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Effects of Colchicine Treatments on Some Grape Rootstock and Grape Varieties at Cotyledon Stage

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ABSRACT

The present study was conducted to polyploidy possibility induction of grapes in the experiment, apical meristem was treated by colchicine. The factorial experiment based on randomized completely design with tree replications. The factors including different colchicine concentrations (0 as control, 1 gL^{-1}, 2 gL^{-1}, 3 gL^{-1}, 4 gL^{-1}, 5 gL^{-1}, 6 gL^{-1}) and four diploid grape genotypes cv. 'Gök Üzüm' (*Vitis vinifera* L.) which came from conventional ancient time and have a perfect adaptation to the territory, and cv. 'Trakya İlkeren' (*Vitis vinifera* L.) was bred in last two decades in Turkey, and 'Isabella' (*Vitis labrusca* L.) and 41 grape rootstock [Chasselas (*Vitis vinifera* L.) x (*Vitis berlandieri* Planch.)] were used. With this purpose eight colchicine doses were dropped to meristematic part of young plantlets twice a day (at 8.30 am and 18:00 pm) during 3 days when first true leaves emerge in greenhouse (25°C during day and 20°C at night). The morphological and cytological parameters were evaluated 16 weeks after the polyploidization treatments. Based on the size and shape, and density of stomata, the number of chloroplasts in guard cells, and chromosome counting in the root tip-end indicated that these descendants were diploids $2n=2x=38$ like their parents that colchicine-induced mutation was failed in tested grape genotypes for tetraploid induction. On the other hand, only in the cv. 'Trakya İlkeren' an aneuploidy was observed by 5 gL^{-1} colchicine treated plantlet. Aneuploid 'Trakya İlkeren' plantlet was identified as $2n = 2x = 40$. It is seeming that grape genotypes hardly response to colchicine induction for genome doubling. This is the first report for colchicine-induced aneuploid in grapes.

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1. Introduction

Polyploidy is a very common phenomenon in species of angiosperms and vascular plants (Wendel, 2000; Moghe ve Shiu, 2014). Polyploid species can be classified into allopolyploid and autopolyploid based on the origins and levels of ploidy (Chen, 2010). Autopolyploid results from doubling a diploid genome, while allopolyploids are formed by the combination of two or more sets of distinct genomes. In addition to polyploidy, plant species have been shown to be either intraspecific or interspecific hybrids (Mavárez ve ark., 2006) and many plants showing an apparent diploid inheritance are actually paleopolyploids (ancient polyploids), which derived from at least one event of whole-genome duplication followed by massive gene loss and genomic reorganization through a process known as diploidization (Wolfe, 2001).

Polyploidy induction has been successfully applied to crop, ornamental, and medicinal plants in order to obtain lines exhibiting new agronomical characteristics. This procedure has provided plants that are seedless, with larger fruits and flowers, and with enhanced pest resistance and physical stress tolerance (An ve ark., 2014). The presence in polyploid crop species of larger fruits, leaves, and kernels can improve their marketing in comparison to their diploid counterparts, and because polyploid species exhibit features adaptive to the presence of various biotic and abiotic stresses-such as drought, salinity, extreme temperatures, and resistance to various pathogenic diseases-these plants have the potential to adapt to future climate changes (Brochmann ve ark., 2004; Hahn ve ark., 2012). Thus, cultivation of polyploid species could reduce economic losses.

The induction of polyploidy in horticultural and agronomic plant species is based on the application of dinitroaniline antimicrotubule drugs such as colchicine, oryzaline, and triflurarin.

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The treated seedlings were first examined morphologically and then analyzed by root tip-end slicing to verify the ploidy structure of the meristematic cells from. The numbers of chromosomes vary mainly in two ways. These are changes in euploid (in the chromosome set) and aneuploid (decrease or increase of some chromosomes in the genome) (Park et al., 1999, 2002, 2010; Acanda et al., 2013; Maheshwari et al., 2015; Sattler et al., 2016; Lee et al., 2017; Midin et al., 2018).

In this study, the effects of colchicine on grape genotypes seedlings were studied for induction polyploidy or aneuploidy in vivo.

2. Material and Method

2.1. Plant material

In this study 41 B [Chasselas (*Vitis vinifera* L.) x (*Vitis berlandieri* Planch.)], 'Gök Üzüm' (*Vitis vinifera* L.), 'Trakya İlkeren' (*Vitis vinifera* L.) collected from Selcuk University Faculty of Agriculture Department of Horticultre collection vineyard and 'Isabella' (*Vitis labrusca* L.) collected from Ondokuz Mayis University Faculty of Agriculture Department of Horticultre collection vineyard seeds were used.

2.2. Colchicine

Colchicine is an alkaloid extracted from meadow saffron (*Colchicum autumnale* L.) and the most widely used antimitotic agent for polyploidy induction (Planchais ve ark., 2000). The mechanism of action of colchicine involves its binding to a- and b-tubulin dimers, inhibition of micro-tubule polymerization during the cell cycle and prevention of chromosome/chromatid migration during anaphase. Consequently, cytokinesis will also be compromised, resulting in the formation of cells with doubled chromosome number. Colchicine has low affinity for plant tubulins and must be used at millimolar levels for effective polyploidy induction in plants (Dhooghe ve ark., 2011). Besides, through the use of colchicine, artificial plant polyploidy may also be achieved with other classes of antimitotic agents, such as the herbicides dinitroanilines (trifluralin and oryzalin) and phosphoric amides (amiprophos-methyl and butamiphos). These substances have higher affinity for plant tubulins. Therefore, micromolar concentrations of such agents might produce the same results as colchicine treatment (Planchais ve ark., 2000, Sattler et al., 2016).

2.3. Seedling treatment

In the experiment, the seeds were planted in planting trays. At the emergence of cotyledon stage, the seedlings were immerged in the colchicine doses (0 gL^{-1}, 1 gL^{-1}, 2 gL^{-1}, 3 gL^{-1}, 4 gL^{-1}, 5 gL^{-1}, 6 gL^{-1}) for 72 h. In each treatment, 30 seedlings were used. The treated seedlings were washed with distilled water and carefully planted in the pots filled with a mixture of leaf mold: sand: loam soil (1:1:1). The pots were put in greenhouse with 16 h light period, 25/20°C day/night temperature and 65% humidity.

2.4. Stomatal observations

For this purpose, ten diploid seedlings (control) and ten colchicine treated plants were randomly selected. Measurement and scoring were performed for four well-expanded leaves of each plant. Three samples of epidermal cells were obtained from lower surface by nail varnish technique. A small area of the abaxial side of leaves was covered with a thin layer of clear nail polish and left to dry. Then, it was removed with a pair of ne tip forceps. The polish strips were mounted on a microscope slide and then evaluated for the density and size of leaf stomata under the light microscope (Olympus BX40, Shinjuku, Tokyo, Japan) at 40× and 100× magnification (Hamill et al. 1992; Saharkhiz 2006; Ghani et al. 2014).

2.5. Chloroplast count

The chloroplast counting was performed to determine the ploidy level of the grape seedlings. Preparates were prepared to perform chloroplast counting from plant material by modified the protocol of Yuan et al. (2009) in the study. Firstly, fresh leaf was discoloured in Carnoy solution, then immersed in sterile water for 2-5 min, and then stained with 1% I-KI solution for 30 s. Finally, chloroplast number was counted under the light microscope (Olympus BX40, Shinjuku, Tokyo, Japan) at 40× and 100× magnification.

2.6. Chromosome count

Seedlins that had 0.5–1.0 cm long roots were pretreated with 0.002 M 8-hydroxyquinoline (8-HQ) for 4 h at room temperature. The roots were then washed in distilled water for 5 min. The root tips were fixed in Carnoy solution (ethyl alcohol and acetic acid, 3:1) at room temperature for 24 h. The samples were rinsed twice with distilled water and stored in 70% ethanol at 4°C for further cytological analysis. The roots were hydrolyzed in 1N HCl at 60°C for 12 min, and then squashed on slides containing a drop of 1% acetocarmine staining solution (Sakhanokho et al. 2009). A photomicroscope (Olympus BX40) was used for chromosome observations.

2.7. Statistical analysis

This experiment was a factorial arrangement based on a completely randomized design with tree replications. The numerical data obtained the tests were compared with Duncan multiple comparison test at 0.05 significance level using SPSS 17.0 and JMP 7 statistical programs.

3. Results and Discussion

3.1. Apical meristem treatments

The morphological parameters were evaluated 16 weeks after the polyploidization treatments. Morphological characteristics were observed all treated plants (Figure 1 and 2). There were significantly ($p<0.05$) differences in surviving rates of shoot tips of the induced plants. In treated 41B seedlings were survived more then Gök Üzüm, Trakya İlkeren and Isabella. There were no shoots tip failed in control seedlings, and 1 gL^{-1}, 2 gL^{-1}, 3 gL^{-1}, 5 gL^{-1} colchicine treated 41 B, and 1 gL^{-1} treated Gök Üzüm. The lowest percentage of survival was 68.21% in 41B by 4 gL^{-1} treatmet, in Isabella 67.86% by 1 gL^{-1}, 85.33% in Gök Üzüm by 6 gL^{-1}, and 87.23% in Trakya İlkeren by 3 gL^{-1} and 6 gL^{-1} treatments were observed respectively (Figure 1).

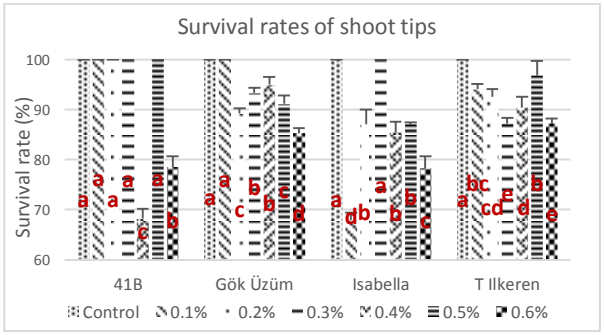

Figure 1
Survival rates (%) of shoot tips

3.2. Shoot length

The results showed that different colchicine dosages had significant effects ($p<0.05$) on the shoot lengt of of the grape seedlings in all grape genotypes. There were not contant shoot lengths by colchicine dosage in grape genotypes. The maximum shoot lengts were recorded conrol seedlings in all tested materials that were 23.50 cm in 41B, 22.48 cm in Gök Üzüm, 23.28 cm in Trakya İlkeren, and 22.85 cm in Isabella. The lowest shoot lengts were measured in 4 gL^{-1} colchicine treted 41 B as 20.21 cm, in 4 gL^{-1} colchicine treated Gök Üzüm as 19.20 cm, in 3 gL^{-1} colchicine treated Trakya İlkeren as 16.92 cm, and in 1 gL^{-1} colchicine treated Isabella as 18.13 cm respectively (Figure 2).

Similar results were reported by Motosugi et al. (2002) they indicated that tetraploid plants show weaker development and tetraploid Gloire and St George shoots were shorter than diploids.

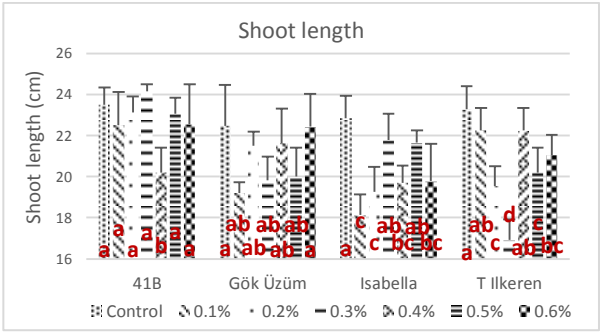

Figure 2
Shoot lengths (cm)

3.3. Stomatal characters

3.3.1. Stomatal lengths (µm)

The treatmes were significanty affected stomat legth in cvs. Isabella and Trakya İlkeren but nonsignificant in cvs 41B and Gök Üzüm (Figure 3). The range was between 27.66±0.08 µm (2 gL^{-1}) and 30.17±0.30 (5 gL^{-1}) in Isabella and 27.60±0.56 µm (2 gL^{-1}) and 30.21±0.17 (5 gL^{-1}) in Trakya İlkeren.

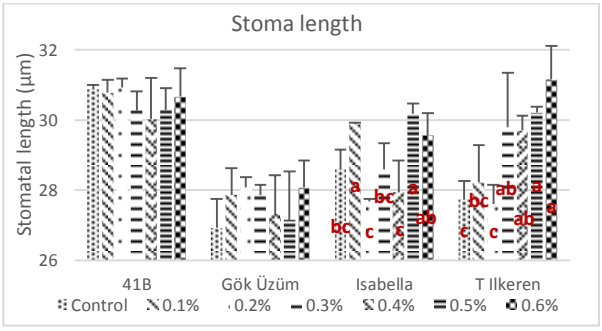

Figure 3
Stomatal lengths

3.3.2. Stomatal width (µm)

The treatmes were significanty affected stomat width in cvs. 41 B and Isabella and but nonsignificant in cvs Gök Üzüm and Trakya İlkeren (Figure 4). The range was between 16.17±0.29 µm (Control) and 18.30±0.49 (3 gL^{-1}) in 41B and 15.28±0.35 µm (2 gL^{-1}) and 16.97±0.72 (1 gL^{-1}) in Isabella.

In previous studies indicated that tetraploid plants have wider stomata than the diploids (Motosugi et al., 2002; Jun et al., 2009;), and stomata size is a suitable predictor of genome size for induced autopolyploid grapes (Yang et al., 2006; Sinski et al., 2014).

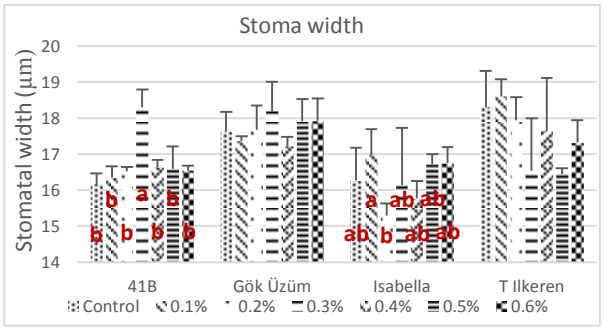

Figure 4
Stomatal widths

3.3.3. Stomatal area surface (µm^{-2})

The treatmes was significanty affected stomatal area surfaces only in cvs. Isabella and but in the other genotyes were nonsignificant (Figure 5). The range was between 425.59±8.35 µm^{-2} (2 gL^{-1}) and 510.38±22.29 µm^{-2} (1 gL^{-1}) in Isabella. There were not stabil stomatal area surfaces variation by colchicine dosage in grape genotypes.

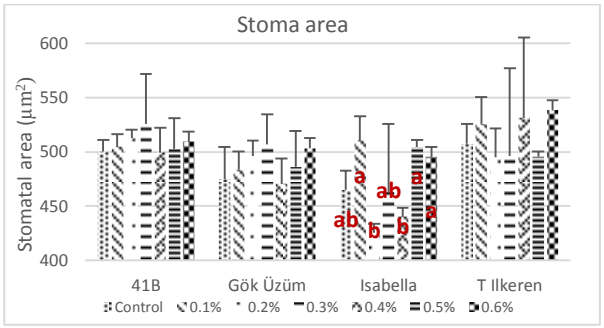

Figure 5
Stomatal area surfaceses

3.3.4. Stomatal density (stomata mm^{-2})

The colchicine applications were significanty affected stomatal density in cvs. Isabella and Trakya İlkeren but nonsignificant in cvs 41B and Gök Üzüm (Figure 6). The stomatal density was dose dependant. It was between 436.83±4.37 stomata mm^{-2} (3 gL^{-1}) and 478.49±17.81 stomata mm^{-2} (1 gL^{-1}) in Isabella and 387.69±12.29 stomata mm^{-2} (2 gL^{-1}) and 435.05±8.31 stomata mm^{-2} (Control) in Trakya İlkeren.

Several researchers were reported the increase of stomata sizes and reduces the stomata density (Yang et al., 2006; Bilir, 2010; Ma et al., 2014; Sinski et al., 2014; Xie et al., 2015) by the ploidy increas. Stomata area values were significantly differed due to the colchicine applications. A similar results of colchicine treatments was reported by Kara et al., (2018) on stomatal differences without genome doubling.

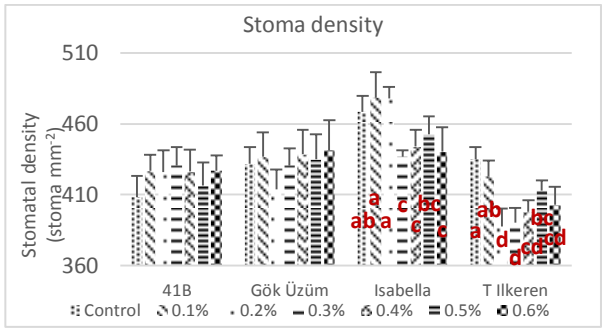

Figure 6
Stomatal density

3.4. Chloroplast numbers

The chloroplast numbers of stomatal guard cells were significantly differed between the colchicine treated diploid grape genotypes, the range was 18-20 in all treatments and it was 38-40 in tetraploid 'Kyoho' (Table 1). Previous studies were observed a correlation between the ploidy level and chloroplast number in stomatal guard cells in some crops (Yang and Yang, 1990; Zhang et al., 2007; Yuan et al., 2009; Xie et al., 2015). There were no statically difference between the colchicine treated plants average chloroplast numbers that were similar to previous studies (Xie et al., 2015). As a result, in all colchicine treated plants were described as diploid.

Table 1
Chloroplast numbers[a]

[a:] Mean separation within columns by Duncan Multiple Test, p<0.05.

Doses	41 B	Isabella	T İlkeren	Gök Üzüm	Kyoho
Control	19.83±0.30^{b}	19.41±0.83^{b}	19.63±0.33^{b}	19.70±0.28^{b}	39.74±0.44a
1 gL^{-1}	19.73±0.29^{b}	19.71±0.27^{b}	19.85±0.27^{b}	19.78±0.38^{b}	
2 gL^{-1}	19.47±0.40^{b}	19.45±0.66^{b}	19.57±0.38^{b}	19.88±0.21^{b}	
3 gL^{-1}	19.76±0.22^{b}	19.90±0.10^{b}	19.89±0.20^{b}	19.74±0.33^{b}	
4 gL^{-1}	19.42±0.17^{b}	19.49±0.50^{b}	19.79±0.36^{b}	19.94±0.10^{b}	
5 gL^{-1}	19.78±0.22^{b}	19.83±0.30^{b}	19.77±0.30^{b}	19.36±0.12^{b}	
6 gL^{-1}	19.74±0.43^{b}	19.59±0.24^{b}	19.77±0.22^{b}	19.89±0.18^{b}	

3.4. Chromosome observation

Determination of chromosome number is difficult in grape genotypes because of the thickness of the roots and very small sizes of the chromosomes.

The chromosome count of the root tips of colchicine treated grape genotipes and conrol seedlings showed that all non trated seedlings ploidy levels were diploid (2*n*=2*x*=38). There were no tetraploid plants were confirmed. The ploidy inducktion by colchicine only effected grape cv Trakya İlkeren as 5 gL^{-1} colchicine for aneuploidy that was (2*n*=2*x*=40) (Figure 6).

Lee et al., (2017) indicated that economic usefulness of aneuploid plants is rarely evaluated be-

cause aneuploid plants often exhibit developmental abnormalities, sterility, or lethality. Park et al. (1999) reported that five aneuploidy plants, with chromosome numbers ranging from 51 to 59, were recovered from various crosses among 184 different triploid hybrid grape vines through the use of immature seed culturing and subsequent embryo cultures. Similarly, grapes with different ploidy levels, such as haploid, diploid, tetraploid and aneuploid, were consistently obtained using the same approaches (Guo et al., 2011b; Ji et al., 2013b; Park et al., 2002; Wakana et al., 2003; Yang et al., 2007).

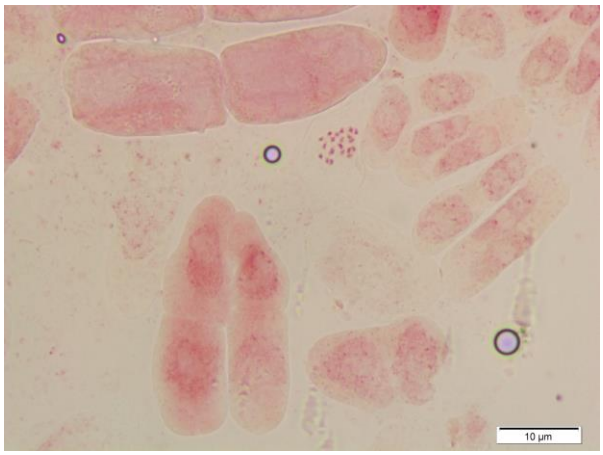

Figure 6
Chromosome number of Aneuploid Trakya İlkeren

4. Conclusion

There were so many important morfological and stomatal differences but no genome doubling were detected in colchicine treated grape seedling as *Vitis vinifera* L. cvs. Gök Üzüm, Trakya İlkeren, and *Vitis labrusca* L. cv. Isabella, and rootstock 41 B.

The reported diversity may be explained by cellular aneuploidy (abnormal number of chromosomes in a cell, Latorre et al., 2016). Colchicine pleiotropic effects predicted by model fitting to higher order designs are represented by the almost four-fold increase in aneuploidy, in comparison to oryzalin, especially at higher concentrations of the antimitotic agent (Sinski et al., 2014).

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5. References

Acanda Y, Prado MJ, González MV, Rey M (2013). Somatic embryogenesis from stamen filaments in grapevine (Vitis vinifera L. cv. Mencía): changes in ploidy level and nuclear DNA content. *In Vitro Cellular & Developmental Biology-Plant*, *49*(3), 276-284.

An F, Fan J, Li J, Li QX, Li K, Zhu W, Wen F, Cavalho LJ, Chen S (2014). Comparison of leaf proteomes of cassava (Manihot esculenta Crantz) cultivar NZ199 diploid and autotetraploid genotypes. *Plos one*, 9 (4), e85991.

Bilir, E. H., 2010, Trakya İlkeren ve Flama Seedless üzüm çeşitlerinde Co60 ve kolhisin kullanılarak mutasyon ve poliploidi oluşturma olanakları, Doktora Tezi, Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Adana.

Brochmann C, Brysting, A, Alsos I, Borgen L, Grundt H, Scheen AC, Elven R (2004). Polyploidy in arctic plants. *Biological journal of the Linnean society*, 82 (4): 521-536.

Chen ZJ (2010). Molecular mechanisms of polyploidy and hybrid vigor. *Trends in plant science*, 15 (2): 57-71.

Dhooghe E, Van Laere K, Eeckhaut T, Leus L, Van Huylenbroeck J (2011). Mitotic chromosome doubling of plant tissues in vitro. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 104 (3): 359-373.

Ghani A, Neamati SH, Azizi M, Saharkhiz MJ, Farsi M (2014). Artificial Autotetraploidy Induction Possibility of Two Iranian Endemic Mint. *Notulae Scientia Biologicae*, 6 (2): 185.

Guo Y, Zhao Y, Li K, Liu Z, Lin H, Guo X and Li C (2011). In vitro embryo rescue culture of F1 progenies from crosses between tetraploid grape and Vitis amurensis Rupr. African Journal of Agricultural Research 6(21): 4906-4909.

Hahn MA, van Kleunen M, Müller-Schärer H (2012). Increased phenotypic plasticity to climate may have boosted the invasion success of polyploid Centaurea stoebe. *Plos one*, 7 (11), e50284.

Hamill SD, Smith MK, Dodd WA 1992. In vitro induction of banana autotetraploids by colchicine treatment of micropropagated diploids. Aust J Bot. 40(6):887–896.

Ji W and Wang Y (2013). Breeding for seedless grapes using Chinese wild Vitis spp. II. In vitro embryo rescue and plant development. Journal of the Science of Food and Agriculture, 93(15), 3870-3875.

Jun C, XiaoPing T, XiaoHe M, QiFeng Z, and FuQingL (2009). Identification of the ploidy structure of bud sport of Red Globe grape cultivar. Journal of Fruit Science, 26(5), 619-622.

Kara Z, Doğan O, Yazar K, and Sabır A (2018). 41 B asma anacına in vivo kolhisin uygulamalarının morfolojik ve sitolojik etkileri. Selçuk Tarım ve Gıda Bilimleri Dergisi, 32(1), 8-13.

Lee MG, Park YS, Jeong, SH and Park SM (2017). Production of Hypo-and Hyper-tetraploid Seedlings from Open-, Self-, and Cross-Pollinated Hypo-and Hyper-tetraploid Grape. *Journal of the Korean Society of Horticulture* 152-153.

Ma X, Dong Z, Zhao Q, Li X, Tan W, Tang X, Chen J (2014). A series of Polyploid grape cultivars and their structural identification of ploidy character. *XI International Conference on Grapevine Breeding and Genetics 1082*: 63-68.

Maheshwari S, Tan EH, West A, Franklin FCH, Comai L and Chan SW (2015). Naturally occurring differences in CENH3 affect chromosome segregation in zygotic mitosis of hybrids. *PLoS genetics*, 11(1), e1004970.

Mavárez J, Salazar CA, Bermingham E, Salcedo C, Jiggins CD, Linares M (2006). Speciation by hybridization in Heliconius butterflies. *Nature*, 441 (7095): 868.

Midin MR, Nordin MS, Madon M, Saleh MN, Goh HH, and Mohd Noor N (2018). Determination of the chromosome number and genome size of Garcinia mangostana L. via cytogenetics, flow cytometry and k-mer analyses. *Caryologia 71*(1): 35-44.

Moghe GD and Shiu SH (2014). The causes and molecular consequences of polyploidy in flowering plants. *Annals of the New York Academy of Sciences*, 1320 (1): 16-34.

Motosugi H, Naruno T, Komazaki S and Yamada M 2002, Resistance of autotetraploids of grapevine rootstock cultivars to phylloxera (*Daktulosphaira vitifoliae* Fitch), Vitis - Journal of Grapevine Research 41(2): 103-106.

Park SM, Wakana A and Hiramatsu M (1999). Most hypotetraploid seedlings from self-pollinated tetraploid grapes (*Vitis complexes*) have abnormal cotyledons. *Journal of the Faculty of Agriculture, Kyushu University* 44(1-2): 81-89.

Park SM, Wakana A, Kim JH and Jeong CS (2002). Male and female fertility in triploid grapes (Vitis complex) with special reference to the production of aneuploid plants. *Vitis* 41(1): 11-20.

Park YS, Kim IJ and Park SM (2010). Selection of hypo- and hyper-tetraploid seedlings from abnormal cotyledons seedlings obtained during crossing of tetraploid grapes (Vitis complexes). *Korean Journal of Horticultural Science and Technology* 28(5): 810-817.

Planchais S, Glab N, Inzé D, and Bergounioux C (2000). Chemical inhibitors: a tool for plant cell cycle studies. Febs Letters 476(1-2): 78-83.

Saharkhiz MJ. 2006. The effects of some environmental factors and ploidy level on morphological and physiological characteristics of feverfew (*Tanacetum parthenium* L.) medicinal ornamental plant [PhD Thesis]. Iran: Tarbiat Modares University: 173.

Sakhanokho HF, Rajasekaran K, Kelley RY, Islam-Faridi N (2009). Induced polyploidy in diploid ornamental ginger (*Hedychium muluense* RM Smith) using colchicine and oryzalin. *HortScience* 44(7): 1809-1814.

Sattler MC, Carvalho CR and Clarindo, WR (2016). The polyploidy and its key role in plant breeding. *Planta* 243(2): 281-296.

Sinski I, Dal Bosco D, Pierozzi NI, Maia JDG, Ritschel PS and Quecini V (2014). Improving in vitro induction of autopolyploidy in grapevine seedless cultivars. Euphytica 196(2): 299-311.

Wakana A, Hiramatsu M, Park SM, Hanada N, Fukudome I, Yasukochi K (2003) Seed abortion in crosses between diploid and tetraploid grapes (*Vitis vinifera* and *V. complex*) and recovery of triploid plants through embryo culture. J Fac Agric Kyushu U 48:39–50.

Wendel JF (2000). Genome evolution in polyploids, In: Plant molecular evolution, Eds: Springer, 225-249.

Wolfe KH (2001). Yesterday's polyploids and the mystery of diploidization. *Nature Reviews Genetics*, 2 (5): 333.

Xie X, Agüero CB, Wang Y and Walker MA 2015, In vitro induction of tetraploids in Vitis x Muscadinia hybrids, Plant Cell, Tissue and Organ Culture 122(3): 675-683.

Yang D, Li W, Li S, Yang X, Wu J, Cao Z (2007) In vitro embryo rescue culture of F1 progenies from crosses between diploid and tetraploid grape varieties. Plant Growth Regul 51: 63–71.

Yang XM, Cao ZY, An LZ, Wang YM, Fang XW (2006) In vitro tetraploid induction via colchicine treatment from diploid somatic embryos in grapevine (Vitis vinifera L.). Euphytica 152, 217–224.

Yang, J. and Yang, X. (1990). Analysis of the relationship between the chloroplast number and the ploidy level in mulberry. Journal of Zhejiang Agricultural Sciences 5, 238-240.

Yuan SX, Liu YM, Fang ZY, YangLM, Zhuang M, Zhang YY Xie and Sun PT (2009). Study on the relationship between the ploidy level of microspore-derived plants and the number of chloroplast in stomatal guard cells in Brassica oleracea. Agricultural Sciences in China 8(8): 939-946.

Zhang J P, Gong Z H, Liu K K, Huang W, Li D W, Zhang Z X. 2007. Rapid determination of ploidy level of chromosome in pepper. Journal of Northwest A&F University (Natural Science Edition) 35, 121-124.