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STUDIES OF SOME CYTOLOGICAL FEATURES ON TWO MORINGA SPECIES (*M. OLEIFERA* AND *M. STENOPETALA*) CULTIVATED IN EGYPT.

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ABSTRACT

Moringa oleifera and *Moringa stenopetala* are belonging to a monogeneric family of shrubs and trees and cultivated in Egypt for different benefits especially those of medicinal. The present work aimed at study some cytological features in plants of these two species of Moringa. For instance, cell shape and size, mitotic chromosomes and nucleolus appearance. Active meristematic cells could be categorized into three types. Type I is including dividing cells, type II meristematic cells with diffused chromatin and those of type III that are representing cylinderic cells with diffused chromatin. Interestingly, the percentages of dividing cells may indicate to the mitotic index (MI). Hence, the MI is considerably high at cells of *M. oleifera* than that of *M. stenopetala.* The scored diploid number (2N) of many metaphase plates of the plants of the two studied Moringa species is mostly = 28. Appearance of large nucleolus at metaphase and even at anaphase or telophase occurred exclusively at cells of *Moringa stenopetala*. In contrast, there was no appearance of nucleolus body mitotic cells of *Moringa oleifera* except those of interphase and some prophases.

Key words: Moringa species, cytological studies, chromosome number

INTRODUCTION

Moringaceae are old-world perennial soft-wood trees that are distributed in tropical regions of the world. These trees indigenous to the western and sub-Himalayan tracts, including India, Pakistan, Asia Minor, Africa, and Arabia (Somali *et al.*, 1984), but have now spread to other regions of the world, including the Philippines, Cambodia, Central America, North and South America, and the Caribbean Islands (Anwar *et al.*, 2007). A total of thirteen tropical and subtropical species of the Moringa genus are known, and of these, many are in danger of extinction, including *M. arborea*, *M. borziana*, *M. longituba*, *M. rivae*, *M. ruspoliana*, and *M. stenopetala* (Stephenson and Fahey, 2004). Mendieta-Araica *et al.* (2012) reported that *Moringa* contains large amounts of crude protein, iron, zinc, and high concentrations of vitamins A, B, and C in its foliage sample which makes it a very good feed and fodder for animals.

M. oleifera is belonging to a monogeneric family of shrubs and trees, the Moringaceae (Ramachandran *et al.*, 1980 and Muluvi *et al.*, 1999). Other species of genus Moringa such as *M. stenopetala* is an important crop in Kenya and Ethiopia (Verdcourt, 1985) and commonly called the cabbage-tree (along with a number of other species), is a tree in the Moringa genus of flowering plants, It is a multipurpose tree producing edible leaves, seeds used for the purification of water, and traditional medicinal products (Leone *et al.*, 2015).

Cytological studies revealed that plants of *Moringa oleifera* has 2c genome size of 1.2 pg (Ohri and Kumar, 1986). Diploid number (2N) of *M. oleifera* is 28 as reported by Mendioro *et al.* (2005). The meiotic behavior of *M. oleifera* plants that was carried out by Silva *et al*. (2013) revealed 14 bivalents in diakinesis of all studied plant. Meiotic abnormalities were rare and metaphase I was the most affected phase. Pollen viability was superior to 88%. Tripolar spindles in metaphase II, leading to the formation of unreduced gametes.

The present work aimed at study cytological behavior of plants of two cultivated Moringa species (*Moringa oleifera and Moringa stenopetala*) in Egypt.

MATERIALS AND METHODS

Two Species of Moringa (*Moringa oleifera and Moringa stenopetala*) were used in the present work. Seeds of the two species (Fig. 1) were kindly provided by the Ornamental Branch, Horticulture Department,

Faculty of Agriculture, Minia University.

To study some mitotic parameters of the above mentioned Moringa species, growing root tips of 1-2 cm were collected and pretreated in 0.05% colchicines at room temperature for three hours. The colchicines-treated roots were immediately fixed with Farmer's fixative solution (absolute Ethyl alcohol and Glacial acetic acid 3:1 v/v) for 24h and stored in 70% ethanol at 4ºC. Before cytological examination roots were incubated in the acetocarmin dye for overnight then, acetocarmin-squashed preparations were made from the root tips. Stained metaphase plates with well-spread chromosomes were chosenand photographed microscopically using CCD camera (Olympus C-4040). To determine the cell shape and size, length and width of 120 examined somatic cells from roots were measured and statistically analyzed according to Gomez and Gomez (1984) using MSTAT program (version 4.0) edited in 1985 by the MSTAT development team, Michigan University and Agricultural University of Norway.

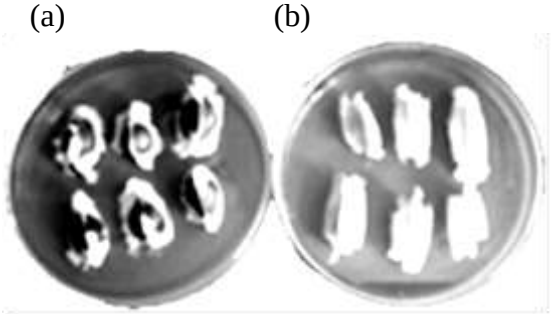

Fig. (1): seeds of the two Moringa species, (a) brown seeds of *M. oleifera* and (b) white seeds of *M. stenopetala*

RESULTS

1. Cell size and shape of *Moringa oleifera and Moringa stenopetala*

Length and width of the examined cell types were measured to determine the cell shape and size in fixed root tips of plants of the two studied Moringa species (*M. oleifera and M. stenopetala*). Accordingly, the examined somatic cells were categorized into three types. Data in Table (1) and Figure (2) showed the numbers and percentages as well as samples of examined cells per category. Type I is including dividing cells, type II meristematic cells with diffused

chromatin and those of type III that are representing cylinderic cells with diffused chromatin. Interestingly, chromatins like bodies of interphase nuclei in non-dividing cell were appeared especially in those of cylinderical and some of meristematic cells. The percentages of dividing cells may indicate to the mitotic index (MI). Hence, the MI is considerably high at cells of *M. oleifera* than that of *Moringa stenopetala.*

Data in Table (2) showed that cylinderic cells have the highest lengths (more than 50 microns) in both studied species. Generally, statistical analysis indicates that there were no significant differences in size of the different cell types between the two Moringa species of the present study.

Table (1): Frequency and percentages of the cell types observed in tissues of the root tips of the two Moringa species which categorized according to cell size and shape (Type I, mitotic dividing cells; Type II, meristematic with diffused chromatin and Type III, cylinderic cells with diffused chromatin).

Species	Type (1)		Type(2)		Type (3)	
	No.	%	No.	%	No.	%
Moringa oleifera	48	6.6	238	33.1	434	60.3
Moringa stenopetala	28	3.2	235	27.2	602	59.6

2. Mitotic chromosomes of *Moringa oleifera and Moringa stenopetala*

The chromosome movements during different mitotic stages were cytologically investigated and examined (Fig. 3). The scored diploid number (2N) of many metaphase plates of plants of the two studied Moringa species is mostly = 28. Indeed, it was very difficult to confirm this chromosome number in almost all examined cells. As shown in Fig (3), all examined stages of mitotic cells exhibited normal and regular metaphases and ana-telophases.

3. Nucleolus

As usual the appearance of nucleolus is contemporarily occurred with initiation of mitotic division events. Notably, appearance of large nucleolus at interphase, prophase and metaphase even at anaphase or telophase occurred at cells of *Moringa stenopetala*. In contrast, there was no appearance of nucleolus body mitotic cells of *Moringa oleifera* except those of interphase and

some prophases as shown in Fig. (4).

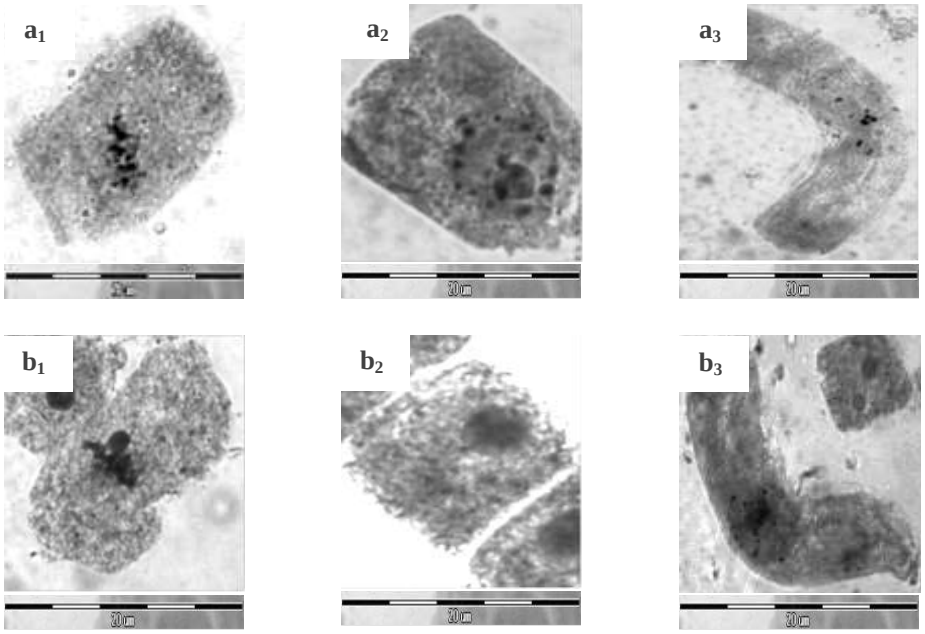

Fig (2): Samples of the three cell types observed in tissues of the two Moringa species root tips (a_1, a_2 and a_3 *M. oleifera* and b_1, b_2 & b_3 *M. stenopetala*), a1 & b_1: Type I (mitotic dividing cells); a_2 & b_2: Type II (meristematic with diffused chromatin) and a_3 & b_3: Type III (Cylinderic cells with diffused chromatin).

Table (2): Lengths and widths of the three cell types observed in root tips of *M. oleifera* and *M. stenopetala*.

Cell Types	Cell length (in microns)		Cell width (in microns)	
	M. olifera	*M. stenopetala*	*M. olifera*	*M. stenopetala*
Type 1	27.277	27.956	18.041	14.871
Type 2	26.715	27.254	14.704	16.472
Type 3	51.548	57.145	19.463	13.666
L. S. D. at 0.05	6.043	7.991	2.603	3.443

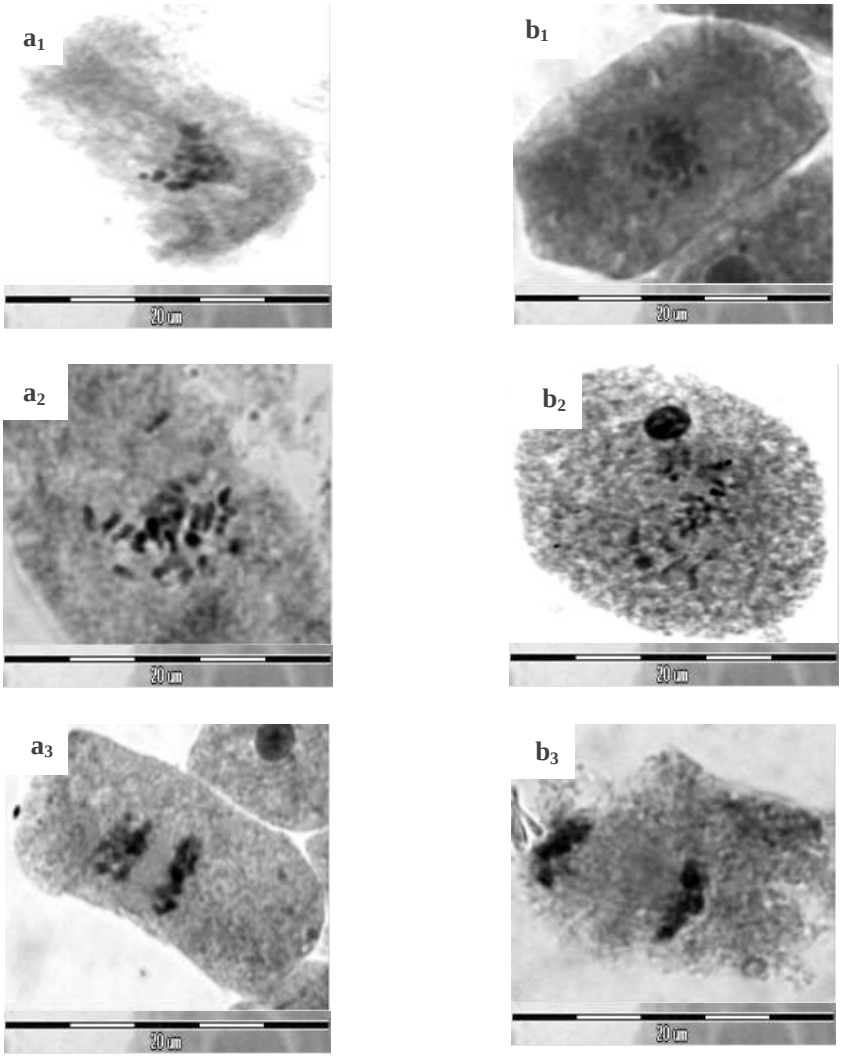

Fig. (3): Cells at different mitotic stages of two Moringa species (a_1, a_2 & a_3 *M. oleifera* and b_1, b_2 and b_3 *M. stenopetala*), Prophase (a_1 & b_1), Metaphase (a_2 & b_2) and Anaphase Telophase (a_3 & b_3).

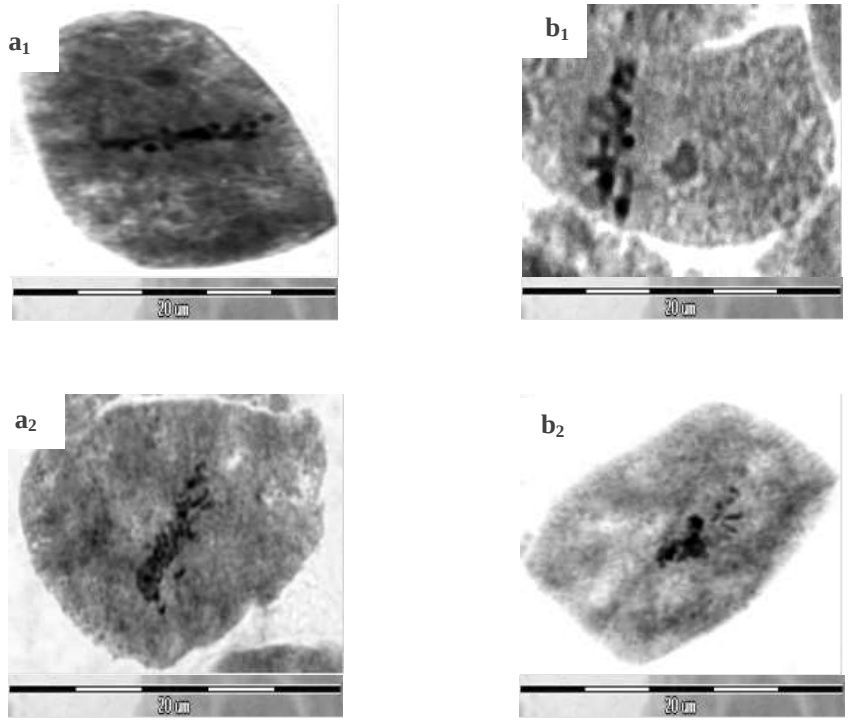

Fig. (4): appeareance of nucleolus during mitotic division of *Moringa stenopetala* (a_1 & a_2), in contrast with plants of *Moringa oleifera* (b_1 & b_2).

DISCUSSION

Cytological studies of plants belonging to Moringa species are very difficult because of the high number with small sized chromosomes in their genomes. The present work revealed that the active meristematic cells of root tip are small sized as well. Investigated tissues in root tips of plants of *Moringa oleifera* and *Moringa stenopetala* revealed that there are three types of active examined cell. Two types are meristematics, dividing and non-dividing. The frequency of dividing cells or so called mitotic index is considerably higher at cells of *M. oleifera* than that of *Moringa stenopetala*. This may due to the differences in genetic control set of cell cycle between the two studied congeneric species of Moringa. So, the inhibition of mitotic division in plants has been attributed to a number of factors (Shehata *at el.*, 2000 and Deysson, 1968). The two major reasons are the inhibition of both protein synthesis (Kim and Bendixen, 1978) and DNA amount

and replication (Beu *et al.*, 1976 and Badr, 1983). DNA content and the mitotic index in the root meristem are negatively correlated. The higher proportion of cells entering mitosis in the meristem of plants with a lower amount of DNA is not the result of alterations of the duration of the mitotic cycle, which was found to be quite comparable with largely differing genome sizes (Minelli *et al.*, 1996).

Second type of meristematic are those of non-dividing cells and seems to have condensed chromatin regions but dispersive within the interphase nucleus. This may due to the aggregation of several heterochromatic regions of different sizes along the relaxed and dispersed chromatin at interphase (Silva *et al*., 2013). The third type of active cells is the largest one and cylindrical shaped contained sharp visible condensed chromatin bodies embedded at interphase nucleus. The interpretation of this chromatin bodies aggregation is as mentioned by Silva *et al*. (2013). The concepts as well as recent additions of how cells construct cell walls of a given shape and the underlying processes were Summarize by Ivakove *et al*. (2013). These processes include cell wall synthesis, activity of the actions and microtubule cytoskeletons, in particular their regulation by microtubule associated proteins, actin-related proteins, GTPases and their effectors, as well as the recently elucidated roles of plant hormone signaling and vesicular membrane trafficking.

The diploid numbers of chromosome are often 28 in plants of the two Moringa studied species. It wasn't easy to determine crucial chromosome number of these taxa because of small sized chromosomal set. In the present study, regardless of some ploidy cell, it is clear that 28 chromosomes are found in metaphase plates of plants of both *M. oleifera* and *M. stenopetala*. This chromosome number became crucial after agreement with those reported by Ohri and Kumar, (1986), Mendioro *et al.* (2005) and Silva *et al*. (2013). The finding that nucleolus body appeared in metaphase and ana-telophase cells of *M. stenopetala* and disappeared in those of *M. oleifera* may be due to the species specific differences. These data may provide further cytological marker to distinguish the plants of the two Moringa congeneric species. As usual the occurrence of nucleolus interpreted the activity of so called housekeeping genes or different families of rRNA genes.

It could be concluded that the merstematic and mitotic activity of root tip tissue in plants of two

Moringa species (*M. oleifera* and *M. stenopetala*) are significantly different particular in mitotic index and nucleolus appearance. It is possible to use the cytological marker to distinguish between plants of the two Moringa congeneric species. Interestingly, these plant taxa exhibited some cell types contained condensed and aggregated chromatin at interphase nucleus. These dark stained chromatin aggregates may due occurrence of small region of heterochromatin distributed along the chromosome arms.

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الملخص العربى

دراسات لبعض النواحى السيتولوجية على نوعين من المورينجا (***oringa oleifera*** **and** ***Moringa stenopetala***) المنزرعين فى مصر

جيهان محمد انور عبد الغنى
قسم الوراثة – كلية الزراعة – جامعة المنيا

تعتبر الـ *M. oleifera* والـ *M. stenopetala* من الشجيرات والأشجار التى تنتمى لعائلة Moringaceae المنزرعة فى مصر للحصول على العديد من الاغراض خاصة الطبية. وتهدف هذه الدراسة إلى التعرف على بعض الملامح السيتولوجية في النباتات من هذين النوعين من المورينجا مثل شكل الخلايا وحجمها، والكروموسومات ومدى ظهور النويات. ولقد أمكن تقسيم الخلايا الجسدية النشطة إلى ثلاثة أنواع، النوع الأول هو الخلايا المنقسمة والثاني الخلايا المريستيمية الغير منقسمة أما النوع الثالث فهو الخلايا البرميلية أو الأسطوانية وبها تجمعات كروماتينية داكنة. وقد أوضحت الدراسة أن نسب الخلايا المنقسمة والتى يمكن أن يطلق عليها المؤشر الميتوزى (MI) كانت مرتفعة إلى حد كبير في خلايا الـ *M. oleifera* عن تلك الموجودة فى الـ *M. stenopetala*. وبخصوص العدد الزوجى من الكروموسومات فإن النباتات من نوعى المورينجا تحت الدراسة كانت فى الغالب = 28. وقد أظهرت الدراسة وجود نوية كبيرة فى مرحلة الإستوائى والإنفصالى والنهائى فى نباتات الـ *M. stenopetala* وفي المقابل، لم يكن هناك أي ظهور لهذه النويات فى هذه الأدوار فى نباتات الـ *M. oleifera* .