J. of Plant Production, Mansoura Univ., Vol. 15 (9): 519 - 523, 2024

Journal of Plant Production

Journal homepage & Available online at: www.jpp.journals.ekb.eg

Effectiveness of Colchicine in Inducing Polyploidy in 'Balady' Mandarin

El-Shereif, A. R. * **and Doaa M. Abou Elyazid**

Horticulture Department, Faculty of Agriculture, Kafrelsheikh University, 33516 kafr El-Sheikh, Egypt.

Cross Mark

ABSTRACT

Lateral buds of potted mandarin plants (*Citrus reticulata* Balnco cv 'Balady') were treated with four concentrations of colchicine (0.0, 0.1, 0.5 and 1.0%) for five consecutive days in order to investigate colchicine effectiveness in producing tetraploid plants of important mandarin cultivar in Egypt. These tetraploid plants can later be used as parents for producing seedless triploid plants through hybridization with diploid ones. Colchicine treatments decreased bud sprouting percentage and reduced shoot length. Furthermore, increasing colchicine concentration reduced the number of stomata per unit area compared to the control; however, stomatal length and width increased proportionally. Based on chromosome counting, the highest tetraploid percentage (42.2%) was obtained at 1.0% colchicine followed by the treatment of 0.5% (35.6%), whereas 0.1% concentration achieved the lowest tetraploid percentage (11.3%). The tetraploid shoots exhibited a broader leaf area and bigger floral buds expressed in length and width compared to the diploid mother plants. The results indicate that 1.0% colchicine is the most effective concentration for producing tetraploid plants.

Keywords: Chromosome doubling; *Citrus reticulata*; tetraploid; stomata number.

INTRODUCTION

Citrus is one of the world's most vital fruit crops, contributing significantly to the economic development of various regions. Egypt is one of the important growing areas that located in the northern hemisphere, where 68% of world production occurs (Chen et al., 2019). Mandarins are the world's second most important group of citrus plants and in Egypt as well.

Balady mandarin variety has alternate bearing and fruits containing a large number of seeds. Because seedless fruits are highly desirable among consumers, it is critical to improve this cultivar by reducing or eliminating the seeds. One of the most efficient techniques for producing seedless fruits is produce triploids through interploid crossing of diploid and tetraploid genotypes (Grosser & Chandler, 2004; Reforgiato *et al*., 2005). However, there are few tetraploid types available to citrus breeders and constitute only a small portion of the germplasm required for exploiting the potential of seedless citrus breeding (Barrett, 1974; Wakana *et al*., 2005; Zeng *et al*., 2006).

Over the past century, plant breeding programs have focused on improving plant material through induced polyploidy (by doubling the number of chromosomes in somatic cells) or natural polyploidy (due to the formation of unreduced gametes). Polyploid organisms are preferred over diploids due to their higher vigor and performance (Sattler *et al.* 2016; Sadat Noori *et al*., 2017).

Colchicine, a mitotic inhibitor, is commonly used for chromosome doubling due to its relative safety and the lack of need for specialized equipment (van Harten, 1998; Urwin 2014). Doubling the entire chromosome set can lead to an increase of cell volume and, consequently in an increase of plant parts (particularly the vegetative ones). This can be a useful tool in breeding and selecting for larger fruit size. Furthermore, tetraploidization in citriculture has been reported as an effective way to improve abiotic stress tolerance (Tan *et al*., 2015) such as drought (Allario *et al*., 2013), salinity stress (Ruiz *et al*., 2016), chromium toxicity (Balal *et al*., 2017), and chilling stress (Oustric *et al*., 2017).

Because cell responsiveness to physical and chemical mutagens is genotype dependent and influenced to varying degrees by numerous biological, environmental, and chemical factors (Noor et al., 2009, Abou Elyazid & El-Shereif, 2014), this study aimed to investigate the effectiveness of colchicine as a chemical mutagen in inducing polyploidy in 'Balady' mandarin, which may aid in the improvement of this important cultivar.

MATERIALS AND METHODS

Plant material: Potted two-year-old "Balady" mandarin plants that were purchased from a private nursery, approximately uniform in size and vigor and grafted on volkamer' lemon (*Citrus volkameriana* Ten.) were used in this study as plant material. The shoots of these mandarin plants were bent approximately 14-16 inches above the ground into a loop and fastened to the stem (Fig. 1). Three buds on the loop of each shoot were treated with colchicine.

Fig. 1. Mandarin plant prepared for colchicine treatments

* **Corresponding author.**
E-mail address: aelshereif@agr.kfs.edu.eg
DOI: 10.21608/jpp.2024.314029.1368

Colchicine treatment: A small piece of cotton was fastened to each selected bud to extend the time it was in contact with the colchicine solution. For five consecutive days, four concentrations of colchicine (0.0, 0.1, 0.5, and 1.0%) were applied twice a day to the selected buds with a dropper, enough to wet the without allowing the solution to run down the stem. The fastened cotton pieces were removed, and the treated buds were left to grow, while any growth from untreated buds was continuously removed. Each treatment was represented with 10 plants.

Phenotypic evaluation: Sprouted buds were observed after cotton removal at 10-day intervals for up to 40 days. The rate and percentage of sprouted buds were calculated. Shoot length was measured after 1, 3 and 6 months of treatment**.**

Thirty approximately typical mature leaves were collected from each treatment in three replicates. Then, the width and length of the leaves were measured and leaf shape index (width/length) was calculated.

Nearly full-grown flower buds that were initiated on shoots developing from treated buds were observed, and their length and width were measured.

Stomata Morphology: To conduct the stomatal studies, the leaf lower surface was coated with a thin layer of a clear nail polish, allowed to dry, then peeled off, placed on a microscope slide, and a drop of safranine was added to stain the stomata before being examined using a light microscope (Leica DM 1000) equipped with a digital camera (Abou Elyazid & El-Shereif, 2014). Images were captured at a magnification of 400 X and then processed using Leica Image Manger software to obtain stomata number, length, and diameter.

Cytological Examination: Floral buds (2-3 mm in length) were harvested in the early morning (8-11 am) for chromosome counting. The buds were fixed in absolute ethanol-acetic acid solution (3:1) for 24 hours at room temperature, and then the buds were preserved in ethanol 70% at 5ºC. Chromosome number was examined according to the method described by Belling (1920) by squashing the anthers in acetocarmine (2%) onto slides and left for 24 hours before being examined under a light microscope at 1000X magnification.

Data Analysis: The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor & Cochran (1980) and means comparison at probability of 5% was performed according to Duncan (1955) using MSTAT program.

RESULTS AND DISCUSSION

Colchicine-treated buds took longer time to sprout compared to the control especially under the highest concentration, where no sprouting was observed 10 days after treatment. The bud sprouting rate increased under control and 0.10% treatments, peaking 20 days after treatment before declining. In contrast, the 0.50 and 1.0% treatments showed continuous increase until 30 day then decreased (Fig. 2).

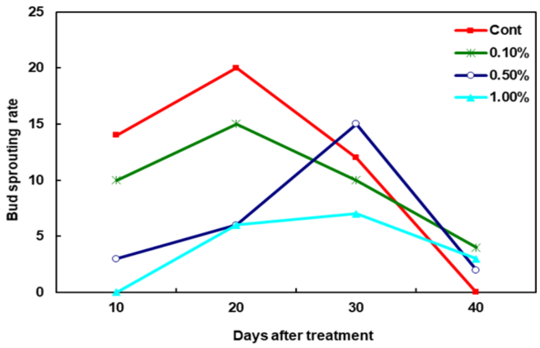

Fig. 2. Bud sprouting rate as affected by colchicine treatments

As the concentration of colchicine increased, the percentage of sprouting buds decreased (Figure 3). The control had the highest percentage (92%), while the treatment with 1.0% colchicine had the lowest percentage (32%).

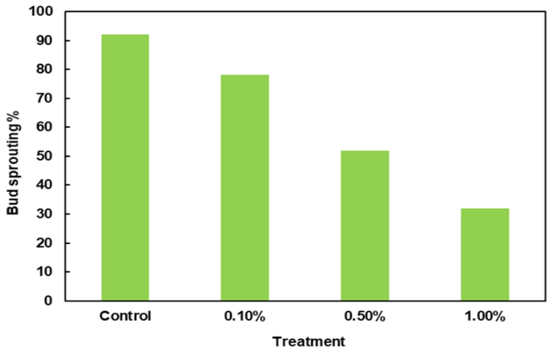

Fig. 3. Effect of colchicine treatment on total sprouted bud percentage

The initial sign of colchicine's effect on buds of Balady mandarin was a noticeable delay in the emergence of new growth from treated buds. This–delay became more pronounced by increasing the colchicine concentration. Similar results were found by Barrett (1974), Fatima (2004), Wakana *et al.* (2005) and Usman *et al.* (2008).

Meiotic studies confirmed the tetraploids by counting the number of chromosomes in the pollen mother cells of flower buds (Fig. 4).

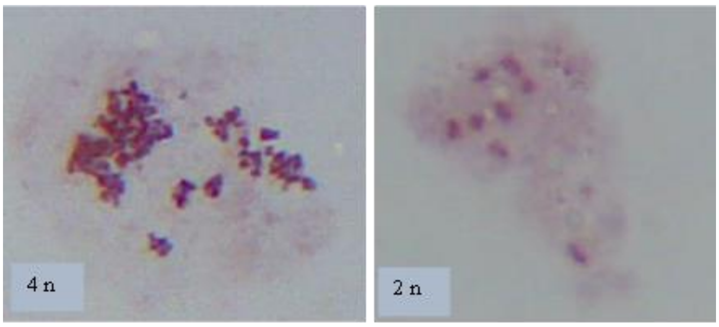

Fig. 4. Chromosome number of diploid (2n=9 bivalents) and tetraploid (4n=18 bivalents) in pollen mother cells of Balady mandarin

Figure (5) shows the ploidy level percentages under the four colchicine treatments. The results demonstrated that 1.0% colchicine produced the highest tetraploid percentage (42.2%), followed by 0.5% (35.6%), while 0.1%

concentration produced only 11.3% tetraploid percentage. Furthermore, mixploid shoots with diploid and tetraploid buds on the same shoot were found. The percentage of mixploid shoots was 7% at 0.1% colchicine, 6% and 2.4% at 0.5 and 1.0% treatments, respectively.

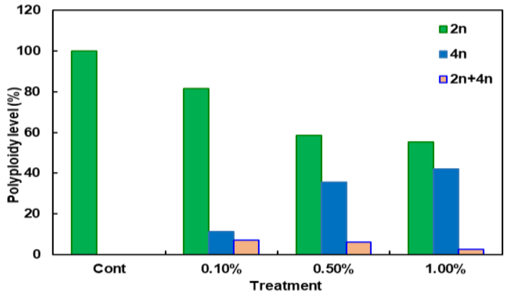

Fig. 5. Effect of colchicine treatment on polyploidy level percentage

The increase in polyploidization efficiency with increasing colchicine concentration was reported in citrus when seeds were treated (Abou Elyazid & El-Shereif, 2014) and in kiwi when leaves were treated *in vitro* (Li et al., 2019), and it also increased when treatment time was extended.

Data in Fig. (6) clearly show that colchicine treatments inhibited shoot growth. Increased colchicine concentration resulted in greater growth reduction. This inhibitory effect persisted throughout the experiment. After six months of treatment, the untreated buds (control) produced longer shoots than treated buds.

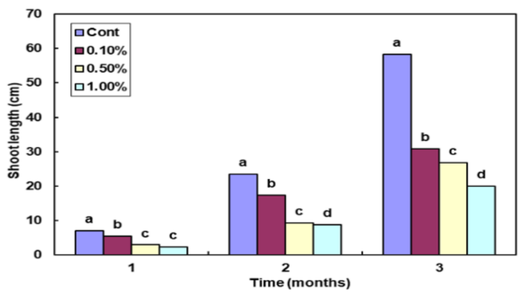

Fig. 6. Shoot length as affected by colchicine treatments of 'Balady' mandarin plants. In each month, means followed by the same letter are not significantly different at 5% level by DMRT.

The growth inhibition caused by colchicine can be attributed to its toxic effects, which is proportional to the concentration used. The high concentration of colchicine or the too long duration of treatment affect protoplast viability, kill the meristems and the new shoots tend to be hyperploid and may eventually die (Sanford, 1983; Zeng *et al*., 2006).

Stomata number significantly decreased by increasing colchicine concentration when compared to the control (Table 1). The lowest values were recorded at the 0.5 and 1.0% treatments without significant difference. On contrary, both stomata length and width were directly proportional to the colchicine concentration. The differences among treatments were significant. It is well known that the number of stomata per unit area is less numerous and stomata size is larger in tetraploids than in diploid (Barrett, 1974; Oiyama & Okudai, 1986; Usman *et al*., 2008; Li *et al*., 2019; Sabzehzari *et al*., 2019; Cimen, 2020; Lin *et al*., 2023; Narukulla *et al*., 2023).

Table 1. Effect of colchicine treatments on stomata number and size of 'Balady' mandarin plants

Treatments	Stomata No/mm^2	Stomata length (µm)	Stomata width (µm)
Control	26.80 a	96.03 c	87.65 c
0.1 %	19.94 b	119.85 b	103.9 b
0.5 %	16.89 c	130.30 a	113.52 a
1.0 %	15.73 c	131.33 a	113.37 a

Means followed by the same letter at the same column are not significantly different at 5% level by DMRT

Data in Table (2) show that, leaf length and width increased by increasing colchicine concentration from 0 to 0.5%, then decreased by treatment of 1.0%, but still higher than the control. However, colchicine treatments had no effect on the leaf shape index. Figure (7) shows the difference in leaf dimension between diploid and tetraploid shoots.

Table 2. Effect of colchicine treatments on leaf dimension of 'Balady' mandarin plants

Treatments	Leaf length (cm)	Leaf width (cm)	Leaf shape index (W/L)
Control	5.29 d	2.02 c	0.38 a
0.1 %	6.3 c	2.5 b	0.41 a
0.5 %	7.4 a	3.2 a	0.43 a
1.0 %	6.64 b	2.57 b	0.39 a

Means followed by the same letter at the same column are not significantly different at 5% level by DMRT

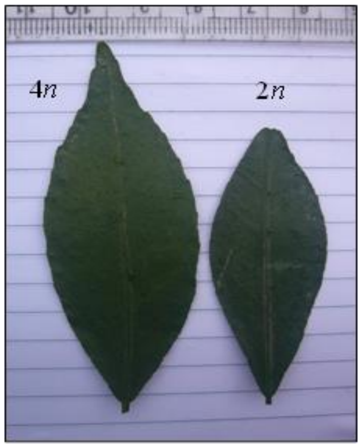

Fig. 7. A photo showing the difference between leaf of the diploid and tetraploid shoots as affected by colchicine treatments.

Colchicine application resulted in a significant increase in both flower bud length and width compared to the control (Table 3). However, the differences among colchicine concentrations were not significant. The colchicine-induced tetraploid shoots produced larger flower buds, petals and pistils (Fig. 8).

Table 3. Flower bud dimension as affected by colchicine treatment of 'Balady' mandarin plants

Treatments	Floral bud length (cm)	Floral bud width (cm)
Control	0.51 b	0.34 b
0.1 %	0.78 a	0.50 a
0.5 %	0.80 a	0.52 a
1.0 %	0.81 a	0.52 a

Means followed by the same letter at the same column are not significantly different at 5% level by DMRT

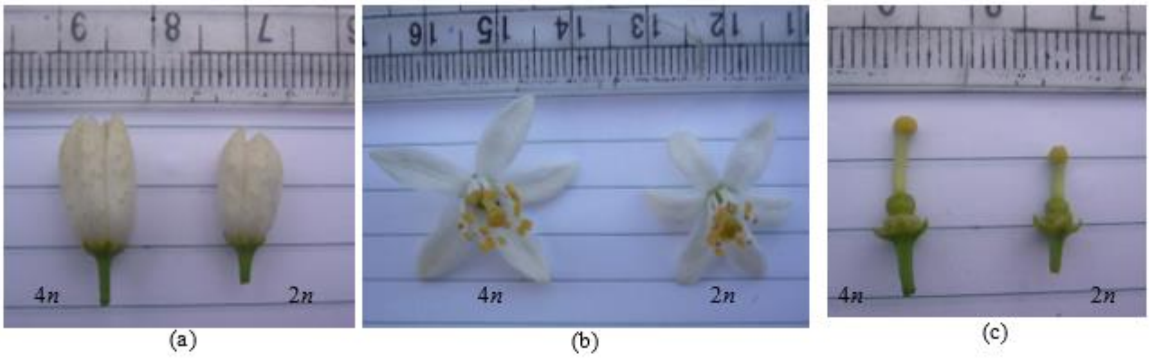

Fig. 8. Size of flower bud (a), flower (b) and pistil (c) of Balady mandarin as affected by colchicine treatments.

These changes in leaf and flower bud size are associated with the induction of tetraploids, where it is reported that tetraploids had larger and thicker leaves, larger flower buds, anthers and pistils (Oiyama & Okudai, 1986; Aranada *et al*., 1997; Wakana *et al*., 2005; Sabzehzari *et al*., 2019; Cimen, 2020; Lin *et al*., 2023; Narukulla *et al*., 2023). Furthermore, Polyploidy-induced phenotypic and genetic alterations in plants can be attributed to the increase in cell size, allelic diversity (level of heterozygosity), gene silencing and gene dosage effects, or epigenetic and genetic interactions (Manzoor *et al*., 2019).

CONCLUSIONS

The results indicate that a 1% colchicine concentration is the most efficient for producing tetraploids of 'Balady' mandarin. The tetraploids obtained exhibited reduced shoot growth and number of stomata, increased of stomata size, broader leaves, bigger flower buds and longer pistils. The performance of colchicine-induced tetraploids needs to be further evaluated. Additionally, these tetraploids will be valuable as parent plants for interploid hybridization to produce commercially valuable seedless triploid mandarin.

REFERENCES

Abou Elyazid, D.M. and El-Shereif, A.R. (2014). *In vitro* induction of polyploidy in *Citrus reticulata* Blanco. American Journal of Plant Sciences, 5: 1679-1685.

Allario, T., Brumos, J., Colmenero-Flores, J.M., Iglesias, D.J., Pina, J.A., Navarro, L., Talon, M., Ollitrault, P. and Morillon, R. (2013). Tetraploid Rangpur lime rootstock increases drought tolerance via enhanced constitutive root abscisic acid production. Plant Cell Environ. 36 (4): 856–868.

Aranada, R.R., Bondada, B.R., Syvertsen, J.P. and Grosser, J.W. (1997). Leaf characteristics and gas exchange of diploid and autotetraploid citrus. Annals of Botany 79: 153-160.

Balal, R.M., Shahid, M.A., Vincent, C., Zotarelli, L., Liu, G., Mattson, N.S., Rathinasabapathi, B., Martínez-Nicolas, J.J. and Garcia-Sanchez, F. (2017). Kinnow mandarin plants grafted on tetraploid rootstocks are more tolerant to Cr-toxicity than those grafted on its diploids one**.** Environ. Exp. Bot. 140: 8–18.

Barrett, H.C. (1974) Colchicine-induced polyploidy in citrus. Bot. Gaz. 135: 29-41.

Belling, J. (1920). The iron-acetocarmine method of fixing and staining chromosomes. Bio-Bull, 50: 160-162.

Chen, Y., Barzee, T., Zhang, R. and Pan, Z. (2019). Citrus. In: Integrated Processing Technologies for Food and Agricultural By-Products. Z. Pan, R. Zhang, and S. Zicari (Eds). Elsevier Science and Technology Books.

Cimen, B. (2020). Induction of polyploidy in C35 Citrange through in vitro colchicine treatments of seed-derived explants. International Journal of Fruit Science, 20 (sup3), S1929–S1941.

Duncan, D.B. (1955). Multiple ranges and multiple tests. Biometrics, 11: 1-24.

Fatima, B. (2004). Morphological and genetic characterization of citrus polyploids. Ph D thesis, Fac. of Agri., Faisalabad Univ., Pakistan.

Grosser, J.W. and Chandler, J.L. (2004). Production of twelve new allotetraploid somatic hybrid citrus breeding parents with emphasis on late maturity and cold-hardness. J. Amer. Pomol. Soc. 58: 21-28.

Li, S., Liu, X., Liu, H., Zhang, X., Ye, Q. and Zhang H. (2019). Induction, identification and genetics analysis of tetraploid *Actinidia chinensis*. *R.* Soc. open sci. 6: 191052.

Lin, J., Zhang, B., Zou, J. *et al.* (2023). Induction of tetraploids in Paper Mulberry (*Broussonetia papyrifera* (L.) L'Hér. ex Vent.) by colchicine. BMC Plant Biol 23, 574.

Manzoor, A., Ahmad, T., Bashir, M.A., Hafiz, I.A. and Silvestri, C. (2019). Studies on colchicine induced chromosome doubling for enhancement of quality traits in ornamental plants. Plants, 8, 194.

Narukulla, V., Lahane, Y., Fiske, K., Pandey, S. and Ziogas, V. (2023). Induction of polyploidy in citrus rootstocks through *in vitro* colchicine treatment of seed-derived explants. Agronomy, 13, 1442**.**

Noor, N.M., Clyde, M.M., Rao, V.R. and Jeevamoney, J. (2009). Radiosensitivity and *in vitro* studies of *Citrus suhiensis*. IAEA-TEC DOC. 1615: 17-32.

Oiyama, I. and Okudai, N. (1986). Production of colchicine-induced autotetraploidy plants through micrografting in monoembryonic citrus cultivars. Japan. J. Breed., 36: 371-376.

Oustric, J., Morillon, R., Luro, F., Herbette, S., Lourkisti, R., Giannettini, J., Berti, L. and Santini, J. (2017). Tetraploid Carrizo citrange rootstock (*Citrus sinensis* Osb.×*Poncirus trifoliata* L. Raf.) enhances natural chilling stress tolerance of common clementine (*Citrus clementina* Hort. ex Tan). J. Plant Physiol. 214: 108–115.

Reforgiato, R.G., Russo, G. and Recupero, S. (2005). New promising citrus triploid hybrids selected from crosses between monoembryonic diploid female and tetraploid male parents. HortSci. 40: 516-520.

Ruiz, M., Quiñones, A., Martínez-Cuenca, M.R., Aleza, P., Morillon, R., Navarro, L., Primo-Millo, E. and Martínez-Alcántara, B. (2016). Tetraploidy enhances the ability to exclude chloride from leaves in carrizo citrange seedlings. J. Plant Physiol. 205: 1–10.

Sabzehzari, M., Hoveidamanesh, S., Modarresi, M. and Mohammadi V. (2019). Morphological, anatomical, physiological, and cytological studies in diploid and tetraploid plants of *Plantago psyllium*. Plant Cell Tiss Organ Cult, 139**:** 131–137.

Sadat Noori, S.A., Norouzi, M., Karimzadeh, G. *et al.* (2017). Effect of colchicine-induced polyploidy on morphological characteristics and essential oil composition of ajowan (*Trachyspermum ammi* L.). Plant Cell Tiss Organ Cult, 130: 543–551.

Sanford, J.C. (1983). Ploidy manipulations. In: J. N. Janik (eds.) Advances in fruit breeding. Purdue Uni. Press, West Lafayelle, Indiana, pp. 100-123.

Sattler, M.C., Carvalho, C.R. and Clarindo, W.R. (2016). The polyploidy and its key role in plant breeding. Planta 243 (2): 281–296.

Snedecor, G.W. and Cochran, W.G. (1980). Statistical Methods. 7th ed. The Iowa State Univ. Press, Ames.

Tan, F.-Q., Tu, H., Liang, W.-J., Long, J.-M., Wu, X.-M., Zhang, H.-Y. and Guo, -W.-W. (2015). Comparative metabolic and transcriptional analysis of a doubled diploid and its diploid citrus rootstock (*C. junos* cv. Ziyang xiangcheng) suggests its potential value for stress resistance improvement. BMC Plant Biol. 15: 89.

Urwin, N.A. (2014). Generation and characterization of colchicine-induced polyploid Lavandula × intermedia. Euphytica 197 (3): 331–339

Usman, M., Fatima, B., Gillani, K.A., Khan, M.S. and Khan, M. (2008). Exploitation of potential tissues to develop polyploids in citrus. Pak. J. Bot., 40: 1755-1766.

Van Harten, A.M. (1998). Mutation Breeding: Theory and Practical Applications. Cambridge Univ. Press, Cambridge.

Wakana, A., Hanada, N., Park, S.M., Fukudome, I. and Kajiwara, K. (2005). Production of tetraploid forms of acid citrus cultivars by top grafting of shoots with sprouting axillary buds treated with colchicine. J. Fac. Agri., Kyushu Univ., 50: 93-102.

Zeng, H.Z., Chen, C.W., Hong, L., Liu, J.H. and Deng, X.X. (2006). *In vitro* induction, regeneration and analysis of autotetraploids derived from protoplasts and callus treated with colchicine in citrus. Plant Cell Tissue and Organ culture 87: 85-93.

فعالية الكولشيسين فى إستحداث التضاعف فى اليوسفى البلدى

على رمضان الشريف و دعاء محمود أبو اليزيد

قسم البساتين – كلية الزراعه – جامعة كفر الشيخ - مصر.

الملخص

تم معاملة البراعم الجانبيه لأشجار يوسفى بلدى منزرعه فى أصص بأربعة تركيزات من الكولشيسين وهى صفر، 0.1، 0.5، 1% وذلك لمدة خمسة أيام متتاليه بهدف دراسة فعالية الكولشيسين على استحداث نباتات رباعيه من هذا الصنف الهام فى مصر، والتى يمكن استخدامها لاحقا فى إستحداث نباتات ثلاثيه عديمة البذور من خلال التهجين مع النباتات الثنائيه . أدت معاملات الكولشيسين إلى انخفاض نسبة البراعم المتفتحه كما اظهرت إنخفاض فى طول الأفرع. علاوة على ذلك وجد أنه بزيادة تركيز الكولشيسين إنخفض عدد الثغور فى وحدة المساحه بالمقارنه بالكنترول، فى حين أنه بزيادة التركيز إزداد طول الثغور وعرضها. بناءا على الفحص السيتولوجى لعدد الكروموسومات وجد أن أعلى نسبة نموات رباعيه (42.2%) تم الحصول عليها تحت المعامله بتركيز 1% تلتها المعامله بـ 0.5% (36.6%) ثم المعامله بـ 0.1% والتى أظهرت اقل نسبة تضاعف (11.3%). أظهرت النموات الرباعيه مساحة اكبر للأوراق وحجم اكبر للبراعم الزهريه من حيث الطول والعرض مقارنة بالثنائيه (الكنترول). بناءا على النتائج المتحصل عليها فإن أكثر المعاملات كفاءة فى إستحدات نباتات رباعيه هى المعامله بتركيز 1%.

الكلمات الدالة: التضاعف الكروموسومى ، اليوسفى ، النباتات الرباعيه ، عدد الثغور.