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Research Article

Chromosomes Behaviour In Three Species Of Capsicum Genus

Adamson Daniel [1] and Mathias Fidelis[1]

[1] *Department of Plant Science, Faculty of Life Sciences, Modibbo Adama University, Yola*

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Corresponding author:
Adamson Daniel
adamsondaniel102@gmail.com

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. Anthers 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 60° 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, the result showed that diplonema, zygotene, leptotene, pachytene and diakinesis at Prophase I. Non-oriented chromosomes at Metaphase II were observed. One lagging chromosomes as well as chromatid separation and Diakinesi 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 % viable, 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 unravelling the different evolutionary trends in the genus.

Key Words: meiotic, chromosomes, behavior, capsicum, zygotene, leptotene, genus, species.

1 Introduction

The Solanaceae family includes the genus Capsicum (Bosland and Votava 2000). There are 35 species in the genus Capsicum, five of which are widely domesticated and farmed in tropical and subtropical areas of the world. They include Capsicum baccatum L., Capsicum frutescens L., Capsicum pubescens R., Capsicum annum L., Capsicum chinenses Jacqs., and Capsicum frutescens L. (Garcia *et al.* 2016). In addition to their commercial significance, capsicum species are highly prized for their superior nutritional content (Xiao-min *et al.* 2016). In order to maintain a constant amount of genetic material upon the fusing of gametes, cells undergo a process called meiosis in which the somatic to the gametic content of the cell's genetic makeup is lowered (Barlow and Hult´en 2015). One of a plant genome's most active processes is meiosis (Prusicki *et al.* 2019). The meiotic cell undergoes two cell divisions after one round of DNA replication in order to produce a reductional division (Mercier *et al.* 2015). Meiosis is one of the most delicate phases in the life cycle of seed plants because it maintains gametic viability and is a complex process with great evolutionary fidelity and genetically encoded cellular activities (Tantry *et al.* 2021). The behaviour of meiotic chromosomes in the Capsicum genus has not been extensively explored through cytological investigations, which have focused mostly on the processes of divisions and chromosome numbers distinctive of the various species (Olatunji and Afolayan 2019). The main reason that these prospective genetic resources have not been successfully utilized for Capsicum breeding or evolutionary studies is a lack of fundamental knowledge regarding chromosome counts and meiotic behaviour. Over time, morphological, chemical, and anatomical descriptors have largely served as the foundation for identification and taxonomy of the cultivated Capsicum species (Hamant and Cande 2006). However, the importance of the current study lies in its ability to offer solutions for the potential genetic resources that have not been effectively used for Capsicum breeding or evolutionary studies, primarily as a result of a lack of fundamental knowledge regarding chromosome counts and meiotic behaviour. Comparative meiotic chromosomal behaviour in the Capsicum genus can be used to achieve this. The many evolutionary patterns in the genus were better understood thanks to this study.

2 Materials and Methods

Experimental Site

The study was conducted at Modibbo Adama University at Yola's College of Life Sciences and Department of Plant Science. The three Capsicum species, C. annum L., C. chinenses Jacqs., and C. frutescens L., are the subjects of the study, which examines chromosomal behaviour and pollen viability.

Collection of Plant Samples

The growers in Girei Local Government Area provided the pepper samples directly to us. The plant samples were transported to the Department of Plant Science, where the conserved specimen voucher will be used for identification. Three pepper species—C. annum L., C. chinenses Jacqs., and C. frutescens L.—were examined and categorized in accordance with (Purseglove 1968).

Germination of Plant Samples

Before to spreading the seeds, the plant sample seeds were submerged and rinsed with tap water to remove contamination from the exterior surface. Six to nine days after being sown, the seeds began to grow. The plants were grown to maturity in buckets made of plastic.

Preparation of Pollen Grains

According to (Falusi 2006), the following protocol was followed: Using tweezers, pollen grains from crushed anthers were extracted. The vitality of the light coloured pollens was evaluated to be low, whereas that of the dark coloured pollens was considered good. The pollen was collected between 9:30 and 10:00 in the morning. the time frame during which the pollen grains' meiocytes were actively spreading during meiosis.

Pre-treatment of Pollen Grains

Using the method outlined by (Falusi 2003), anthers from blooming, healthy flowers were chosen and removed. The removed anthers spent 4 hours submerged in 0.02% Colchicine. To replace lost oxygen, the setup was aerated using battery-operated aerator bubbles every 30 minutes. **Fixation of the Pollen Grains**

Here are some examples of how (Falusi, Salako, and Falusi 2005).'s approach was applied: The pre-fixed flower buds were kept fixed for 24 hours by being placed directly in a solution of Carnoy's Fluid (a 3:1 mixture of glacial acetic acid and absolute ethanol). The roots were then cut off and kept in 70% ethanol until cytological examination was needed

Hydrolysis and Squash Method

This is how Olorode's method (Olorode 1973) was applied: The anthers were then rinsed in distilled water at room temperature before being immersed in 1N HCL at 60° C for 5-8 minutes in a water bath. The hydrolyzed flower buds were rinsed in water before being squished in aceto-orcein stain, one anther at a time. For illustration, photomicrographs of appropriate cells were acquired. At diplonema, zygotene, leptotene, pachytene, and diakinesis, records of the number of cells with interconnections and the number of bivalents involved per cell were also kept.

Statistical Analysis

With SPSS version 17, it was possible to calculate the standard deviation, coefficient of variation, and mean differences.

3 Results

Fig1, Fig2 illustrate the meiotic chromosomes of the three species, along with pollen viability and non-pollen viability Fig3. Chromosome composition and meiotic behaviour in three species of capsicum Fig4. Comparative estimation of pollen vitality Fig5. (Capsicum spp.) pepper description Table 1, The Capsicum Species' chromosomal counts and meiotic activity, as well as three Capsicum Species' pollen viability estimations, are shown in Table 2. Table 3.

Chromosomes number, Meiotic Chromosomal Behavior and Percentage Pollen viability in C. annuum L.

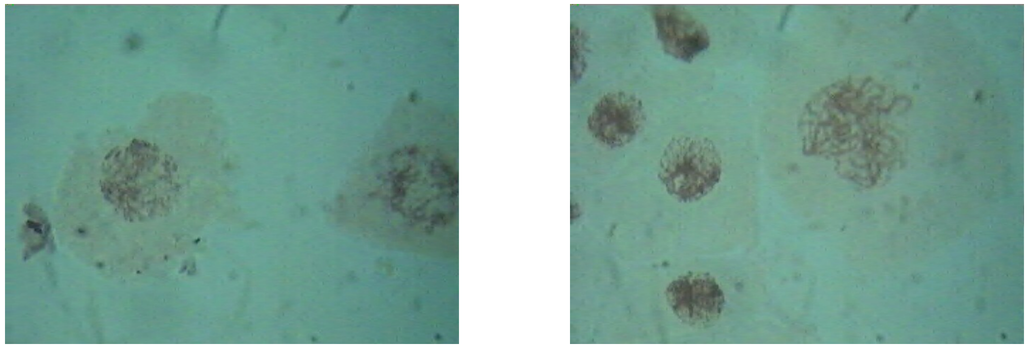
The total number of chromosomes in the three species is 2n=24. On the basis of this discovery, prophase I revealed the presence of zygotene, leptotene, diplotene, and pachytene. The outcomes also demonstrated that the chromosomes were paired, and the stage was distinguished by the pairing of homologous chromosomes, which can be seen at Metaphase I as paired chromatin threads (bivalents). More specifically, Anaphase I and Metaphase II were seen, with a percentage frequency of 23.5% in the meiotic cell. Pollen with a strong red tint was considered viable, and pollen that was colourless was considered nonviable. 71.3% of the pollen in C. annuum L. was viable.

Chromosomes number, Meiotic Chromosomal Behavior and Percentage Pollen viability in C. chinenses Jacqs.

The number of chromosomes is 2n=24. The following things were noticed According to the findings of the current study, pachytene caused the chromatins to become thick and the chromatin threads to shorten and seem thick. It is possible to see pairs of homologous chromosomes. Since there are two chromatids on each chromosome, a bivalent contains four chromatids. Also, the findings of this investigation revealed that chiasmata, which signify the site of the exchange of components between two homologous chromosomes, were seen (i.e. crossing over). The homologous pair of chromosomes seemed more shorter, thick, and conspicuous during diakinesis, indicating separation or movement. Pollen with a strong red colour was considered viable, and pollen that was colourless was considered nonviable, according to staining levels. 68.4% of the pollen of C. chinenses Jacqs. was viable.

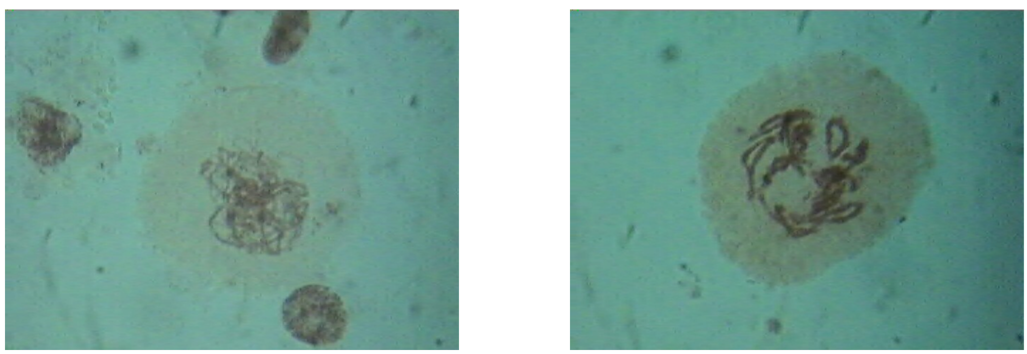
Chromosomes number, Meiotic Chromosomal Behavior and Percentage Pollen viability in C. frutescens L

In prophase I, zygotene, leptotene, diplotene, and pachytene were seen. The outcomes also demonstrated that the chromosomes were paired, and the stage was distinguished by the pairing of homologous chromosomes, which can be seen at Metaphase I as paired chromatin threads (bivalents). There were 22.4% lagging chromosomes at anaphase I. This causes the number of chromosomes to be cut in half. The presence of two chromatids on each chromosome indicates this stage. As a result, variously sized viable and non-viable pollen grains were identified. The ratio of viable grains to total grains was used to calculate the %pollen viability. In C. frutescens L., pollen viability was 84.2%.



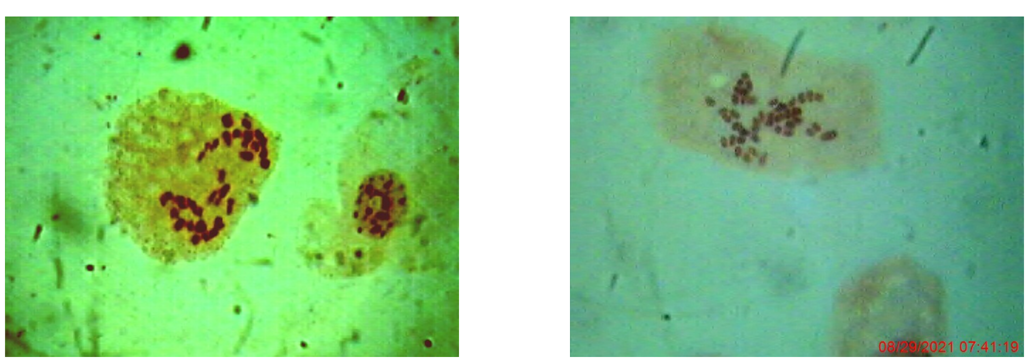
(a) (b)

Figure 1. Prophase I, showing Leptotene chromatin and Zygotene chromatin in C. annum L., C. chinenses Jacqs., C. frutescens L



(a) (b)

Figure 2. Prophase I, Pachytene chromatin and Diplotene-Diakinesis in C. annum L., C. chinenses Jacqs., C. frutescens L.



(a) (b)

Figure 3. Anaphase I with chromatid separation and Diakinesi and scattered chromosomes in C. annum L., C. chinenses Jacqs., C. frutescens L

Table 1. Description of the pepper (Capsicum spp) plants that were used in this study.

Serial number	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.

Species	2n	No. of cells	%Leptot	% Zygotene	No. of cells	%Pachy	%Diplot	%Diakin	No. of cells	%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

4 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.

Chromosomes Number

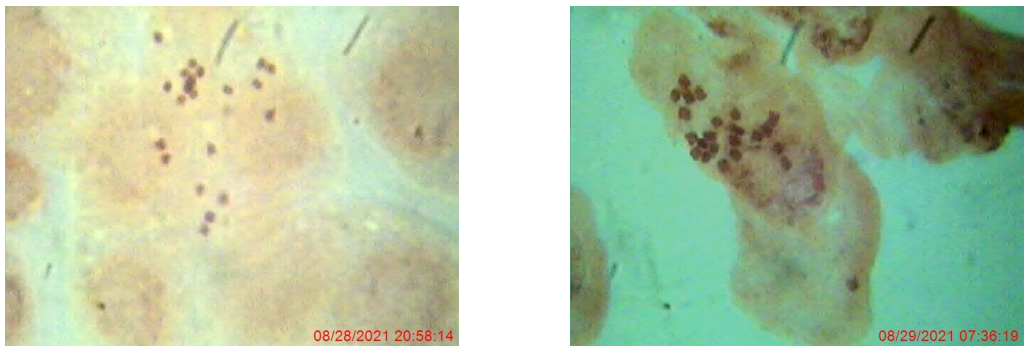
According to (Moscone *et al.* 2007), Capsicum's chromosome basic number would be x = 12, with x = 13 having developed during the evolution of the genus. (Pozzobon, Schifino-Wittmann, and Bianchetti 2006) state that the genus has two lines of independent evolution, with the ancestral number of domesticated species, x = 12, emerging after the loss of a pair of chromosomes, which is consistent with the findings of this study and was confirmed by the presence of 24 chromosomes at mixed Anaphase I and Metaphase-II. The wild species has x=13 chromosomes. According to (María *et al.* 2018; Teodoro-Pardo, García-Velázquez, and Corona-Torres 2007), the meiosis analysis for C. annuum, C. frutescens, and C. chinense verified that chromosome number 2n = 2x = 24. (2015). (Moscone *et al.* 2007) revealed that all three species shared the same chromosomal number. The same chromosomal number was recorded for some wild species by (Pozzobon, Schifino-Wittmann, and Bianchetti 2006; Teodoro-Pardo, García-Velázquez, and Corona-Torres 2007), including C. buforum, C. campylopodium, C. cornutum, C. villosum var. villosum, C. schottianum, and C. pereirae.

Meiotic Chromosomal Behavior during the various Stages of Cell Division

The understanding of numerous genomic differences brought forth by meiosis promotes research into plant genomes and sheds light on the genomic evolution and genetic diversity of plants. Leptotene, Zygotene, pachytene, and diplotene were found in prophase I meiocytes in C. annum L.,

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



(a) (b)

Figure 4. Metaphase II with non-oriented chromosomes and Anaphase I with one lagging chromosomes in C. annum L., C. chinenses Jacqs., C. frutescens L.

C. chinenses Jacqs., and C. frutescens L. The findings indicated that leptotene, zygotene, and pachytene diplonema were present at prophase I of meiosis. At metaphase I and anaphase II in the three species, diakinesis and chromosomal separation were more obvious. Moreover, all three species of the Capsicum genus had higher findings at leptotene, diplonema, zygotene, and pachytene, and there were several links among the heterochromatic zones. The level of intensity was highest in C. frutescence and lowest in C. annuum. In comparison to diplonema, diakinesis has fewer connections. Usually amongst the three greatest bivalents, the linkages were terminal. At metaphase I, chromosomes showed laggardness, disorientation, and separation. The aforementioned outcomes were consistent with what was discovered in (Teodoro-Pardo, García-Velázquez, and Corona-Torres 2007).

Viable and non-viable pollen mother cell (PMC) meiosis

The male reproductive potential of a species is indicated by pollen viability, which is also an important parameter in taxonomic, ecological, and palynological studies that provide fundamentally useful information for genetic conservation and in agriculture for the planning of improvement and cultivation (Teodoro-Pardo, García-Velázquez, and Corona-Torres 2007). The level of staining was used to rate the pollen viability (pollen with bold red colour as viable and colourless as nonviable). The ratio of viable grains to total grains was used to calculate the % pollen viability. C. annum L., C. chinenses Jacqs., and C. frutescens L. had pollen viability of 71.3%, 68.4%, and 84.2%, respectively. Meiosis is one of the most delicate phases in the life cycle of seed plants because it maintains gametic viability and is a complex event with high evolutionary fidelity and genetically programmed cellular mechanisms (Teodoro-Pardo, García-Velázquez, and Corona-Torres 2007; Hamant and Cande 2006; Fuzinatto, Pagliarini, and Valle 2008; Muyt, Mercier, and Mezard 2009).

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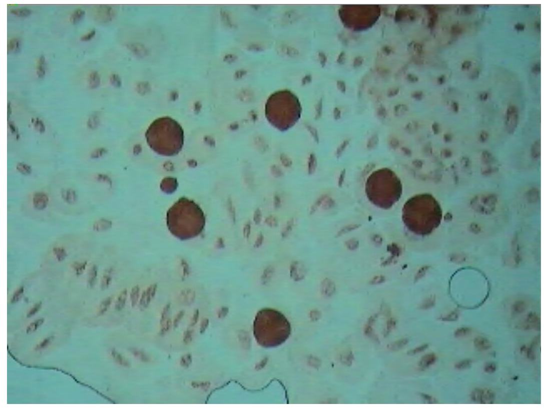

Figure 5. Viable and non-viable pollen mother cell (PMC) meiosis in C. annum L., C. chinenses Jacqs., C. frutescens L.

Conflict of Interest: There is no conflict of interest.

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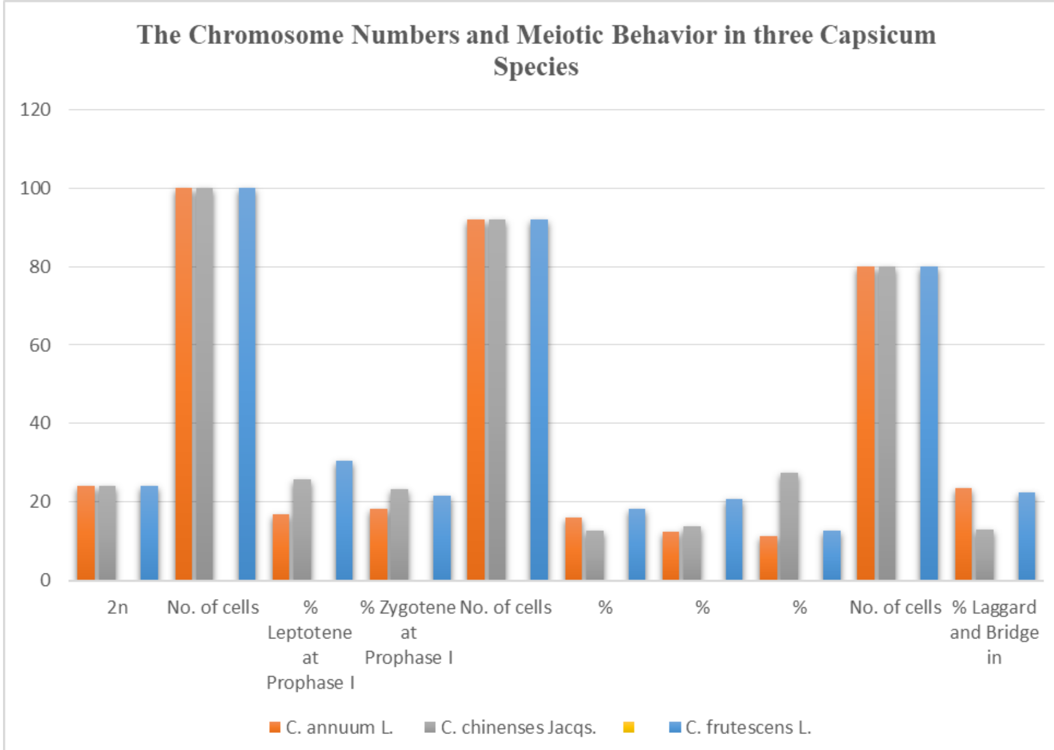

Figure 6. The chromosome numbers and meiotic behavior in three Capsicum Species.

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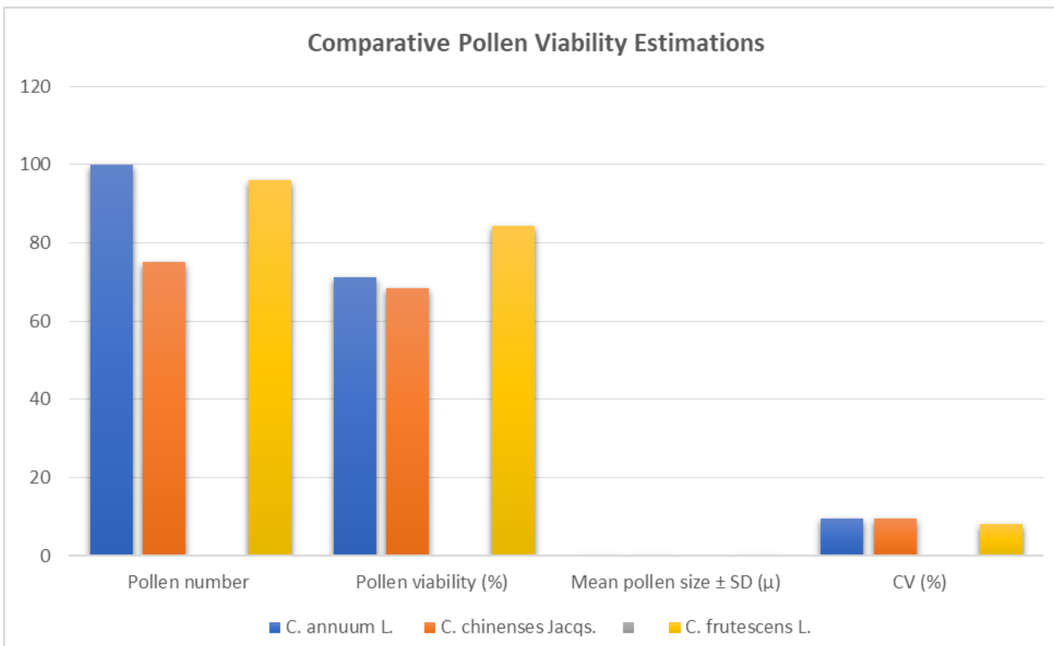

Figure 7. Comparative Pollen Viability Estimations.