatomical descriptors (Hamant *et al.,* 2006) However, this present study is paramount in proffering solutions to the potential genetic resources that have not been properly used for Capsicum breeding or evolutionary studies mainly due to the bottleneck of lack of basic information as chromosome numbers and meiotic behavior. This can be achieved through comparative meiotic chromosomes behavior in the *Capsicum* genus. This study helped in unravelling the different evolutionary trends in the genus.

Materials and methods

The research was undertaken at the Faculty of Life Sciences, Department of Plant Science, Modibbo Adama University, Yola. The study covers chromosomes behavior and pollen viabilty in three Capsicum species viz., *C. annum* L., *C. chinenses* Jacqs. and *C. frutescens* L. The peppers samples were obtained directly from the farmers in Girei Local Government Area. The plant samples were moved down to the Department of Plant Science for identification using the preserved specimen voucher. Three species of pepper C. annum L., *C. chinenses* Jacqs., *C. frutescens* L. were investigated and they were grouped according to Purseglove (1968). The seeds of the plant samples obtained were immersed and washed with tap water to remove the contamination from the outer surface prior to sowing of the seeds. The seeds germinated between 6-9 days after sowing. The plants were raised to maturity in plastic buckets. Pollen grains were prepared according to Falusi (2006). Anthers pollen were collected by crushing with tweezers to release pollen grains. The dark colored pollens were considered viable, whereas the light pollens were estimated to have poor viability. Harvesting of the pollen was done between hours of 9:30 am and 10:00 am. The period in which meiotic spread of the meiocytes of the pollen grains were active. For pre-treatment of pollen grains the technique described by Falusi *et al.,* (2003) anthers from flowers that blossom and healthy were selected and excised. The excised anthers were immersed in 0.02 % Colchicine for 4 hours. The set up was aerated at 30 min intervals using battery operated aerator bubble so as to replenish loss of oxygen.

For fixation of the pollen grains the technique described by Falusi *et al.,* (2005) were used as follows: The pre-fixed flower buds were fixed directly into Carnoy's Fluid (Glacial acetic acid and absolute ethanol in a 3:1 ratio) solution for 24 hours. The roots were then removed and stored in 70 % ethanol until required for cytological analysis. A technique 'Hydrolysis and Squash Method' by Olorode (1973) were used as follows: The anthers were then washed in distilled water at room temperature, transferred into 1N Hydrochloric acid (HCL) at 60º C in a water bath for 5-8 minutes. The Hydrolysed flower buds were rinsed in water and one anthers at a time was squashed in aceto-orcein stain. Photomicro-graphs of suitable cells were taken for illustration. Records of number of cells with interconnections and the number of bivalents involved per cell at diplonema, zygotene, leptotene, pachytene and diakinesis were also kept. Standard deviation, coefficient of variation and mean differences were determined using SPSS version 17.

Results and discussion

Information on meiotic chromosomes behavior and pollen viability are very vital in ascertaining the evolutionary changes. It is useful in identification and establishing relationships among species as well as genetic diversity and encourages research into plant genomes. Meiotic chromosomes of the three species, presented (Fig. 1a and 1b, 2a and 2b) while pollen viability and non-pollen viability (Figure 3). The chromosome numbers and meiotic behavior in three *Capsicum* species presented in fig. 4 and comparative pollen viability estimate (Fig. 5) presented. Description of the pepper (*Capsicum* spp) presented in table 1. The chromosome numbers and meiotic behavior in the *Capsicum* species (Table 2) and pollen viability estimates in three *Capsicum* species presented in Table 3.

Variation in chromosomes number, meiotic chromosomal behavior and percentage pollen viability in *C. annuum* L.

The chromosomes number in all the three species is 2n=24. Based on this finding zygotene, leptotene, diplotene and pachytene were observed in prophase I. Also, the results showed that the chromosomes were paired and the stage was characterized by the pairing of the homologous chromosomes which can be seen as paired chromatin threads (bivalents) at Metaphase I. More so, Anaphase I and Metaphase II were observed and the percentage frequency of it appearance in the meiotic cell was 23.5 %. The pollen viability was scored according to staining level (pollen with bold red color as viable and colorless as nonviable. pollen viability in *C. annuum* L. were 71.3 % viable.

The findings according to Moscone *et al.,* (2007), revealed that 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 and 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 which connotes with the results obtained from this research and was confirmed from the presence of 24 chromosomes at mixed Anaphase I and Metaphase-II. The meiosis analysis for C. annuum, *C. frutescens* and *C. chinense*, confirmed chromosome number 2n = 2x = 24, similarly to reported by María et al. (2018); Pozzobon *et al.,* (2015). In addition, Moscone *et al.,* (2007), reported the same chromosome number for the three species. Pozzobon and Wittmann (2015) and Teodoro *et al.,* (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*.

Variation in chromosomes number, meiotic chromosomal behavior and percentage Pollen viability in *C. chinenses* Jacqs.

The chromosomes number is 2n=24. The following were observed Pachytene which according to this present research results revealed that the chromatins were thick and the chromatin threads get condensed and appear shortened and thick. Pairs of homologous chromosomes can be seen. Each chromosome has two chromatids and thus each bivalent consists of four chromatids. Also, the results based on this present study showed that chiasmata were observed representing the site of exchange of the parts between two homologous chromosomes (i.e. crossing over). Diakinesis showed that there was separation or movement and the homologous pair of chromosomes appeared more shortened, thick and prominent. The pollen viability was scored according to staining level (pollen with bold red color as viable and colorless as nonviable Pollen viability in *C. chinenses* Jacqs. were 68.4 % viable. Knowledge of various genomic variations induced by meiosis provides insights into plant genomic evolution and genetic diversity and encourages research into plant genomes. Prophase I among meiocytes that included leptotene, zygotene, pachytene and diplotene were observed in *C. annum* L., *C. chinenses* Jacqs., *C. frutescens*.

The results showed, that there were leptotene, zygotene and pachytene diplonema at prophase I of meiosis. Diakinesis and chromosomes separation was more evident at metaphase I and Anaphase II in the three species. Also, results at leptotene, diplonema, zygotene and pachytene were more frequent in all the three species of *Capsicum* genus and many interconnections between the heterochromatic regions. This was most intense in C. frutescence and least in C. annuum. There were fewer interconnections in diakinesis than at diplonema. The interconnections were usually terminal and between the three largest bivalents. Laggard and chromosomes disorientation as well as separation were observed at metaphase I. The above results were in line with findings according to (María *et al.,* 2018).

Variation in chromosomes number, meiotic chromosomal behavior and percentage pollen viability in *C. frutescens* L.

zygotene, leptotene, diplotene and pachytene were observed in prophase I. Also, the resultsshowed that the chromosomes were paired and the stage was characterized by the pairing of the homologous chromosomes which can be seen as paired chromatin threads (bivalents) at Metaphase I. At anaphase I, there were lagging chromosomes at 22.4 %. This results into the reduction of number of chromosomes to half. This stage can be identified by the presence of two chromatids in each chromosome. Consequently, viable and non-viable pollen grains of variable sizes were ascertained resulted. The percentage pollen viability was determined as the ratio of the number of viable grains to the total grain number. Pollen viability in *C. frutescens* L. was 84.2 %.

Pollen viability is an important parameter in plant studies, indicating the male reproductive potential of the species, and also contributing in taxonomic, ecological, and palynological, studies, which provide basic practical information for genetic conservation, as well as in agriculture, for the planning of improvement and cultivation (María *et al.*, 2018). The pollen viability was scored according to staining level (pollen with bold red color as viable and colorless as nonviable).

The percentage pollen viability was determined as the ratio of the number of viable grains to the total grain number. Pollen viability in *C. annum* L., *C. chinenses* Jacqs., *C. frutescens* L were 71.3 %, 68.4% and 84.2 %, respectively. Meiosis, a complicated event of high evolutionary fidelity and genetically programmed cellular processes, ensures gametic viability and is regarded as one of the most sensitive stages in the life cycle of seed plants (Teodoro-Pardo *et al.,* 2007); (Hamant *et al.,* 2006); Fuzinatto *et al.,* 2008) and (de Muyt *et al.,* 2009).

Table 1: Description of the pepper plants that were used in this study

Source	Local name	Botanical name	Description
1.Girei LGA, Adamawa State	"Tatase"	*C. annuum* L.	Small annual plant, medium size, bell-shaped fruits with mild taste one pedicel per node.
2. Girei LGA, Adamawa State	"Ata-rugu"	*C. chinenses* Jacqs.	Medium sized annual plant, small oblong and wrinkled fruits with hot taste one pedicel per node.
3. Girei LGA, Adamawa State	"Ata-wewe"	*C. frutescens* L.	Large perennial shrub, small pointed fruit with very hot taste, 2-4 pedicels per node.

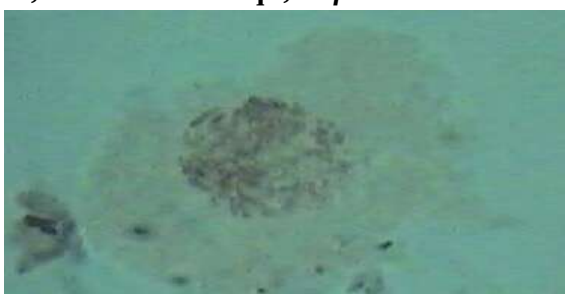
Table 2: The chromosome numbers and meiotic behavior in the capsicum species in Leptotene (Lep), Zygotene (Zyg), Pachytene (Pac), Diplotene (Dip) and Diakinesis (Dia)

Species	2n	Cells no.	Lep (%)	Zyg (%)	Cells no.	Pac (%)	Dip (%)	Dia (%)	Cells no.	Laggard and bridge (%)
***C. annuum* L.**	24	100	16.8	18.4	92	16.2	12.5	11.2	80	23.5
***C. chinenses* Jacqs.**	24	100	25.8	23.2	92	12.8	13.8	27.5	80	13.0
***C. frutescens* L.**	24	100	30.4	21.6	92	18.3	20.9	12.6	80	22.4

Table 3: Pollen viability estimations in three capsicum species

Plant	Pollen number	Pollen viability (%)	Mean pollen size ± SD(µ)	CV (%)
***C. annuum* L.**	100	71.3	26.8 ± 2.51	9.44
***C. chinenses* Jacqs**	75	68.4	26.2 ± 2.51	9.58
***C. frutescens* L.**	96	84.2	29.3 ± 2.40	8.18

Fig 1a: Prophase I showing leptotene chromatin and zygotene chromatin in *C. annum* L., *C. chinenses* Jacqs., *C. frutescens* L



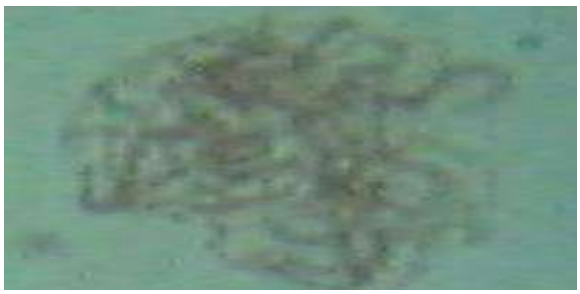


Fig 1b: Prophase I pachytene chromatin and diplotene-Diakinesis in *C. annum* L., *C. chinenses* Jacqs., *C. frutescens* L.

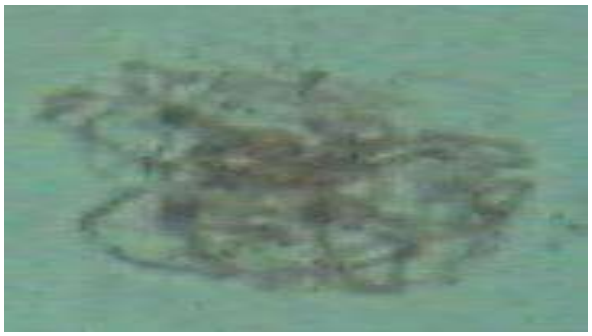



Fig 2a: Anaphase I with chromatid separation and diakinesi and scattered chromosomes in *C. annum* L., *C. chinenses* Jacqs., *C. frutescens* L

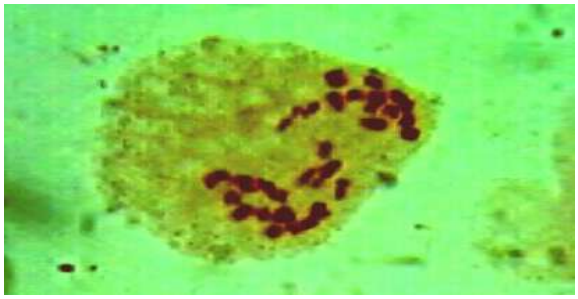



Fig 2b: Metaphase II with non-oriented chromosomes and Anaphase I with one lagging chromosomes in *C. annum* L., *C. chinenses* Jacqs., *C. frutescens* L.

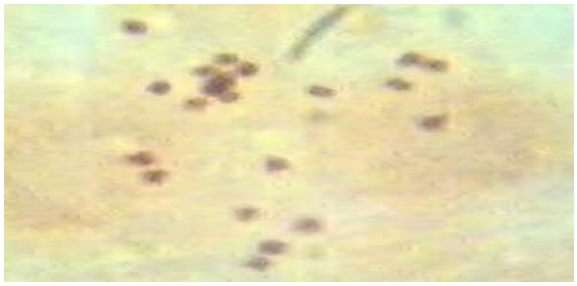
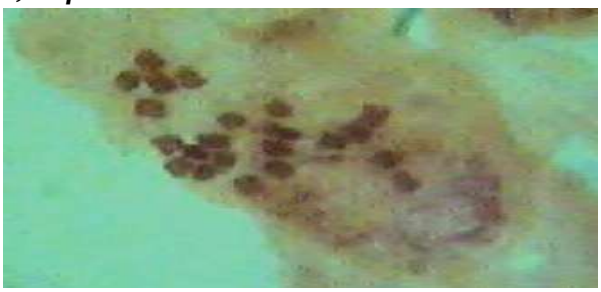



Fig 3: Viable and non-viable pollen mother cell (PMC) meiosis in *C. annum* L., *C. chinenses* Jacqs., *C. frutescens* L

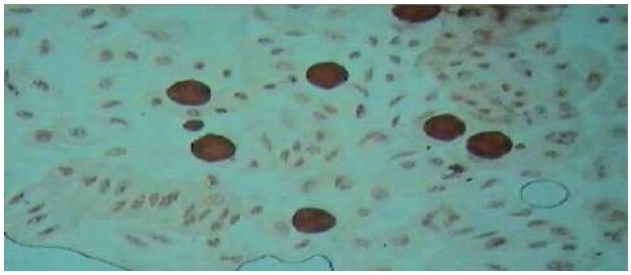


Fig 4: The chromosome numbers and meiotic behavior in three *Capsicum* species

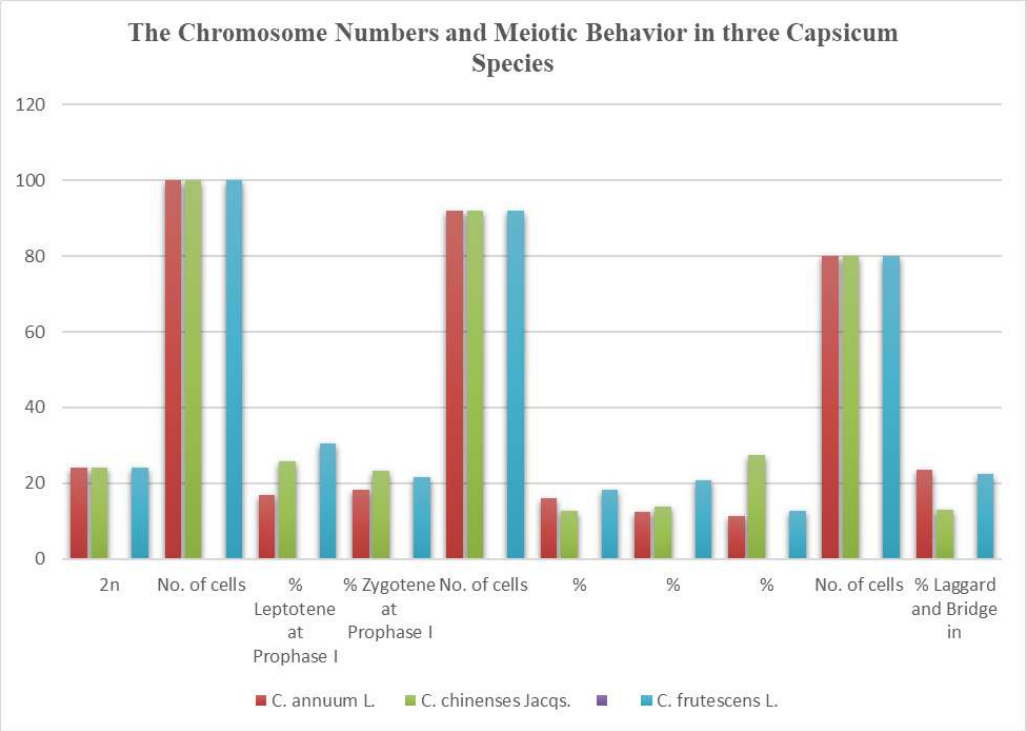
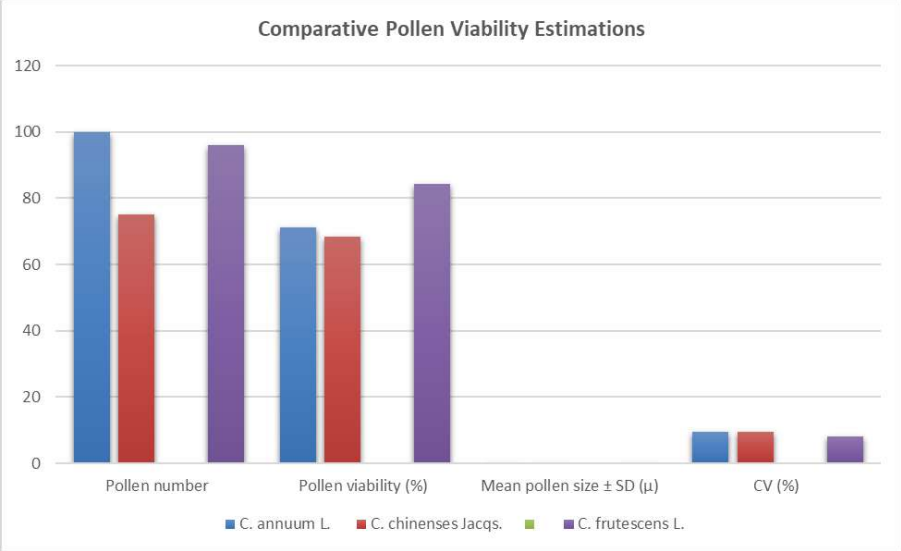


Fig 5: Comparative pollen viability estimations



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