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Induction of mutation by treatment with colchicine and spraying with progesterone for two cultivars of zinnia elegans by using DNA molecular

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***Abstract*---**The study was carried out in the fields of the Department of Horticulture and Landscaping, College of Agriculture and Forestry, University of Mosul, during the agricultural season spring 2021, to study the development of the mutation by treatment with colchicine and spraying with progesterone for two cultivars of zinnia, where two cultivars of zinnia (red and white), and three concentrations of colchicine at 0, 0.05 and 0.1%, and three concentrations of progesterone, which are 0.5, 10 mg / liter. And the study of genetic markers using RAPD technology, where four primers were used. The results obtained are summered up in the occurrence of chromosomal duplication for the two cultivars, and the stomata density was affected as a result of the interaction between cultivars and colchicine, the red cultivar was superior with 0.1% colchicine significantly. And progesterone at 5 mg/L had the highest value in the stomatal area, and the triple interaction treatment between the red variety and 0.1% colchicine and 5 mg/L of progesterone produced the highest value in the stomatal density, and the highest stomatal area resulted when the triple interaction between the white variety and 0% colchicine And 5 mg/L of progesterone. The initiators also showed different bundles in the number and locations, as the primer OPB-10 showed a unique

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bundle for the white cultivar, and the percentage of variation at the OPB-10 primer for the two cultivars and for mutagenic plants, and the highest sites that produced was at the primer OPB-10 in white cultivar, and OPE-19 In red cultivar.

***Keywords*---**Colchicine, Progesterone, Zinnia Elegans, DNA Molecular.

Introduction

Zinnia Jacq. Zinnia elegans is a summer annual plant belonging to the family (Asteraceae). The height of the plant reaches (76-80 cm) approximately when growth is complete. It is one of the plants that are used as cut flowers and can also be used in landscaping due to its attractiveness, multi-colored, and short-term flowering (Esmaeili *et al.,* 2014), and there are varieties grown in flower beds or flowering pots, zinnia plant is sensitive to low temperatures. It is planted after the danger of frost has passed. It is noted that the daytime temperature drops below 15 degrees, which leads to yellowing of the leaves, changing the shape of the plant and decreasing its aesthetic value. It is resistant to drought and soil salinity (Shiravand, 2011). At the beginning of their life, plants need a long day of 12-14 hours of light / day and the temperature is not less than 18oC during the day to encourage vegetative growth, then they are exposed to a short day of less than 12 hours of light to encourage the formation and growth of flower buds. And a temperature of not less than 18oC to obtain large inflorescences of high quality.

The technology of using colchicine in plant breeding to develop mutated plants and double chromosomes on a large scale is to improve traits in various horticultural plants represented in vegetables, ornamentals, and fruits, as plant breeders use it in self-pollinating horticultural plants that have a narrow genetic base (Micke, 1988) and the effects of colchicine were identified. Colchicine is a chemical mutagenic and is used in a simple and easy way in the production of mutagenic ornamental plants. Kazi et al., (2015) showed that treatment of ornamental plants such as crepe myrtle, Cyclamen, Pelargonium, Super flowers, Zinnia, Canna, Iris, and Azalea with colchicine led to a stimulation in the production of doubling plants. Colchicine-treated plants also appear with stunted growth and sometimes produce plants with a larger leaf area than untreated plants, as well as increased petiole diameter and pollen thickness (Zhang et al., 2016). Studies have shown that colchicine helps to develop chromosomal replication in plants, as this substance works to break down the spindle fibers during the meiotic process, and the cells treated with this substance are quadruple chromosomal groups instead of the normal binary group. Colchicine is used in different concentrations and for different periods of time, and plant parts are treated like seeds, or by spraying the growing tops of plants. Where the plants treated with colchicine are of a larger size and have larger leaves, flowers, and fruits than the untreated plants i.e. diploid chromosomal set. Mujib (2005) mentioned that ornamental pineapple plants treated with colchicine produce albino-type plants that have low chlorophyll content. The development of diploid plants using colchicine technology works to create biological diversity in the type and sex of plants treated with colchicine through an increase in the cell size of the vegetative parts and reproductive organs in the plant. Colchicine leads to the

production of plants with multiple chromosomes in a short period of time by interfering with the phases of cell division (Eng and Ho, 2019).

Several studies showed that when treating orchid plants with different concentrations of colchicine, which is 0.1 to 1%, it had a broad effect on increasing the number of chromosomes in the plant (Silva *et al.* 2000, Kime *et al.*, 2003, Vichiato *et al.*, 2007). Adamee and Aliyu (2007) obtained genetic and phenotypic variations in plants of the Zingiberaceae family with different concentrations of colchicine. Kobayashi et al. (2008) obtained when plants from Salvia were treated with concentrations of colchicine 0, 250 and 500 ppm that plants treated with high concentration produced quadruple diploid plants with large size, thick leaves and long flowering inflorescences compared with diploid plants.

Omidbaigi *et al.* (2010) found and attempted to induce chromosomal replication in Dracocephalum moldavicaL. Colchicine was used in two stages, the first when the cotyledons appeared and the second when the first two true leaves appeared on the plant by one drop of the prepared concentrations of 0, 0.05, 0.1, 0.2, 0.5 and 0.75%. It was 5.673 ± 195.684 mm 2, while it was 2.236 ± 395.633 mm 2 in diploid plants, 2x group, and there was a significant increase in the length and width of stomata, which amounted to 1.21 ± 19.97 and 0.258 ± 7.97 m, respectively. As shown by Xing et al. (2011) when treating seeds of Catharanthus roseus with colchicine at concentrations of 0.05, 0.1, 0.2, 0.3 and 0.4% for periods of 12, 24, 46 hours, colchicine had a significant effect on stomata characteristics in terms of size and number of stomata, and in the formation of plants Quad chromosomal group They also studied the molecular markers using 22 primers. The primers showed different bundle lengths. The RTASA-F primer gave the highest weight and bundle length of 339 kkbp.

Kerdsuwan and Te-chato (2012) recorded that orchid plants treated with high concentrations of colchicine produced an increase in stomata in the plant.
In a study conducted by Ravandi *et al.* (2013) for doubling chromosomes and their effect on the morphological characteristics of *Chichorium intybus* L. using colchicine by soaking seeds or treating the growing top of 6-day-old seedlings at concentrations 0, 0.05, 0.10, 0.25 and 0.50%, by three drops and at intervals of 8 And 12 and 24 hours later, the results showed that adding colchicine to the growing top of the seedlings was more responsive to the formation of diploid plants than soaking the seeds with colchicine, and it reduced the stomata density in diploid plants, reaching 80 µm compared to 132 µm in diploid plants. Said *et al.*, (2013) also found when studying the morphological and anatomical characteristics and RAPD-PCR analysis of three types of Malvatheca and using five primers, the primers showed different bundles among them, and the primer OPA-1 gave the highest number of bundles amounted to 17 bundles and 4 bundles similar to the primer, and the weights of the bundles ranged Between 500-2000 kbp for the five prefixes.

Niu *et al.* (2015) indicated when studying the effect of adding colchicine at a concentration of 0, 0.1, 0.2, 0.4, 0.6 and 0.8% to the growing top of Jatroph acurcas seedlings in the stage of cotyledon and one true leaf, and for three times every day at 8:00 and 16:00 and 23:00 for 1, 4, 7 or 10 days, colchicine

significantly reduced stomata in tetraploid and octahedral plants by 24.19% and 67.74%, respectively, than diploid plants. Zhang et al. (2016) observed that when seeds of *Trolliuschinensis bunge* plants were treated with colchicine at 3 days of age at concentrations of 0, 0.05, 0.10, 0.20% and for 12, 24, 36, 48 hours, the plants produced diploid plants and an increase in the length and width of the stomata.

He *et al.*, (2016) found that when treating the growing tip of the branch of *Dendranthema indicum* cultivar aromaticum with colchicine at concentrations 0, 100, 200, 500, 1000 and 2000 $mg.l^{-1}$ for a period of 12, 24, 36 hours, treating the buds with colchicine at a concentration of 1000 $mg.l^{-1}$ for 7 days led to a decrease in stomata density, which was 1.75 ± 9.00 mm2 in Tetrachromosomal 4x plants compared to diploid plants 2x, which amounted to 2.74 ± 22.00 mm2, while the stomata area increased in Tetraploid plants.

Estaji *et al.* (2017) showed in their studying effect of treatment with colchicine in two ways, the first by treating the seeds and the second by adding to the apical meristem after the formation of four real leaves on Salvia leriifolia at five concentrations 0, 0.05, 0.10, 0.20 and 0.50% by adding drops of Each concentration to the cotton balls surrounding the growing apex of the seedling, the colchicine treatment significantly reduced the stomatal density and reached 5.83 ± 77.5 mm 2 in the tetraploid plants compared to 5.83 ± 152.5 mm^2 in the diploid plants.

Talebi *et al.* (2017) showed that when they conducted three experiments using mutagenic substances, Colchcine, Oryzalin and Trifloralin, adding Colchicine in the form of drops to the growing top of the seedling at the stage of forming two true leaves of Agastache foeniculumL. At concentrations of 0, 5000, 12500, 17500 µm to produce diploid plants, they increased both the diameter of the stomata and their length, while the stomata density decreased compared to the diploid control plants.

Wibisono *et al.* (2018) studied the molecular markers in the plant species Vanda tricolor using RAPD-PCR technology and used the primers OPU-03, OPU-16, the results showed that there is genetic affinity in the studied samples and it ranged between 0.4-0.8. Choopeng *et al.* (2019) observed when studying the effect of colchicine on the percentage of plants multiplied by cross-breeding between two orchid cultivars Dendrobium Santana X D. friedericksianum using four levels of 0, 0.01, 0.05 and 0.1% for three periods of 24, 48, 72 hours. The concentrations of colchicine led to an increase in the DNA content from 33-50%, and the increase in the concentration of colchicine led to a decrease in the stomatal density.

Mo *et al.* (2020) studied multiple chromosomal replication using colchicine at concentrations of 0, 0.025, 0.05, 0.1, 0.15, 0.2, and 0.25% for three periods of 24, 48, and 72 hours on Rhododendron fortune Lindl plant. Colchicine treatment led to the development of tetrapolid plants and eight the octoploid plants, and the diploid comparison plants outperformed the tetrapolid plants of the tetrapolis and the octoploid plants of the eight chromosomal group. and 38.5 ± 52.40 mm-2, respectively compared to the diploid plants of 11.85 ± 164.79 mm^2, respectively.

Progesterone is one of the citrogenic sex hormones in mammals and is a cyclic organic compound, and several studies have indicated the presence of such mammalian stimulants in plants (Simons and Grinwich, 1989). Progesterone was discovered in more than 80% of the studied species, and they showed that the mammalian hormone has an effect in causing callus, as well as increasing sugar and protein, increasing growth, reproduction, flowering, number of flowers, the ratio of female flowers to males, pollination and fertility. Known plant steroids have different effects on morphology and physiology including influence on cell division and elongation, stem and photosynthesis, ethylene production and activation of the stress response (Janeczko and Skoczowski, 2005). The researchers, sedaghathoor and Mohammadi (2018) observed in their study the effect of using gibberellic acid at concentrations 0, 100, 200 mg / liter and progesterone at concentrations 0, 5, 10 mg / liter on the growth and development of Zinnia elegans flowers as well as the date of addition to the hormone before planting or in the stage of formation Four leaves on the plant or two months after planting, the best values of chlorophyll content were obtained when treated with progesterone at a concentration of 10 mg / liter after two months of planting, while the best area of leaves was obtained when spraying progesterone at a concentration of 5 mg / liter after Two months of planting.

The aim of the study is to develop mutations in two cultivars of zinnia using colchicine and progesterone in terms of genetic markers under the conditions of Mosul / Iraq.

Materials and Methods

The study was carried out in the fields of the Department of Horticulture and Landscaping, College of Agriculture and Forestry, University of Mosul for the period from April 2021 to September 2021, the seeds of the summer annual Zinnia elegans produced by the Italian company Pagane and obtained from the local market/ Dohuk governorate, Kurdistan region, Iraq were used to study the effect of colchicine. And progesterone in the development of mutation in two types of zinnia plant. The seeds were sown on April 28/04/2021 in seed propagation trays containing peat moss inside the greenhouse. After the seeds germinate and the seedlings grow and reach the appropriate size (2-3), true leaves were transferred to the open plastic house 12/5/2021, as the seedlings were planted directly In the soil of the plastic house, the soil of the house was composed of the river mixture. The study included three factors, two cultivars of zinnia, the first with red flowers, Doppia Rosa scarlatto, and the second with white flowers, Doppia Bianca, and treatment with colchicine with three concentrations of 0, 0.05, 0.1% when the true leaves were formed on the plant, where three drops were added to the growing top Figure (1).

Figure 1. technical Using Colchicine ON Zinnia plant.

For the plant four days after planting in the permanent place, the treatment was carried out in the early morning and for one time The progesterone produced by JSC Farmak was sprayed with three concentrations of 0.5 and 10 mg.l^{-1} after two weeks of permanent solution, and genetic markers were studied using RAPD technology and using four primers as shown in Table (1).

Table 1. The primers use in the study

No.	Primer	Sequence 3'-5'	Length
1	Opc-08-k	-TGG ACC GGT G -	10
2	OPB-10	-CTG CTG GGA C -	10
3	OPE-19	-ACG GCG TAT G -	10

The genomes of the two varieties were extracted. Using a Kit in the extraction process produced by Addprep Genomic DNA Extraction. The RAPD index is used to determine the genetic variation between the studied varieties and the effect of the mutagen colchicine and the progesterone used during the experiment, depending on the appearance of the multiplication bundles from their absence, and the study of the different molecular sizes between the bundles, after the necessary concentrations were calculated and the optimal conditions were created that were adopted in RAPD interactions. Programming the Thermocycler (PCR) and adjusting the number of cycles, temperatures and required time/cycle. Three random prefixes, OPC-08-K, OPB-10 and OPE-19 were used in the study

The experiment was designed using randomized complete blocks within the split split plots, with three replications and seven plants for each replicate. Data were analyzed statistically using Duncan's polynomial test at 5% probability level.

Results and Discussion

Through the effect of treatments with colchicine and progesterone on two cultivars of zinnia, there was a mutation in the flower shape for both cultivars as in Figure (1), where plants appeared bearing flowers with two ovaries completely different from the untreated plants. The stomata density and stomata area for both cultivars were also affected by colchicine and progesterone treatment.(Figure 2).

Figure 2. Shows the occurrence of chromosomal duplication and the development of the mutation in two cultivars of white and red zinnia as a result of treatment with concentrations of colchicine.

Table (2) shows the effect of cultivars, colchicine concentrations and progesterone spray, and the interaction between them on the stomata density of zinnia plants, as the table indicates that there are significant differences between the three factors studied on this trait. As it appears that the varieties do not have any

significant effect on the stomatal density, and the concentrations of colchicine used in the study did not reach the significant level among them by their effect on the stomatal density. 5 mg.L^{-1} had the highest stomata density of 150.1 (stomata/mm^2), and this significantly lagged behind with concentration 0 mg/L of progesterone, which produced the lowest stomata density of 122.6 stomata/mm^2, and there were no significant differences between concentrations 5 and 10, and 0 and 10 mg.L^{-1} . As for the effect of the two interaction treatments between cultivars and colchicine, it appears from Table (3) that the interaction between the red variety and 0.1% colchicine produced the highest stomata density of 155.5 stomatas/mm^2 and this significantly outperformed the interaction coefficients between the red variety and 0 % colchicine and white cultivar and 0.1% colchicine, which produced the lowest stomata density of 120.7 and 123.9 stomata/mm^2 respectively. The dual interaction treatment between the red variety and 5 mg.L^{-1} of progesterone produced the highest stomata density of 161.9 and it differed significantly with the interaction treatment. The bilateral interaction between the red variety and 0 mg.l^{-1} of progesterone, which produced the lowest stomata density of 116.3, the treatment of the bilateral interaction between the concentrations of colchicine and progesterone was superior, as the dual interaction treatment between 0.05% of colchicine and 5 mg.l^{-1} of progesterone produced the highest reading of 153.5 and this outperformed Significantly with the binary interaction treatment between 0% colchicine and 0 mg.L^{-1} of progesterone, which produced the lowest reading in that amounted to 100.2.

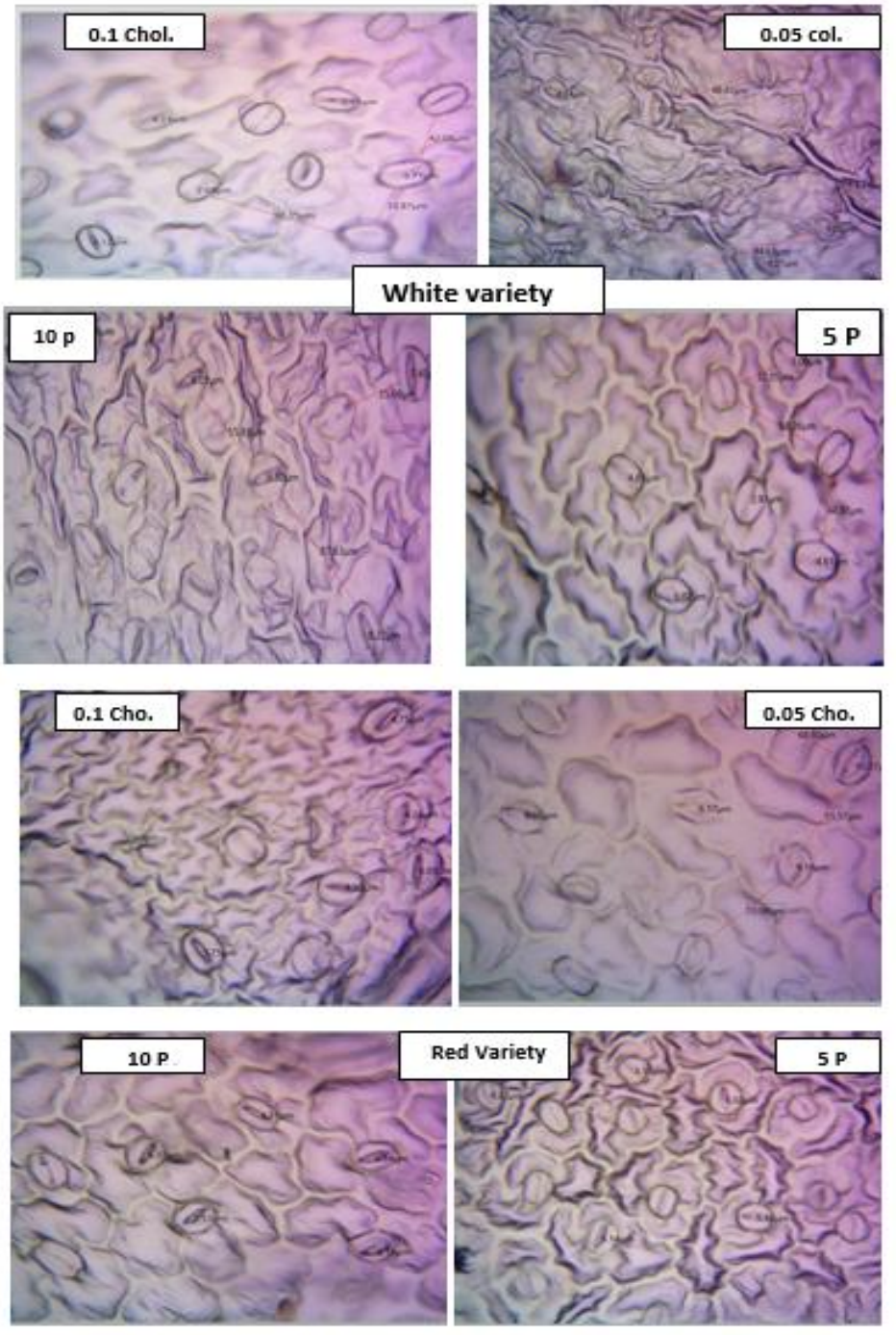


Figure 3. Effect of colchicine and progesterone on the stomata area of two cultivars of zinnia

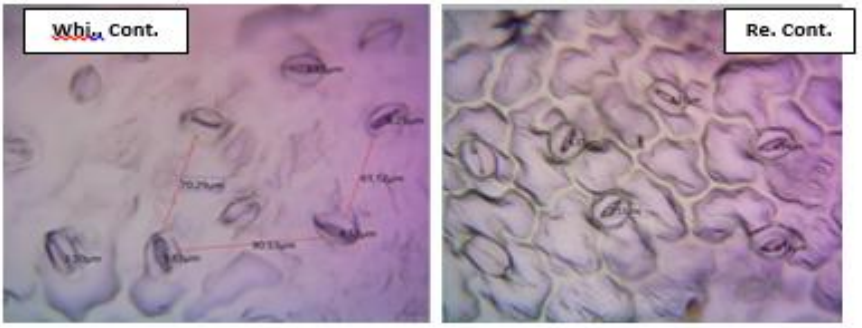


Figure 4. Shows the comparative treatment of two cultivars of zinnia

Table 2. Effect of varieties, colchicine and progesterone and interaction between them on stomata density (stomat /mm^2) for zinnia plant. *

Variety	Con. Of colchicine (%)	Concentration of progesterone (mg/liter)			Variety x colchicine	Variety
		0	5	10		
Red	0	82.0 d	143.7 a-c	136.5 a-c	120.7 ab	138.7 a
	0.05	117.0 b-d	156.0 a-c	146.7 a-c	139.ab	
	0.1	150.0a-c	186.0 a	130.5 b-d	155.5 a	
White	0	118.5 b-d	158.7 ab	157.5 a-c	144.9 ab	136.3 a
	0.05	145.5 a-c	151.0 a-c	124.3 b-d	140.3 ab	
	0.1	123.0 b-d	105.5 cd	143.3 a-c	123.9 b	
Progesterone x variety	Red	116.3 b	161.9 a	137.9 ab	Effect of colchicine	
	White	129.0 ab	138.4 ab	141.7 ab		
Colchicine x progesterone	0	100.2 b	151.1 a	147.0 a	132.7 a	
	0.05	131.2 ab	153.5 a	135.5 a	140.1 a	
	0.1	136.5 a	145.7 a	136.9a	139.7 a	
Effect of progesterone		122.6 b	150.1 a	139.8 ab		

*The values with similar letters for each factor or their interactions individually do not differ significantly according to Duncan's polynomial test under the 5% probability level.

It also appears from the results of the same table that the triple interaction treatments between the cultivars, colchicine and progesterone had a significant effect on this trait. This treatment was significantly superior to the triple interaction treatment between the red variety, 0% colchicine and 0 mg/L of progesterone, which produced the lowest value of 82.0.

It appears from Table (3) that the varieties had no significant effect on the stomatal area, and the different concentrations of colchicine had no significant effect on this trait. mμ and differed significantly with the concentration of 10 mg/l of progesterone, which produced the least alluring area of 5.354 mμ, as it appears from the same table that the binary interaction between the concentrations of colchicine and the cultivars of zina plant did not reach the significant level in that, as for the treatment of the binary interaction between the cultivars and progesterone, it produced The interaction treatment between the white variety

and 5 mg/L of progesterone had the highest stomata area of 6.924 mμ and it differed significantly with the binary interaction treatment between the white variety and 10 mg/L of progesterone, which amounted to 4.760 mμ, but it did not reach the significant level between it and the rest of the bilateral interaction treatments. Concerning the effect of the bilateral interaction between colchicine and progesterone on the stomatal area, the bilateral interaction treatment between 0.05% colchicine and 0 mg/L progesterone produced the highest stomata area of 7.585 μm, and the lowest area Stomata interaction resulted from the dual interaction treatment between 0.05% colchicine and 10 mg/l progesterone, which produced the smallest stomata area amounted to 4.500 mμ, while for the triple interaction effect between the studied factors, the triple interaction treatment produced between the white variety and 0% colchicine and 5 mg/l progesterone.

Table 3. Effect of varieties, colchicine and progesterone and interaction between them on stomata area (μm^2) for zinnia plant. *

Variety	Con. Of colchicine (%)	Concentration of progesterone (mg/liter)			Variety x colchicine	Variety
		0	5	10		
Red	0	5.912 a-c	5.953 a-c	6.433 a-c	6.099 a	6.110 a
	0.05	7.980 a	6.047 a-c	4.947 bc	6.324 a	
	0.1	5.260 a-c	5.993 a-c	6.467 a-c	5.907 a	
White	0	5.195 a-c	7.840 a	5.513 a-c	6.183 a	5.937 a
	0.05	7.190 ab	6.260 a-c	4.053 c	5.834 a	
	0.1	5.993 a-c	6.673 a-c	4.713 bc	5.793 a	
Progesterone x variety	Red	6.383 a	5.997 ab	5.948 ab	Effect of colchicine	
	White	6.126 ab	6.924 a	4.760 b		
Colchicine x progesterone	0	5.553 bc	6.897 ab	5.973 a-c	6.141 a	
	0.05	7.585 a	6.153 a-c	4.500 c	6.079 a	
	0.1	5.627 bc	6.333 a-c	5.590 bc	5.850 a	
Effect of progesterone		6.255 ab	6.461 a	5.354 b		

* The values with similar letters for each factor or their interactions individually do not differ significantly according to Duncan's polynomial test under the 5% probability level.

The highest stomata area was 7.840 mμ and this differed significantly with the triple interaction treatment between the white variety and 0.05% colchicine and 10 mg/l progesterone, which produced the lowest stomata area of 4.053 mμ.

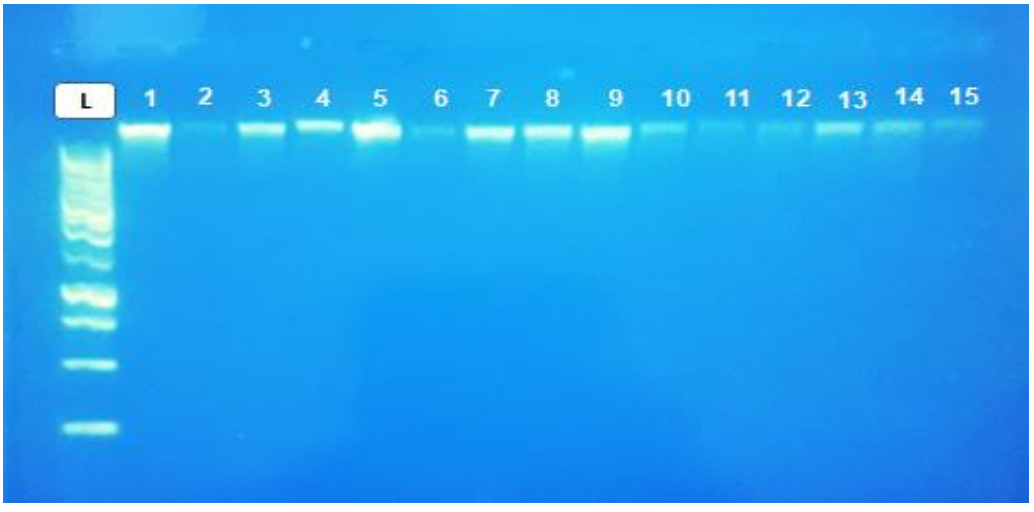
Through the results of Table (2, 3) that there were no significant differences for the effect of varieties and colchicine on the stomatal density and stomatal area, and that high concentrations of it caused a decrease in an insignificant decrease in the stomatal area and stomatal density. This may be explained by the genetic factors in each cultivar of zinnia within the gene complex, which have an overlapping effect with the environment on these traits. These results were in line with what was obtained by (Omidbaigi *et al.*, 2010) on *Dracocephalum moldavica L.*, Majdi *et al.*, , 2010 on *Tanacetum parthenium*, Kerdsuwan and Te-chato, 2012 on orchids, Ravandi *et al.* 2013 on *Chichorium intybus* L., Niu *et al.* 2015 on *Jatroph acurcas*, Zhang *et al.* 2016 on *Trolliuschinensis bunge*, He *et al.* 2016 on

Dendranthema indicum and Talebi *et al.*, 2017, on *Agastache foeniculum* L., Choopeng *et al.* 2019, on *Dendrobium Santana X D. friedericksianum orchid*, Mo *et al.,* 2020, on *Rhododendron fortune*) who indicated the concentrations used From colchicine, it caused an increase in both stomata diameter and length, while the stomata decreased and formed tetraploid plants compared to the diploid control plants, and decreased stomata density.

As for progesterone, the high concentration of it significantly affected the stomatal density and stomata. This result may explain the great role that this hormone, which is the sex hormone, plays in increasing sugars and protein and increasing growth and reproduction, which greatly affected the characteristics of the flower and the stomata traits. Two types of zinnia plant challenged the study.

Genetically study

Measurement of the quality and quality of the extracted DNA (genome study) , This step is considered one of the essential steps to ensure the appearance of the genome and that the DNA extract is of good quality, as DNA was extracted from young leaves of two cultivars of zinnia, the white variety Doppia Bianca and the red variety Doppia Rosa scarlatto, treated with different concentrations of colchicine and progesterone in addition to their interactions according to the Kit method, so 10 microliters of the extracted DNA samples were removed on the agarose gel by an electrophoresis device, and the concentration of the gel was 1.5%. The gel was colored with safestain dye, as in Figure (4).



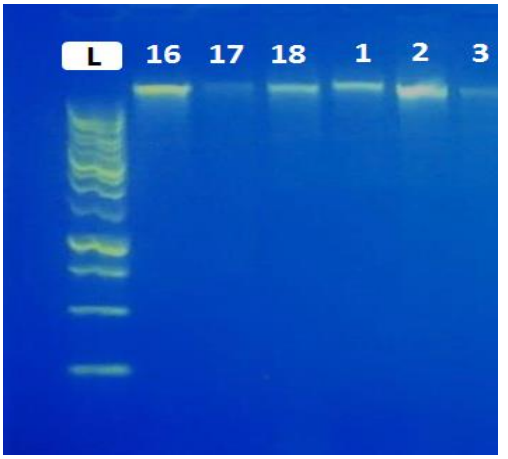


Figure 4. Zinnia genome transferred onto 1.5% agarose gel by RAPD-PCR

*1-9 for white variety. 1=0p. X0 chl. , 2=0px 0.05 chl. , 3= 0px 0.1 chl., 4=10p x 0chl., 5= 10p x0.05 chl. , 6= 10p x0.1 chl. , 7= 5p x 0 chl., 8= 5p x0.05 chl. , 9= 5p x 0.1 cl.
*From 10-18 for red variety, 10=5px0.05chl., 11=5px 0 chl., 12= 5p x0.1 chl., 13=0p x0chl., 14=0p x0.05 chl. , 15= 0p x0.1 chl. , 16= 10p x0.05 chl. , 17=10p x0.1 chl., 18 = 10p x0 chl.
(1, 2, 3) for mutation plant, 1= white variety (10px 0.1 chl.) 2= red variety (10p x0.1 chl.) , 3=red variety (5px 0,05 chl.)

Results of RAPD-PCR molecular

The primers showed different bundles in the number and positions of the polymorphic band between the two cultivars and the different treatments, which showed the genetic variance, which is considered one of the foundations on which to find the genetic relationship. The locations of these prefixes on the genome of samples reached 31 bundles, 21 of which are general sites and 10 varying bunds. It was found that the total number of total bunds that were produced from those sites amounted to 279, including 194 main bunds and 85 polymorphic bunds, as shown in the table (8).

First primer Opc-08-k:-

The primer (Figure 5) showed 32 distinct bunds for both white and red cultivars, 16 bunds for each cultivar, and their molecular sizes ranged between 100-500kbp for both cultivars. The number of sites produced by the primer swallowed 5 bunds for each cultivar, including 4 bunds for the number of general sites and one band for the number of varying sites in the white versus the red variety.

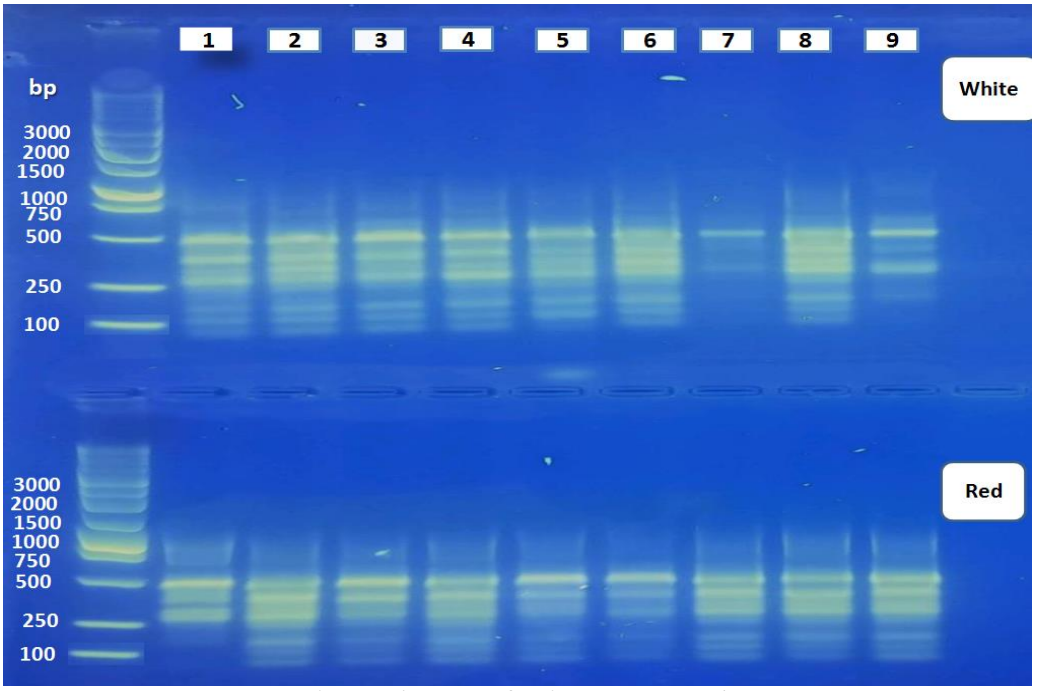


Figure 5. PCR of Primer Opc-08 k

1=0p. x0 chl. , 2=0px 0.05 chl. , 3= 0px 0.1 chl., 4=10p x 0chl., 5= 10p x0.05 chl. , 6= 10p x0.1 chl. , 7= 5p x 0 chl., 8= 5p x0.05 chl. , 9= 5p x 0.1 cl.

The white cultivar Doppia Bianca showed a different bundle site from the red cultivar Doppia Rosa scarlatto, which was 250 kbp, while the red cultivar showed a different site that was 350 kbp from the white cultivar, and the number of bundles was The total for the primer was 45 bundles, 29 bundles of which were general and 16 bundles varied in the white variety, and the red variety achieved the same result, and no unique bundles appeared for the primer % as shown in the table (4a , 4b),

Table 4a. Primer OPC-08-K *

	White variety								
Kbp	T1	T2	T3	T4	T5	T6	T7	T8	T9
500	1	1	1	1	1	1	1	1	1
300	1	1	1	1	1	1	0	1	0
250	0	0	0	0	1	1	0	1	1
240	1	1	1	1	1	1	0	1	0
100	1	1	0	0	0	0	0	0	0

(4b)

	Red variety								
Kbp	T1	T2	T3	T4	T5	T6	T7	T8	T9
500	1	1	1	1	1	1	1	1	1
300	1	1	1	1	1	1	1	1	1
250	1	1	0	0	0	0	0	1	1
240	0	1	0	1	0	0	1	1	1
100	0	0	0	0	0	0	1	0	1

*(1) Unigue band, (0) absent bunds
1=0p. X0 chl., 2=0px 0.05 chl., 3= 0px 0.1 chl., 4=10p x 0chl., 5= 10p x0.05 chl. , 6= 10p x0.1 chl. , 7= 5p x 0 chl., 8= 5p x0.05 chl. , 9= 5p x 0.1 cl.

while the percentage of variation was 20.00% in the white variety and the percentage increased In the red variety, it doubled, reaching 40.00%, and the efficiency of the primer was 31.25% in the white variety, compared to 33.33% in the red variety, while the discriminatory ability of the white variety reached 32.00% and increased in the red variety by 15.71%, reaching 47.71%, as shown in the Table (8).

Second primer OPB-10

The results of the primer represent in figure 6, the appearance of 39 bunds varying in site and in their molecular sizes, which ranged between 100-1500 kbp, and the number of sites produced by this primer ranged between 5-6 bunds. Which produced 4 bundles for the primer , two bundles for general sites and two bundles for divergent sites, and one unique bundle appeared in the white variety at a molecular size of 1000 kbp and when treated with colchicine at a concentration of 0.05% and without spraying progesterone. At the same level, they are 250, 500, 750, and 1000 kbp, while the white variety showed bundle locations that are absent from the red variety are 100 and 1500 kbp, and the total number of primer bunds reached 54, including 27 general bunds and 27 different bunds when studying the white variety against 36 total bunds .

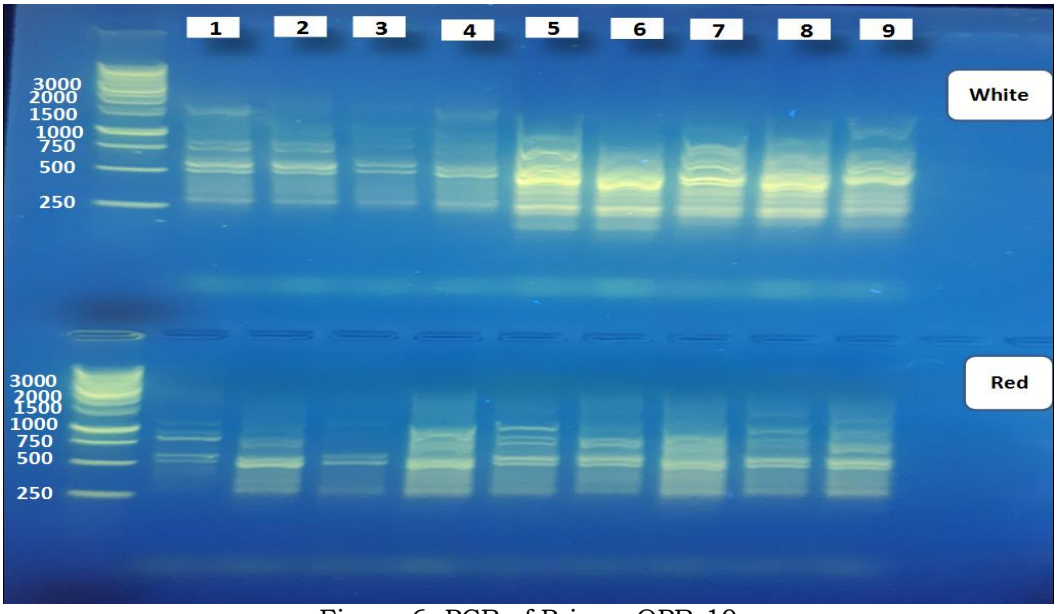


Figure 6. PCR of Primer OPB-10

1=0p. X0 chl., 2=0px 0.05 chl., 3= 0px 0.1 chl., 4=10p x 0chl., 5= 10p x0.05 chl. 6= 10p x0.1 chl. , 7= 5p x 0 chl., 8= 5p x0.05 chl. , 9= 5p x 0.1 cl.

The primer had 24 general bundles and 12 differentiated bundles as shown in Table (5a and b), and the percentage of variation was 33.33% for the white variety and 50.00% for the red variety. The efficiency of the primer for the white variety was 37.50% compared to 26.67% for the red variety, while the ability Discrimination for the primer amounted to 34.29% and increased by 19.71% in the white category, as shown in Table (8).

Table 5a. Primer OPb-10

	White variety								
Kbp	T1	T2	T3	T4	T5	T6	T7	T8	T9
1500	1	0	0	1	0	0	0	0	0
1000	0	0	0	0	1	0	0	0	0
750	1	1	0	0	1	0	1	0	0
500	1	1	1	1	1	1	1	1	1
250	1	1	1	1	1	1	1	1	1
100	0	0	0	0	1	1	0	0	0

Table 5 b.

	Red variety								
Kbp	T1	T2	T3	T4	T5	T6	T7	T8	T9
1000	0	0	0	1	1	0	1	1	1
750	1	1	0	1	1	1	1	1	1
500	1	1	1	1	1	1	1	1	1
250	0	1	0	0	0	0	0	0	1

1=0p. x0 chl. , 2=0px 0.05 chl. , 3= 0px 0.1 chl., 4=10p x 0chl., 5= 10p x0.05 chl. , 6= 10p x0.1 chl. , 7= 5p x 0 chl., 8= 5p x0.05 chl. , 9= 5p x 0.1 cl.

The third primer OPE-19:-

The results of the RAPD-PCR reaction for this primer in figure 7 were 14 bunds of varying site and molecular size, their sizes ranged between 240-1000kbp, and the number of sites

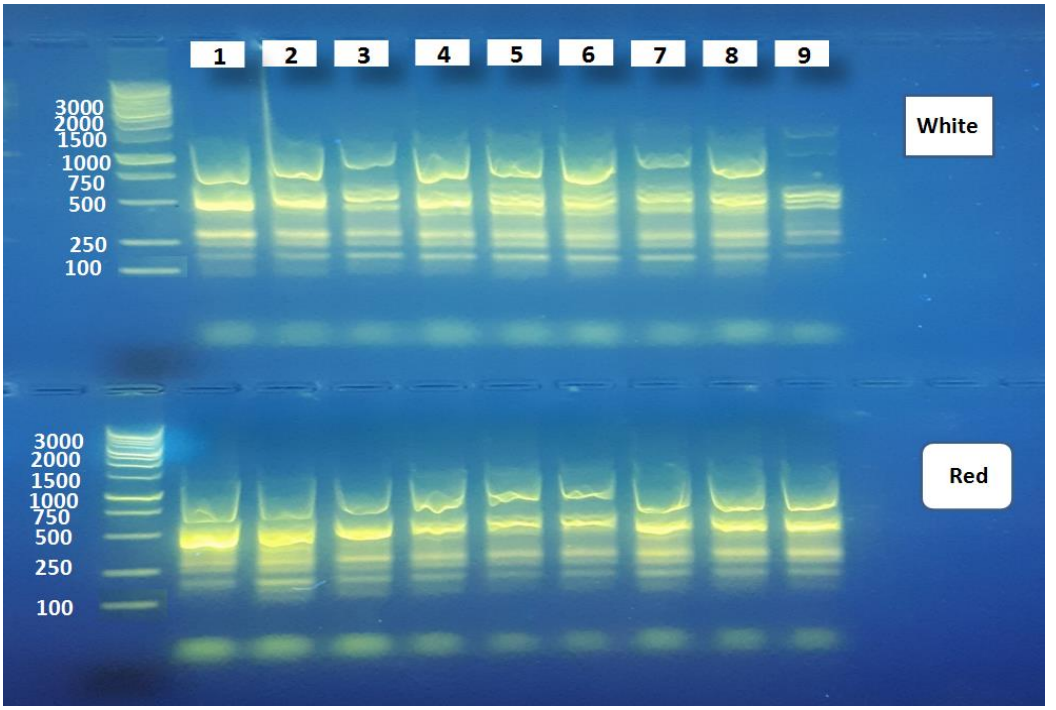


Figure 7. PCR of Primer OPE-19

1=0p. x0 chl. , 2=0px 0.05 chl. , 3= 0px 0.1 chl., 4=10p x 0chl., 5= 10p x0.05 chl. , 6= 10p x0.1 chl. , 7= 5p x 0 chl., 8= 5p x0.05 chl. , 9= 5p x 0.1 cl.

Produced by the primer ranged between 5-6 bunds, the white variety had the least number of sites, 5 bunds for the primer 4 sites. General and one dissimilar bundle, while the red variety produced 6 bundles, 4 bundles for the general sites and 2 bundles for the dissimilar sites. The white variety was distinguished by the absence of bunds for the molecular size of 300 kbp from the red variety. The total number of primer bundles and the number of general bundles in the white variety was 45 and 38 bundles. Respectively, while the values increased in the red variety, reaching 54 and 47 bundles, respectively, and the two types were similar in the number of different bundles, as it reached 7 bundles for each category, as shown in Table (6a and b). In the white variety, the values reached 20.00%, 31.25 and 14.00%, respectively, in contrast to the red variety, in which the percentages increased and reached 33.33%, 40.00% and 20.00%, respectively, as shown in Table (8).

Table 6 a primer OPe-19

	White variety								
Kbp	T1	T2	T3	T4	T5	T6	T7	T8	T9
1000	0	0	1	1	1	1	1	1	0
750	1	1	0	1	1	1	0	1	0
500	1	1	1	1	1	1	1	1	1
250	1	1	1	1	1	1	1	1	1
240	1	1	1	1	1	1	1	1	0

Table 6 b

	Red variety								
Kbp	T1	T2	T3	T4	T5	T6	T7	T8	T9
1000	0	0	0	1	1	1	1	1	1
750	1	1	1	1	1	1	1	1	1
500	1	1	1	1	1	1	1	1	1
300	0	0	1	1	1	1	1	1	1
250	1	1	0	1	1	1	1	1	1
240	1	1	1	0	1	1	1	1	1

1=0p. x0 chl. , 2=0px 0.05 chl. , 3= 0px 0.1 chl., 4=10p x 0chl., 5= 10p x0.05 chl. , 6= 10p x0.1 chl. , 7= 5p x 0 chl., 8= 5p x0.05 chl. , 9= 5p x 0.1 cl.

Mutagen plants of the three primers

When studying the characteristics of vegetative and flowering growth and following up on the experiment, (figure, 8), it was noted that plants appeared different in their morphological characteristics from their similar ones grown in the experiment ground and

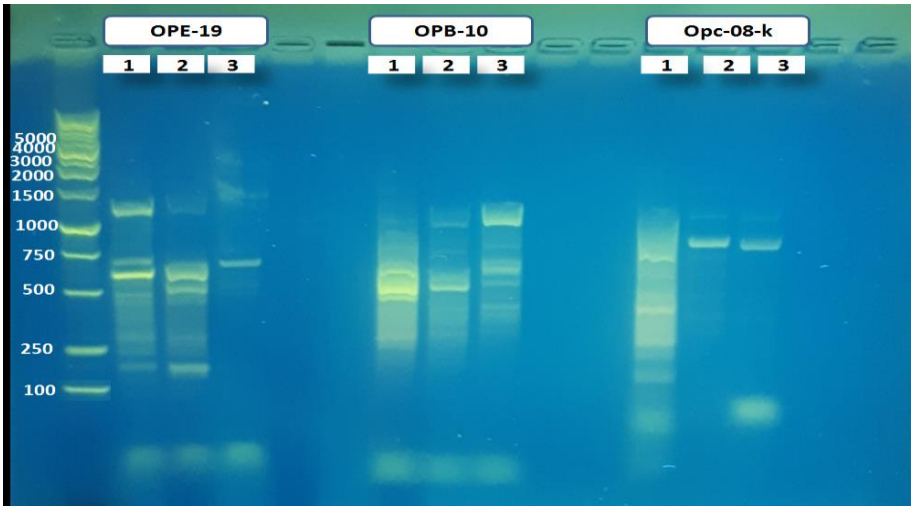


Figure 8. PCR of primer OPE-19, OPB-10, OPC-08-K, for mutation plant

1= white variety (10px 0.1 chl.) 2= red variety (10p x0.1 chl.), 3=red variety (5px 0, 05 chl.)

were considered as mutagenic plants as a result of treatment with the mutagenic substance colchicine and the hormone progesterone. The second sample was from the red variety and treated with a concentration of 0.1% colchicine, which was sprayed with progesterone at a concentration of 10 mg.l-1, while the third sample was from the red variety and treated with colchicine at a concentration of 0.05%, which was sprayed with progesterone at a concentration of 5 mg.l-1.

After the DNA was doubled using the primers Opc-08-k, OPB-10 and OPE-19, the results of the RAPD-PCR index showed clear differences confirming the occurrence of mutations and the morphological changes that were observed.

Table 7. Primers for plant mutation.

	OPE-19		
Kbp	0.1 chl. X 10 Pre. White variety	.1 chl. X 10 Pre. Red variety	0.05 chl. X10 Pre. Red va.
900	0	1	1
750	1	0	0
300	1	0	0
250	1	0	0
100	1	0	0
50	0	1	1

	OPB-1O		
kbp	0.1 chl. X 10 Pre. White variety	.1 chl. X 10 Pre. Red variety	0.05 chl. X10 Pre. Red va.
1000	0	0	1
750	1	0	1
500	1	1	1
300	0	0	1

OPC-08-K			
kbp	0.1 chl. X 10 Pre. White variety	.1 chl. X 10 Pre. Red variety	0.05 chl. X10 Pre. Red va.
1200	1	0	0
750	1	1	1
600	1	1	0
500	1	1	0
200	1	1	0

The results of the primer Opc-08-k showed that the molecular sizes ranged between 50-900kbp and the number of sites produced by the primer was 6 and 4 bunds general and 2 bunds divergent packets. Unique, and the percentage of discrepancy was 33.33%, the efficiency of the primer was 40.00%, and his discriminatory ability was 52.38%, as shown in Table (8)

The OPB-10 primer indicated that its molecular sizes ranged between 300-1000kbp, and the primer produced 4 bunds sites, 2 general sites, 500 and 750 kbp in the mutagenic plants of the white cultivar, when treated with colchicine at a concentration of 0.1%, and sprayed with progesterone at a concentration of 10 $mg.L^{-1}$, and in the mutagenic plants of the red variety. Treatment with colchicine at a concentration of 0.05% and progesterone at a concentration of 5 $mg.l^{-1}$ and 2 contrast sites are 300 and 1000 kbp, while the total number of bundles for the primer was 12 bundles 7 general and 5 distinct bundles, and the primer showed the presence of two unique bundles in the mutated plants of the red variety treated with colchicine At a concentration of 0.05% and progesterone at a concentration of 5 $mg.l^{-1}$ at molecular sizes of 300 and 1000 kbp, the primer was characterized by an increase in the percentage of variation over the primer Opc-08-k and the primer OPE-19, which amounted to 50.00%, and the primer

efficiency was 26.67% and the discriminatory ability was 23.81% as in the table (8).

While the results of the primer OPE-19 and the doubling of the genetic material DNA showed that the number of sites produced by the primer was 5 bundes, and their molecular sizes ranged between 200-1200 kbp 4 bundes, including general sites and one divergent site. A unique one at a molecular size of 300kbp in plants belonging to the red variety and treated with colchicine at a concentration of 0.05% and progesterone at a concentration of 5 $mg.l^{-1}$.

Table 8. Results of the primers used in RAPD reactions

No.	Primer name	kbp	The number of location has produced	Number of Origina Location	Number of variation location	Total number of fragment for prime	Number of original fragments	Number of variation location	Number of unique fragment	Number of absent fragment	Variation Percentage (%)	Primer efficiency (%)	Discriminatory ability of the primer (%)
White variety (Doppia Bianca)													
1	OPC-08-K	100-500	5	4	1	45	29	16	0	1	20.00	31.25	32.00
2	OPB-10	100-1500	6	4	2	54	27	27	1	0	33.33	37.50	54.00
3	OPE-19	240-1000	5	4	1	45	38	7	0	1	20.00	31.25	14.00
Total			16	12	4	144	94	50	1	2		100	100
Red variety (Doppia rosa scarlatto)													
1	OPC-08-K	100-500	5	3	2	45	29	16	0	1	40.00	33.33	47.71
2	OPB-10	250-1000	4	2	2	36	24	12	0	2	50.00	26.67	32.29
3	OPE-19	240-1000	6	4	2	54	47	7	0	0	33.33	40.00	20.00
Total			15	9	6	135	100	35	0	3		100	100
Mutation plant													
1	OPC-08-K	50-900	6	4	2	18	7	11	5	0	33.33	40.00	50.00
2	OPB-10	300-1000	4	2	2	12	7	5	2	0	50.00	26.67	25.00
3	OPE-19	200-1200	5	4	1	15	10	5	1	0	20.00	33.33	25.00
Total			15	10	5	45	24	21	8	0		100	100

The primer led to a decrease in the percentage of variance compared to the other primer s used in the experiment, which amounted to 20.00% and the efficiency of the primer was 33.33%. The discriminatory ability was it was similar to the primer OPB-10 and amounted to 23.81% as in Table (8).

By isolating the DNA of the effects of colchicine and progesterone on two types of white and red zinnia under study according to the Kit method, which is characterized by efficiency and ease during the isolation processes, the technology of extracting and isolating DNA in plants is relatively more difficult compared to other organisms, due to the presence of a cell wall in the cell The plant that surrounds the plasma membrane as well as containing different amounts of phenolic compounds, sugars and other compounds that affect the purity of the genetic material (DNA) extracted and also affect the PCR reactions based on RAPD indicators (Pandey, *et al.*, 2008). RAPD indicators were used because they are simple and have low cost and are considered one of the preferred methods in less advanced laboratories (Yaday *et al.*, 2014). Young leaves of the two cultivars were used according to the treatments used in order to avoid the content of sugars, phenols and other secondary metabolic compounds that will interfere and affect DNA extraction (Tahir *et al.*, 2015).

Through this study, three random primers (table) were used if different bunds appeared when studying the genomes of the two varieties (Figure 4) through the effect of colchicine and progesterone treatments. The results of the primers shown in Table (8) showed different patterns of bunds,

For the first white half (Doppia Bianca), it showed different features of the bundles, and the overall total of the sites identified by the primers on the genome of samples was (16) sites at a rate of 4 bundles for each primer, including a general site at a rate of (4) packets for each primer and at a rate of (1) bundles for each First, the primer OPC-08-K and OPE-19 had the fewest number of sites (5). And the total sum of the apparent packages for this category as a result of the effect of the transactions produced by those sites shown in Table (8) is (144) of which 94 are general packages and 50 are differentiated. At the primer OPC-08K, which reached 29 and the overall variance ratio of the primer s was 73.33%, and the primer OPB-10 had the highest efficiency and the highest discriminating ability which reached 37.50 and 54.00, respectively.

As for the red variety (Doppia rosa scarlatto), different sites of the bunds were shown. The overall total of the sites identified by the primers on the genome of the samples was (15) sites with a rate of (3) bunds for each primer. The primer OPE-19 was distinguished by the highest number of sites that reached (6) . The total sum of the packages appearing in this category as a result of its influence on the transactions produced by those sites (Table 8) is 135, of which 100 are general packages and 35 are differentiated packages. The general variance ratio for the primer s was 123.00%, and the highest efficiency was for the primer OPE-19 amounted to 40.00, and the highest discriminating ability was for the primer OPC-08K which amounted to 47.71.

As for the mutagenic plants, it appears from Table (8) that the total of the general traits that the primer s have identified are 17 sites, including 12 general sites and 5 different sites, and that the total number of visible bunds in these mutant plants reached 45 sites, including 24 general bunds and 21 different bunds . The general variance ratio of the primers was 103.33%, and the discriminant ability amounted to 50.00% for the primer OPC-08K. This result came with (Omidbaigi *et al.*, 2010 on D. moldavial, and Ravandi *et al.*, 2013 on *Chichorium intybus*).

The indicators of the RAPD depend on the foundations, which is the number of fragment shown by a gene of any model, which in turn represents the number of

sites that the primer finds and connects with them. The sequence of the nitrogenous bases of DNA and the variation occurs as a result of the occurrence of induced mutations or self-mutations that affected the area between the sites that occur naturally during the evolutionary process of living organisms, and the differences may have occurred as a result of deletion, replacement or addition in the sites to which the primer (Al-Karkhi 2018; Al-Sakmani *et al.* 2018).

It appears from the same table that the effect of colchicine and progesterone in two types of zinnia. Distinguished bunds (Unigue band and Absent bunds) appeared that there was one unique band for the white variety and two absent bunds, and there were no unique bunds for the red variety, but there were 3 bunds Absent in it, as for the mutagenic plants, it was found from the same table that there are 8 unique bundles, but no bundle was absent in these mutant plants.

The appearance of this discrepancy in these bundles, which is one of the traits and variations as a result of the effect of the concentrations of colchicine and progesterone in the two cultivars of zinnia, we find that a unique bundle appeared at the primer OPC-08 K at a molecular size of 100 kbp as a result of the effect of treatment No. (7) Figure (5a) A unique band appeared at the primer OKBP-10 at a molecular size of 250 kbp for the effect of treatment (2).

Thus, when these bundles appear in one treatment without the other, it indicates the presence of a sequence in the genomic DNA as a result of the effect of treatments with colchicine and progesterone for two varieties of Zinnia. This was noted by Bukhari *et al.* (2018), which indicates a site-specific mutation that led to the identification of the primer and the emergence of the unique package (Tingey, 1993, and Al-Asi 2002).

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