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Genetic Diversity of Some*Citrus* (L) Genotypes As Revealed By Meiosis

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Abstract

The Delta region especially in Qalubia governorate of Egypt is reported to be the new origin place and rich in diversity of *Citrus* (L.) species, where some species of *Citrus appeared* in their natural habitat. To have comprehensive information about the extent of genetic variability and the occurrence of cryptic genomic hybridity between and within various *Citrus* species, a combined approach involving cytogenetical approaches was adopted in the present study. Cytogenetic approaches are applied to five parents and their four crosses. Male meiotic studies showed a gametic chromosome number of n = 9, without any evidence of numerical variations. Bivalents outnumbered all other types of associations in pollen mother cells (PMCs) analyzed at diplotene, diakinesis and metaphase I. Univalents were frequently encountered in nine genotypes presently studied, though their presence appropriately did not influence the distributional pattern of the chromosomes at anaphases I and II.

Keywords: *Citrus* species; Cytogenetic approaches; pollen mother cells (PMCs); genotypes.

Introduction

Commercially, the genus Citrus L., is considered the main source of the Citrus fruits, belongs to the orange subfamily Aurantioideae of the family Rutaceae and is cultivated in tropical and subtropical areas of the world (**Webber and Batchelor (1948)**. The genus Citrus includes the most commercially important fruits like mandarin (*Citrus reticulata Blanco*), sweet orange (*Citrus sinensis* (L.) Osbeck), grapefruit (*Citrus paradisi* Macf.) and lemon (*Citrus limon* (L.) Burm. f.). Egypt begin to set a remarkable area in the "Citrus belt of the world" due to her rich wealth of Citrus genetic resources, cultivated (**Malik *et al*., 2006; Nair and Nayar, 1997**). The delta region of Egypt is the best region of different Citrus species especially El-Qanater El-Khairia and Moshtohor. Natural populations of Citrus gene pool detected due to natural pollination which ensures the assumption that this area might be the center of origin of several Citrus species in Egypt. Many Citrus species, their cultivars and artificial hybrids are found to have originated in the Qalubia governorate (**Bhattacharya and Dutta, 1956 and Amar, 2019**). Citrus is the most important fruit crop of Egypt for exportation and the cultivated area is 456082 feddan (**Ministry of Agriculture Statics, 2019**). Annual production of Citrus species especially sweet orange 306665 Tons of fresh fruits.

Meiotic divisions in Citrus species and its inter-specific and inter-generic hybrids are interesting. However, most of the meiotic behavior in Citrus is regular means irregularities are rare (**Iwamasa 1966 and Agarwal 1989)**. Observing and analysis of meiotic chromosome pairing in hybrids is a classical and authentic approach to knowing the species relationships, besides understanding the genetic stability of polyploids (**Yant *et al*., 2013**). Four cultivated Citrus species have been hybridized with Clementine to introduce desirable traits, mainly resistance to pests and pathogens (**Barrett, 1977 and Motomura *et al*., 1995**). The main goal of this study is the identification of chromosomes of different genomes which could be a simple method of identifying Citrus hybrids and resolving the cryptic hybridity of the Citrus species under study in addition to its importance for future work (**Cameron and Frost, 1968**).

Materials and Methods

Plant materials

Five parents and their four crosses were collected in the form of flower buds. The samples used in the present study are authenticated and are being maintained at the Cytogenetic lab, At Moshtohor scientific station, the trees were marked and appropriately labeled before flowers were collected from them, which formed the basic material for detailed male meiotic studies.

Meiotic studies

Flower buds of appropriate size (0.5–1 cm in diameter) were harvested from mature trees of Citrus species and their hybrids and fixed with freshly prepared 1:3 glacial acetic acid: 95% ethanol mixture for a minimum of 24 h at room temperature and later stored in 70% ethanol at the refrigerator. Anthers were squashed in 1% acetocarmine solution

with ferric chloride solution as mordant. 25–30 PMCs were analyzed at diplotene/diakinesis/metaphase I beside Anaphase I, II and Telophase I, II to estimate the range of chromosome associations and recombination frequencies through detecting chiasma formation. On average The analysis of anaphase I/II to study the distributional pattern of chromosomes and chromatids. Photomicrographs of cytological preparations were taken from temporary slides with a CCD camera (Germany) attached to Labomed LX 400 brightfield microscope.

Table 1. Plant material used in this study as common and cultivar name.

Species name	Common or cultivar name
Citrus limon (L.) Burmf.	Lemon
Citrus reticulata Blanco	Cleopatra mandarin
Citrus paradisi Macf.	Grapefruit
Citrus sinensis (L.) Osbeck	Succari
Citrus clementina L.	Clementine
Citrus species	Lemon x Clementine
Citrus species	Cleopatra mandarin x Clementine
Citrus species	Grapefruit X Clementine
Citrus species	Succari X Clementine

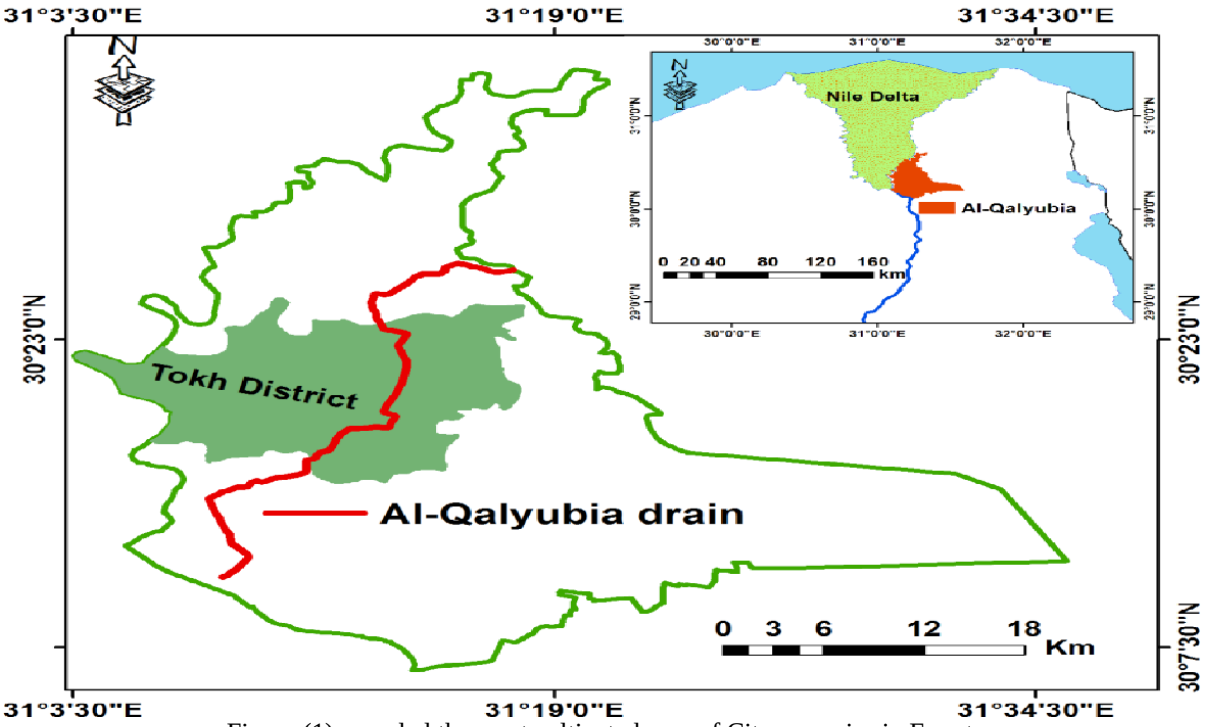

Figure (1) revealed the most cultivated area of Citrus species in Egypt.

Results and Discussion

Meiotic studies

The meiotic divisions were studied in the five cultivated Citrus species and resulted from four F_1 populations. The details regarding the nine Citrus species analyzed in the present investigation, the total number of PMCs analyzed and their association at prophase I and metaphase 1 are summarized in Table 2. The distribution pattern of the chromosome at anaphase I have been detailed in Table 3. Most of these observations are illustrated in Fig. 1. From the data summarized in Table 1, it is clear that all the species presently investigated were characterized by revealing nine bivalents at prophase 1 especially diplotene and metaphase I in the majority of the examined pollen mother cells. The present study carried out on 9 species of Citrus (5 parents and 4 crosses) showed a gametic number of n = 9, without any variation (Fig. 1). The meiotic chromosome behavior in the nine Citrus species studied was normal where bivalent associations outnumbered other types of associations in pollen mother cells (PMCs) studied at diplotene and metaphase I. The mean value for ring bivalents ranged between 1 to 2 (C. limon, C. paradisi, C. sinensis) and 1 to 3 (Citrus reticulata and C clementina) while that of rod bivalents ranged between 1 to 4 (C. limon, C paradisi

and C. sinensis) and 1 to 5 (C. reticulata and C clementina). It was found also meiotic irregularities like univalents, quadrivalents, laggards, bridges and chromosome stickiness, though at low frequency in the PMC. Univalents were frequently encountered in most of the species namely Citrus limon, C. reticulata, C. sinensis and Citrus clementina where a maximum of 3 univalents per PMC was found in C. paradisi. C. reticulata was unique in having one univalent. 1–3 bivalents were observed to be associated with the nucleolus per PMC. Ring bivalents per PMC ranged from 7 to 9 while 1–7 rod bivalents were also recorded per PMC.

Table 2. The chromosomal behavior during prophase I in the five parents and their four F_1 pollen mother cells.

Genotypes	No. of Cells	Bivalents range	Univalent range	Ring chr range	Rods chr
Citrus *limon*	113	8-9	1-2	1-2	1-4
Citrus *reticulata*	96	8-9	1-2	1-3	1-5
Citrus *paradisi*	143	8-9	1-3	1-2	1-4
Citrus *sinensis*	109	8-9	1-2	1-2	1-4
Citrus *clementina*	132	8-9	1-2	1-3	1-5
F1 (1 x 5)	87	8-9	1-2	1-5	1-8
F1 (2 x 5)	92	8-9	1-2	1-3	1-7
F1 (3 x 5)	69	7-9	1-4	1-3	1-6
F1 (4 x 5)	90	8-9	1-2	1-4	1-6

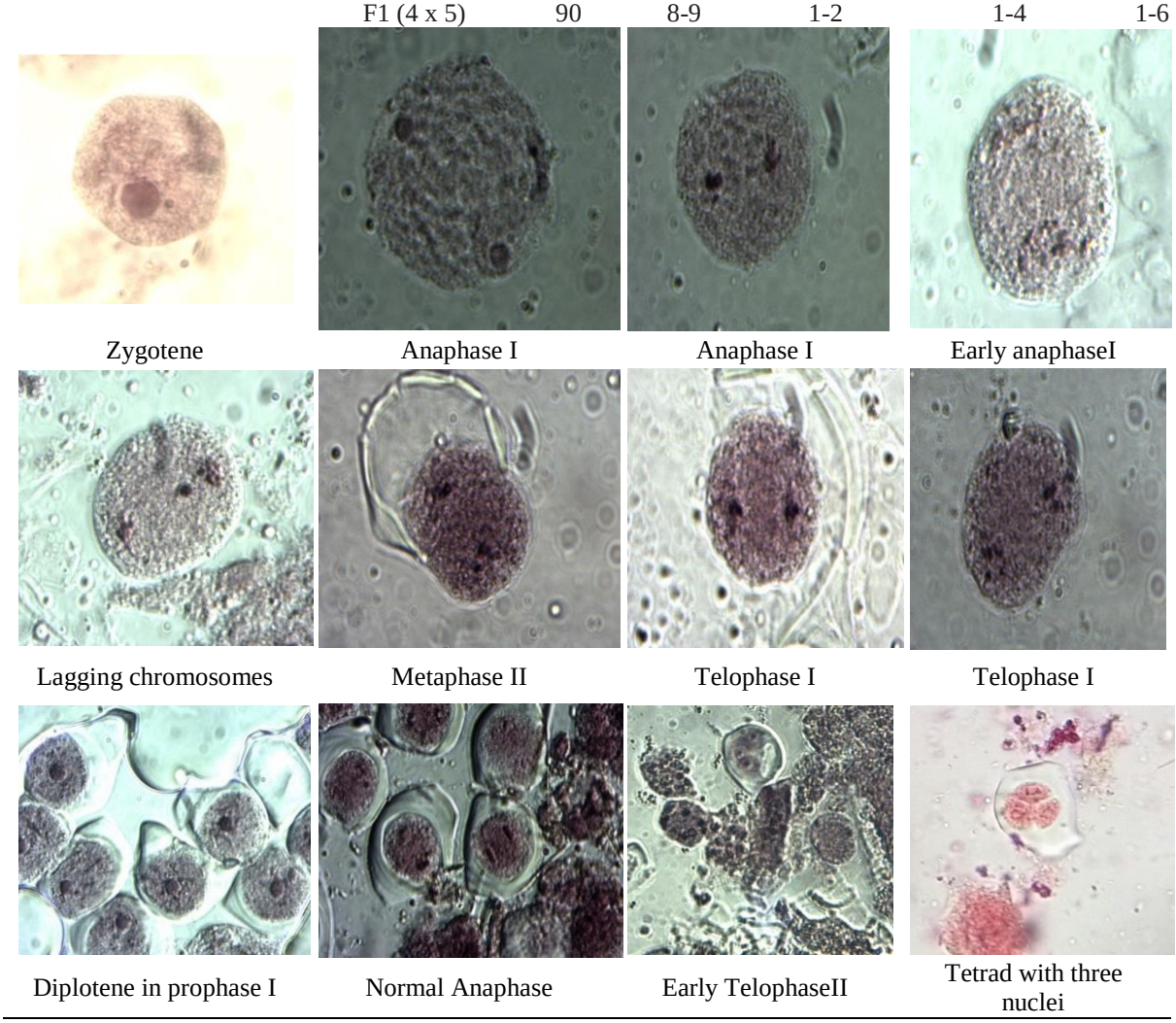

Fig. 2. Different male meiotic stages in the nine Citrus species. 1–3: diplotene, diakinesis & metaphase I in C. limetta; 4–5: diakinesis & metaphase I in C. medica; 6–8: diplotene, diakinesis & metaphase I in C. latipes; 9–10: dilpotene & diakinesis in C. limon; 11–12: diplotene & metaphase I in C. sinensis; 13–14: diplotene & metaphase I in C. reticulata; 15–16: diplotene & diakinesis in C. macroptera.

Secondary associations of the chromosomes were also observed in 1–2 species. Pollen stainability in the ten species studied ranged from 44.4 to 90%. *C. medica*, *C. jambhiri* and *C. limon* exhibited a low percentage of pollen stainability with 44.4%, 59.4% and 63% respectively

Discussion

This study was an attempt to differentiate between the Citrus parents and their hybrids through analysis of chromosomal associations and their behavior during meiosis. Interestingly it was found, by and large, normal for all the Citrus species investigated where bivalent associations outnumbered other types of associations in pollen mother cells (PMCs) studied at diplotene, diakinesis and metaphase I, the presence of regular bivalent formation in these species indicates that the genome of the species is homologous and does not have large structural differences. Univalents were frequently encountered in most of the species studied. **Raghuvanshi (1962)** detect univalents in 17 out of 25 Citrus species analyzed. The presence of univalents in some of the species studied indicates a certain degree of structural heterogeneity in the genetic makeup of the bivalents. C. lemon, C. reticulata and C. sinensis which recorded the highest number of univalents per PMC confirm the heterogeneity within their genomes, which could be intermediate. Early separation of synapsed homologs is generally the reason for the regular occurrence of univalents in many of the tree species (Kumar et al., 2002; Singh, 1993). Univalents may cause unequal distribution at anaphase and consequently a decrease in fertility (Khazanehdari and Jones, 1997). The distribution of chromosomes at anaphases I and II in all the species studied was normal indicating that the pollen sterility in these species is genic controlled (**Agarwal, 1987**).

The association of some bivalents (**Abkenar *et al*., 2004; Agarwal, 1984, 1987, 1989**) with nucleolus in the majority of the PMCs analyzed at diplotene/ diakinesis might be indicative of the nucleolar nature of representative chromosomes. Further, the maximum grouping of these bivalents in groups of three indicates the basic chromosome number for Citrus as three, as has been reported earlier by **Banerjee (1954)** and **Agarwal (1984)**. Meiotic behavior of somatic hybrids provides valuable information for their practical utilization in Citrus breeding programs (**Khan, 2007**). Meiotic abnormalities such as chromosome bridges and chromosomes orientated away from the equatorial plate are frequently observed in hybrids resulting in different sizes of the pollen grain and generally abnormal tetrad formation and irregular chromosome behavior with univalents or multivalent pairing which occur in somatic hybrid plants (**Chen *et al*., 2004**). The relationship between the genomes of the parental species has a great influence on the determination of the process of chromosome pairing and recombination and thus the extent of meiotic irregularities and viability of the gametes (**De Jong *et al*., 1993**).

The presence of the maximum number of ring bivalents indicates the homology and stability of their genome. Univalents were recorded in all parents but are low in numbers and there was a total absence of univalents in C. reticulata, all these findings support the position of the three basic species.

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التنوع الجيني لبعض الطرز الوراثية للموالح وتوضيحها من خلال الانقسام الاختزالي

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تعتبر منطقة الدلتا وخاصة في محافظة القليوبية في مصر هي المنشأ الرئيسي للموالح حيث أنها غنية بألانواع العديدة من الحمضيات، حيث ظهرت بعض أنواع الحمضيات في بيئتها الطبيعية. ومن أجل الحصول على معلومات شاملة حول مدى التباين الجيني وحدوث التهجين الجيني بين أنواع الحمضيات المختلفة، تم اعتماد نهج مشترك يتضمن مناهج الوراثة الخلوية في هذه الدراسة. حيث تم تطبيق مناهج الوراثة الخلوية على خمسة آباء وأربعة هجن.، أظهرت الدراسات الانتصافية الذكرية أن عدد الكروموسومات المشيجية (n = 9)، دون أي دليل على وجود اختلافات عددية. في حين فاق عدد العناصر الثنائية التكافؤ جميع الأنواع الأخرى من الارتباطات في الخلايا الأم لحبوب اللقاح (PMCs) التي تم تحليلها في مرحلة الدبلوتين، والتشكيل، والمرحلة الأولى. تمت مصادفة الأنواع أحادية التكافؤ بشكل متكرر في تسعة أنماط وراثية والتي تمت دراستها حاليًا، على الرغم من أن وجودها بشكل مناسب لم يؤثر على النمط التوزيعي للكروموسومات في الطور الأول و والثاني.