J. Plant Production, Mansoura Univ., Vol. 10 (8): 721- 730, 2019

Influence of Colchicine Treatment on Morphological, Physiological and Anatomical *Cercis siliquastrum* L. Seedlings Growth

Suliman, H. H[1] and H. S. Asander[2]

[1]Department of Forest-Collage of Agriculture Engineering Science-University of Duhok, Kurdistan Region-Iraq

[2] Department of Field Crops- Collage of Agriculture Engineering Science-University of Duhok, Kurdistan Region-Iraq

ABSTRACT

The present study was conducted to assess the effect of polyploidy induction on different properties of *Cercis siliquastrum* plant. Experiment was conducted in RCBD with 15 treatments and 3 replications. The seeds were treated with 0.0, 0.5, 1.0, 1.5 and 2.0 % colchicine solution at room temperature for various exposure times 12, 24 and 48 h. Observations on morphological variations were recorded on each plant in each treatment. Various in seed germination, morphological and growth characteristics observed due to effects of interactions between colchicine concentration and soaked period. By increasing of the colchicine concentration the seed germination rate decreased significantly from 80% to 50%, the effects on seed germinate were most evident at the higher colchicine concentrations (1.5 to 2.0 %). Longer stem and root, thicker stem and root diameter, higher number of leaves, branch and roots per transplant, were width area, thick and greener leaves, variable anatomy wood stem were achieved and development as compared to control plants. The length and width of stomata (21.6 and 10.3µm) respectively were significantly increased and induced in 0.1% of colchicine for 48h, whereas stomata density was decreased to 68 per area in 0.2% of colchicine at 48h. in contrast with initial diploid plants. However 1.5 % and 0.2% concentrations of colchicine at different soaked time were more effective for producing variation in plants. These polyploids plants may be helpful for further development and improvement of ornamental tree.

Keywords: *Cercis siliquastrum* L. variation, colchicine, polyploid breeding; stomata characteristics

INTRODUCTION

The *Cercis* genus (Fabaceae: Caesalpinoideae: Cercideae), also known as redbud, is a valuable commodity in the North American landscape industry and can also be found growing in temperate environments across the globe (David and Dennis 2016). *Cercis* L. consists of about 6-10 species, (Davis *et al.,* 2002 and Fritsch *et al.,* 2009) Only *Cercis siliquastrum* L. (2n=2X=14), species is native to Kurdistan region and whole Iraq (Shahbaz, 2010 and Barwary, 2015). *Cercis siliquastrum* L. (Judas tree) is a deciduous shrub, beautiful, with attractive and interesting rosy-purple flowers, round-shaped foliage, and form, widely cultivated as ornamental tree. It is well adapted to semi-arid conditions and highly tolerant of urban Pollution (Cejka and AL-Aamiry, 1981). Commonly used as a street tree, gardens and parks as a parking lot island and in highway median. It will even thrive in inner city environments. Furthermore, ornamental-conservational importance, for restoration (Jazirei, 2001), protection against soil losses caused by wind or water erosion, used in reforestation of disturbed lands to improve the landscape, windbreaks and wildlife plantings (Gebre and Karam, 2004; and Unal *et al.,* 2009). Also used as a phytoremediation measure against, (Yaşar *et al*. 2010), it has been valued for other purposes too. The blossoms and leaves of *C. siliquastrum* rich in many kinds of chemical compositions and volatile oils (Amer, J. 2019) also founded antimicrobial and antioxidant effects of *C. siliquastrum* leaves and flowers may contribute significantly to the biological activities and potential medicinal properties such as treatment of various microbial infections. In the other hand, wood of Judas tree is generally having good quality, hard with an attractive grain, coarse-textured, easy to work and finishes smoothly, it is used for making veneers, tool handles, mallet heads, turnery, cabinet making and to produce the high-quality charcoal and used in gunpowder manufacture (Pijut, 2008). Moreover, it is traditionally used as a fodder in the Mediterranean region (Papanastasis *et al.,* 1997). In recent times, polyploidy program had been used to bring about variation in chromosome number; in order to increase genetic variability and improve plant characteristics. Induction of polyploidy is widely recognized as an effective technique among various breeding tools because it has broadened genetic base, development of breeding lines in a short time span (Pereira *et al.,* 2014). The polyploidy induction in most cases is associated with gigantism in different plant organs like leaves, flower, fruits and stomata. More than the induction of polyploidy is a valuable method to obtain useful and novel characteristics that are not found in the diploid progenitor. The improvement of plant material through induced polyploidy has been one of the major targets of plant breeding programmes. There for, Hannweg *et al.* (2016) suggested the main advantage of induced polyploidy is that the plants achieved usually have improved morphological and yield characteristics, such as taller height, larger tuber, rhizome or root size. While Noori, *et al.,* (2017) provided the Polyploidy is an amazing evolutionary event that can be used in plant breeding to improve plant material. Actually, increased vigour and better performance are features that make polyploid organisms more preferred than their diploid relatives (Sattler *et al.* 2016). In the other hand, Sourour *et al.,* (2014), indicated that different parts of plant like seed, apical meristems, flower buds and roots can be used to induce polyploidy, however the best results have been obtained in a seed treatment. While Pirkooi *et al.,* (2011), suggested the success of polyploidy induction depends upon the colchicine application method, plant part used, species, concentration and duration of exposure, and founded that high concentration often leads to abnormalities in developing seedlings.

Colchicine ($C_{22}H_{25}NO_6$), frequently used method of increasing the chromosome number of plant, originally extracted from *Colchicum autumnale*, may induce some morphological, cytological and histological changes, and even changes in the gene expression level (Murali, *et al.,* 2013). Very little research is known about the breeding and improvement method of *C. siliquastrum* L. information is

needed on genotypic and phenotypic variation of different characters. Such Information is needed to guide decision-making on future research, development, and management of the species. Keeping in mind the above views, this study was planned in Judas tree to find the efficient method of inducing by using colchicines to creating more genetic variability, made variation, high yield and novel characters that could be used as parent material in future breeding programs.

MATERIALS AND METHODS

This research was conducted at research area in the laboratory and nursery of the Forestry Department/Collage of Agriculture during 2016-2017. Healthy and Mature pods were collected from five healthy and open-pollinated tree of *C. siliquastrum* selected in November 2016, where growing in the stands of collage of Agriculture, University of Duhok of Kurdistan region-North of Iraq (42°, 52′, 02″ E, 36°, 51′, 38″ N, and altitude 456m. over sea level and average annual rainfall 400 – 450 mm). The seeds were extracted, cleaned of any extra material, air dried in shade for 24 h. putted in moisture-proof containers, such as polyethylene bags and stored in a cooling chamber in a low temperature (2-4C°) until experimentative. Seeds of *C. siliquastrum* have a deep double dormancy and are hard to germinate especially when dried related to the hardness and impermeability of their coat, and by endosperm dormancy due to the presence of ferulic acid around the seed endosperm, acting as chemical inhibitor (Haroni, 2014). To break this dormancy before treating with mitotic inhibitors (colchicine), the seed were treated and soaked in concentrated (98%) H_2SO_4 for 45 min. to scarified the seed coat, after that they wished with distilled water for 15 min. later the seed were soaked in distilled warm water for 24 h at 25 C^0. Those seed which became scratched and visibly swollen were selected and surface sterilised by immersion in in 0.1 percent $HgCI_2$ (Mercuric chloride) for 15 minutes and washed thrice with double distilled water for 5 minutes with gentle swirling. These seed were subsequently used as the starting material for colchicine application.

Colchicine treatment of seeds:-

Seeds immersed directly in aqueous solution of colchicine material, five concentrations are (zero, 500, 1000, 1500 and 2000 mgL^{-1}) and different periods of seeds immersion in aqueous concentrations of colchicine in three periods (12, 18, and 24 hours). The disinfected seeds were placed on filter paper in 90 mm Petri dishes (20 seed in each dish) and provided with an aqueous solution of colchicine, so that the filter paper was fully saturated. After that, the seeds were washed with sterile water and transferred to fresh filter paper. Seeds treated throughout only with distilled water provided the control sample. The three treated seeds with colchicine solution were planted in polyethylene sacks (10 X 30 cm), filled with a sandy soils in February 2017, inside a lath house, where the percentage of light was about 50%. Seedlings of each experiment were lebled, maintained, irrigated and weed whenever it is necessary. In the end of November 2017, we selected best (5) seedlings in each experiment units for all treatments to study the variation between treated and untreated plants in each treatments. A rule (with accuracy 1mm) and Verner digital caliper (with accuracy 0.1 mm) were used in this study.

Measurement of stomata

Stomatal characteristics (numbers/mm^2) and size of stomata (length and width) were measured by the method of Omidbaigi *et al.* (2010). Well expanded, mature and enlarged leaves were taken from both control and treated plants. Nail varnish technique was used to isolate samplings from surface epidermises. The epidermis were mounted on glass slides and a light microscopy "Olympus U-DA" with a DinoXcope digital camera support on MAC, was used to photograph and measure stomata dimensions with magnification of 10x and 40x respectively. The 15 readings for each treatment will scored in addition to the average, the stomata number will count per mm^2 by use a gaged lens**.**

Chlorophyll determinations

Depending on method of Knudsen *et al.,* (1977) the chlorophyll content (a, b, and total) in leaves was evaluated. Pigments were extracted by dissolving 0.5 g of fresh mature leaf sample in 100 ml of absolute ethanol alcohol. The leaves after removing the middle vein cut into small pieces, and putted in flasks of 50ml capacity which include to 30ml of the absolute ethanol alcohol and then they kept in darkness for 24 hrs. The operation of extraction was repeats more than three times to guarantee the chlorophyll extraction completely, after that; the final volume was reached 90ml and the volume complete to 100ml. The absorption of solution was measured in two wavelengths (665 nm and 649 nm) by using Spectrophotometer, which was utilized to study the following characteristics:

1. Chlorophyll a Content (CH a) = (13.70) (A 665 nm) – (5.76) (A 649 nm)

2. Chlorophyll b Content (CH b) = (25.80) (A 649 nm) – (7.60) (A 665 nm)

3. Total Chlorophyll Content = Chlorophyll a + Chlorophyll b

Morphological parameters of seedling characteristics:-

After identification of tetraploid plants, morphological, physiological and anatomically characteristics where mention blew, as well as growth behavior of both tetraploid and diploid plants were recorded in order to characterize the differences and compared with control plants.

The largest leaf of transplant were selected and calculated via (ImageJ 1.52a) program according to (Schneider *et al*, 2012), each sample was counted accurately.

Anatomical parameters of seedlings wood characteristics:-

The wood cells of seedlings in each experiment were separated from each other by using the maceration method (Franklin, 1945). The equal amount of glacial acetic acid (CH_3COOH) and hydrogen peroxide (H_2O_2) by volume 1:1 were used to macerate the small piece of the wood stem of seedling. The macerate samples later they washed by distilled water to remove the remaining of solution, and then stained by Safranine stain (1%). After that, the anatomical characteristics of wood will studies by use Olympus microscope with magnification of 100x and

400x respectively. Fifteen readings for each treatment they taken in addition to the average.

Statistical analysis

This study was conducted using factorial base experiment within randomized complete block design (RCBD). The collected data were analysed with the SAS 9.1 for windows software package (Statistical). Means were compared using Duncan Multi ranged test at the 5% and 1% probability levels.

RESULTS AND DISCUSSION

Data present in table (1) indicated there was wide range of variation response of control and colchicine treated plants, a high variability within seed and seedlings characters the seed germination (%), morphological, physiological and anatomical seedling characteristics. The germination percentage was ranged between 50-90%, seedling length 47.5-139.0 cm, leaf area found to be (10.5-36.7cm^2). This positive variations resulted is a great opportunity to allow the use of such a trait as a tool for estimation and screening and then for the specific selective improvement of plant. The changes in this characteristics study such as plant height, leaves area, chlorophyll content stomata size and stomata numbers were important indicators for the detection of ploidy levels in this species.

Table 1. the minimum, maximum, range, coefficient of variation and mean ± standard deviation of growth, morphological, physiological and anatomical characteristics of *Cercis selegustriu* L. seedling studies.

Characters	Minimum, Maximum and Range	Coefficient of Variation (C.V.)	Mean ± Standard Deviation (SD.V.)
Germination Percentage (%)	50.00 - 90.000 (40.00)	17.229	66.222 ± 11.409
Shoot System Characteristics			
Seedling Length (cm)	47.48 – 139.00 (91.52)	28.390	82.979 ± 23.558
Stem Length (cm)	18.52 - 63.4 (44.88)	34.217	35.021 ± 11.983
Stem Diameter (mm)	3.228 - 7.42 (4.192)	23.001	4.748 ± 1.092
Number of Branches / Transplant	1.00 - 3.400 (2.4)	37.36	1.4044 ± 0.524
Largest Leaf Area (cm^2)	10.552 - 36.712 (26.1596)	31.099	21.7121 ± 6.7524
Number of Leaf / transport	16.00 – 41.00 (25.00)25.611	25.611	22.884 ± 5.877
Leaf Blade Length / Leaf Blade Width Ratio	0.7745 - 1.0299 (0.2553)	5.1227	0.7553 ± 0.04484
Leaf Thickness (mm)	0.268 - 0.6300 (0.362)	20.1798	0.3317 ± 0.0770
Root Systems Characteristics			
Root length (cm)	28.960 - 80.510 (51.540)	29.9421	47.9589 ± 14.3599
Root diameter (mm)	3.2860 - 7.856 (4.5640)	21.570	5.2420 ± 1.1307
Number of secondary roots	1.400 – 7.00 (5.600)	40.6408	3.3511 ± 1.362
Root System Dry Weight (g)	1.1708 - 5.460 (4.2892)	46.022	2.5077 ± 1.154
Physiological Characteristics of Leaves			
Chlorophyll (a) Content (mg/g)	9.0532 - 16.2668 (7.2145)	13.8893	11.784 ± 1.6367
Chlorophyll (b) Content (mg/g)	0.9476 - 4.2902 (3.3426)	41.5801	2.2502 ± 0.9356
Total Chlorophyll Content (mg/g)	10.5565 - 20.5570 (10.00)	15.5086	14.0342 ± 2.1765
Anatomical Characteristics of Leaves			
Stomata Length (µm)	14.276 - 23.698 (9.423)	12.223	17.350 ± 2.1204
Stomata Width (µm)	6.948 - 11.430 (4.482)	12.1087	8.4168 ± 1.0192
Number of stomata / mm^2	65.00 - 190.0 (125.0)	14.912	116.10 ± 17.317
Anatomical Characteristics of Shoot Wood			
Fiber Tracheid Length (mm)	0.5203 - 0.8864 (0.3661)	12.977	0.7258 ± 0.0941
Fiber Tracheid Diameter (µm)	9.1077 - 17.2196 (8.112)	9.8647	13.332 ± 1.315
Double Cell Wall Thickness of Fiber (µm)	4.7803 - 7.4630 (2.6826)	9.774	5.999 ± 0.5864
Vessel Length (µm)	44.740 - 91.5668 (46.8261)	15.127	71.8754 ± 10.72
Vessel Diameter (µm)	7.8258 - 19.261 (11.4353)	25.460	11.1965 ± 2.8507

Seed germination:-

Data presented in table 2, (means ± Standard division value and Duncan multi ranged test) indicated that the germination percentage of treated seed of the Judas tree declined with the increase in the concentration of colchicines dosage used, this evident based one valuations, the highest polyploidy induction efficiency was achieved by applying higher concentrations of colchicines (0.15% - 0.2% for 48h.) were most impacted in seed germination, which decreased in to (56.56 and 53.3%) respectively, per contra the highest germination percentage (80.0%) were recorded in controls treatment. This is because colchicine does not only have an effect on cell division but spreads through the cell, interfering with cellular mechanism and causing toxicity at high concentration. Colchicine apparently impacts the viscosity of cytoplasm so the cell cannot function normally. Han *et al.*, (1999) It has been proved that when high dose of colchicine used as a mutation agent for plant, toxic contamination, phytotoxicity and abnormality became main cause of death in the plants. Similar observations were reported in Japanese mulberry genotypes by (Tojyo, 1966), in tropical mulberry varieties namely by (Rao, 1996), in *Platanus Acerifolia* by (Liu *et al*., 2007), *Robinia pseudoacacia* L. and *Ceratonia silique* L. by (Omar, 2008), and in *Quercus aegilops* L. by (Toma, 2015), were indicated that a high concentration of colchicine and longer duration of immersion provided the reduction the rate of seed germination and plant survival.

Morphological characteristics:-

The aim of this work was to induce variation through colchicine treatment in seed and select some useful variant which can be stabilised and used for developing new variety. The ANOVA results showed that there was significant effect of interaction colchicine concentration × treatment duration on the length of seedling, shoot length,

shoot diameter, the number of branches, leaves area, leaf thickness and the length of roots. In the other hand, non-significant impact were found in germination percent, leaves number, leaf blade, root diameter and number. Means comparison analysis using Duncan multi test presented in table (2 and 3) showed that the highest mean of seedling height, shoot height and diameter (107.17, 46.67cm. and 6.36mm) respectively, was achieved by applying 0.15% colchicine for 12h. The lowest seedling height, shoot height and diameter were related to diploid plant with (56.05, 22.81cm and 4.19mm) sequentially. Based on the means comparison analysis Duncan Multi tests the application of 0.1% colchicine for 48h. produced the maximum mean of branch number per transplant (2.27 branch/transplant), whereas the minimum branch number was recorded in diploid plants with average of 1.00 branch per transplant. Also the leaves in colchiploid plants were border, thicker, darker green and remarkable variations in leaf shape among different ploidy level (Figure, 3), have been reported by many studies (Omar, 2008; Toma, 2015; Talebi *et al.,* 2017; Zinan *et al.,* 2018; and Zhang, *et al.,* 2018) that the Plant height, ground diameter, leaf area, and the photosynthetic parameter of triploid were significantly higher than those of the diploid plant. Were the Sattler *et al.,* (2016), provided that the process of induction polyploidy is called gigas effect, is the most important results of polyploidy are increased in cell size due to the addition of extra gene copies.

Table 2. The effect of different colchicine treatments on seedling traits of *C. siliquastrum*

No. of Treatments	Colchicine mgl^{-1}	Duration (h)	Germination Percentage (%)	Seedling Length (cm)	Shoot Length (cm)	Stem Diameter (mm)	No. of Branches/ Transplant	No. of Leaves/ Seedling
T1	0.0 control	12	80.00 ab ± (10.00)	56.05 e ± (5.93)	22.81 f ± (2.43)	4.19 cd ± (0.25)	1.00 d ± (0.00)	18.27 de ± (0.78)
T2	500		66.67 abcd ± (5.77)	98.99 ab ± (12.36)	45.46 ab ± (5.11)	5.47 abc ± (0.83)	1.20 cd ± (0.20)	23.93 abcd ± (2.11)
T3	1000		70.000 abcd ± (10.00)	85.96 bcd ± (12.95)	32.47 cdef ± (7.06)	4.70 bcd ± (0.43)	1.67 abcd ± (0.58)	24.40 abc ± (4.06)
T4	1500		70.00 abcd ± (10.00)	107.173 a ± (11.08)	46.57 a ± (6.08)	6.36 a ± (0.76)	1.00 d ± (0.00)	19.07 cde ± (3.04)
T5	2000		63.333 abcd ± (5.77)	87.38 abcd ± (11.20)	34.65 cde ± (9.10)	5.14 abcd ± (0.95)	2.20 ab ± (0.42)	29.40 a ± (3.17)
T6	0.0 control	24	76.67 abc ± (11.55)	60.367 e ± (2.48)	25.03 ef ± (2.67)	3.92 d ± (0.55)	1.27 cd ± (0.23)	17.33 e ± (1.53)
T7	500		66.67 abcd ± (15.27)	75.48 cde ± (8.15)	32.48 cdef ± (4.68)	4.69 bcd ± (0.49)	2.27 a ± (0.51)	25.20 ab ± (4.66)
T8	1000		60.00 bcd ± (10.00)	94.79 abc ± (13.17)	32.67 cdef ± (5.76)	4.80 bcd ± (0.81)	1.40 cd ± (0.40)	26.40 ab ± (3.14)
T9	1500		63.33 abcd ± (11.55)	88.66 abcd ± (10.24)	36.17 bcd ± (6.35)	4.99 bdc ± (0.93)	1.00 d ± (0.00)	21.20 bcde ± (2.31)
T10	2000		60.00 bcd ± (10.00)	74.77 de ± (12.55)	29.77 fde ± (5.05)	3.92 d ± (0.51)	1.53 bcd ± (0.12)	23.20 bcd ± (2.55)
T11	0.0 control	48	83.333 a ± (5.77)	59.17 e (3.72)	24.71 ef ± (1.17)	3.93 d ± (0.59)	1.33 cd ± (0.12)	18.27 de ± (1.12)
T12	500		70.00 abcd ± (17.32)	89.12 abcd ± (11.89)	39.20 abcd ± (5.31)	4.88 bcd ± (0.79)	1.40 cd ± (0.69)	26.00 ab ± (4.01)
T13	1000		70.00 abcd ± (17.32)	73.36 de ± (10.17)	30.800 def ± (4.33)	3.97 d ± (0.66)	2.27 a ± (0.42)	21.13 bcde ± (3.12)
T14	1500		56.67 cd ± (5.77)	62.19 e ± (8.99)	24.20 ef ± (3.28)	3.97 d ± (0.43)	1.80 abc ± (0.40)	22.00 bcde ± (3.22)
T15	2000		53.33 d ± (5.77)	100.73 ab ± (14.64)	42.1 abc ± (8.20)	5.55 ab ± (0.75)	1.33 cd ± (0.58)	25.07 ab ± (3.41)
P-value			0.8172	0.0004	0.0024	0.0243	0.0012	0.1386

Note: Means ± standard deviations of 3 independent replications in the same column followed by the same letters do not differ significantly (p<0.05) as determined by Duncan's multiple range test.

Red indicates the highest mean of seed germination and seedling morphological characters

The highest mean of leaf area corresponded to using 0.1% for 12h. Followed it T9 (0.15% for 24 h.) of colchicine concentration and period of soaking, in area (29.55 and 25.67 cm^2) respectively, while and also the lowest mean for this trait was related to diploid plants (T1) in area 14.86 cm^2. Were the thicker leaf produced also in 0.01 of colchicine for 12 h. in other hand the thinner leaves produced in control treatment plants (T11). Depending on ANOVA test non-significant effect of different ploidy level on the leaf blade (leaf length/leaf width) results of the leaf shape of *Cercis siliquastrum* L. it was circular to heart shape (Figure 3), and leaf length and width were very close to it. According to the results of the means comparison analysis using Duncan multi test, the application of 0.1% followed it 0.15 of colchicine led to the highest means of root length (62.13 and 60.60cm) respectively. The lowest means were achieved in 0.0 % of colchicine treatment control in root length (Table 3). The application of colchicine also led to different shapes and sizes of leaves in the *Tetradenia riparia* medicinal plant (Hannweg *et al.,* 2016).

Applying the Duncan Multi test (table, 3). The larger means of leaves number per transplant (29.4 leaves) were recorded in 0.2% for 24 h. of colchicine, whilst

lowest founded in diploid plants (17.33 leaves). The thicker and huge number of roots (6.45 mm and 5.6 roots) respectively, were achieved also in treatment (T5) 0.2% for 24 h. of colchicine solution, conversely thinner root and lowest number of roots present in control treatment plants. Sattler *et al*. (2016), according from cultivation techniques to genetic breeding, founded there was considerable interest in improving the organ size of agriculture and forestry products. Polyploidization is generally recognized as an effective strategy to improve organ size. Handayani *et al*, (2018), investigated that the larger cells produce larger parts of the plant such as leaves, flowers, fruits, and plants. Whereas the increase in leaf area leads to increase the biological processes such as photosynthesis which in turn leads to increase the growth of plant (Beest *et al*., 2012 and Li *et al.*, 2014). Visual evaluations were also showed the increasing effects of polyploidy induction on plant height and leaf area (Fig. 1 and 2).

Figure 1. ***Cercis siliquastrum*** **seedlings grown in greenhouse at 8 months old. (Polyploid plant and Diploid plant**).

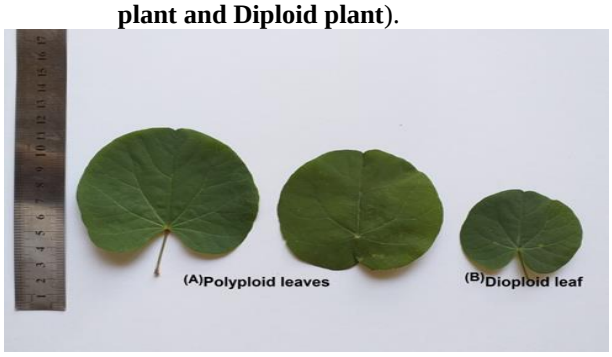

Figure 2. ***Cercis siliquastrum*** **seedling leaves for (A) Polyploid plant, (B) Diploid plant**

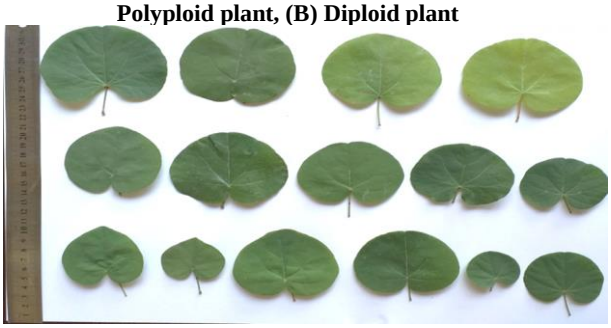

Figure 3. Variation in leaves shape, size and color of ***Cercis siliquastrum*** **L.**

Physiological characteristics:-

Chlorophyll is the green pigments in leaves, is very important in plant life through the process of photosynthesis. It had been not in a number of studies that as ploidy level increases the chlorophyll concentration within the polyploids leaves increases (Romero-aranda *et al.*, 1997). Recently, the quantification of chlorophyll concentration using chlorophyll absorbance will successfully apply to distinguish between diploid and tetraploid.

The accumulation of high chlorophyll contents in the leaves of tetraploid plants may be relevant to increase number of chloroplasts in the stomata guard cells. ANOVA test analysis proved there were significant differences between treated seeds with colchiploidy and untreated control seed on the chlorophyll (a, b and total) content at ($P<0.001$, 0.003 and 0.0001) respectively which showed in table (4). Duncan Multi test also investigated there were significant differences between diploid and tetraploid plants. The results (Table 2) demonstrated that highest chlorophyll content (a, b and total) of the tetraploid plants (15.56 ± 1.01, 3.28 ± 0.51 and 18.74 ± 1.69) respectively, were obtained in T3 and T15 and significantly higher than those of the diploid plants (9.28 ± 0.70, 1.16 ± 0.18 and 10.44 ± 0.88) respectively, were founded in control treatment (T6). The result pointed out that polyploidy plants leaves were darker than diploid plants and they contained higher of chlorophyll a, b and total compere to control plants treatment. The results of chlorophyll were agreement with Omar (2008); Tulay and Unal (2010); Grouh *et al.* (2011); Toma (2015) and others. On the other, the results were disagreeing with the study of both Ariyanto and Supriyadi (2011) and Yildiz (2013) who mentioned that there is a negative correlation between chloroplast number and ploidy level and the chlorophyll a, chlorophyll b and total chlorophyll contents of tetraploid sugar beet genotypes were found to be lower than diploid.

Anatomical characteristics

Stomata characters:-

One of the most appropriate features that can be used as a strong indicator of the ploidy level in plants is stomatal density (Gomes *et al.*, 2014). While Omidbaigi *et al*., (2010) reported that Tetraploid plants could be identified with a fair amount of certainty when the screening was based on the size of stomata and density of stomata.

More then, Zlesak, (2005), detected the size of stomata increases with increased ploidy level, so the size of stomata can be used to test the ploidy of plants, were Sadhukhan, *et al.*, (2014), they used the stomatal lengths as the alternative method for the determination of ploidy in plants. The averages and range of the stomata length, width and density of plants were 17.35 (14.27-23.69), 8.42 (6.95-11.43µm), 116.01 (65.00-190.0 in mm^2) respectively. A comparison of stomata characteristics in diploid and tetraploid plants (Figure, 4) showed the stomata length and width increased while stomata number per area decreased in colchicine-induced tetraploid plants. The ANOVA test in table (4), investigated there were high significant effect of interaction between colchicine concentration and soaked time on stomata size and density in leaf area at ($P < 0.01$).

Table 3. The effect of different colchicine treatments on seedling traits of *C. siliquastrum*

No. of Treatments	Colchicine mgl^{-1}	Duration (h)	Largest Leaf Area (cm^2)	Leaf Blade Length / Leaf Blade Width	Leaf Thicknesses (mm)	Root length (cm)	Root diameter (mm)	Number of secondary roots
T1	0.0 control	12	14.86 e ± (1.11)	0.922 a ± (0.06)	0.32 def ± (0.02)	33.25 d ± (4.21)	4.76 cde ± (0.64)	2.60 bcd ± (0.40)
T2	500		22.91 bc ± (2.54)	0.86 a ± (0.04)	0.38 bcdef ± (0.03)	53.53 ab ± (7.35)	6.23 ab ± (0.54)	4.13 abc ± (1.29)
T3	1000		29.55 a ± (4.16)	0.89 a ± (0.07)	0.51 a ± (0.09)	53.48 ab ± (6.10)	5.64 abcde ± (0.70)	4.47 ab ± (0.70)
T4	1500		21.03 bcd ± (2.34)	0.90 a ± (0.02)	0.38 bcdef ± (0.05)	60.60 a ± (10.10)	6.35 ab ± (1.20)	2.67 bcd ± (1.10)
T5	2000		23.97 abc ± (2.14)	0.86 a ± (0.03)	0.42 abcdef ± (0.05)	52.74 ab ± (7.41)	6.45 a ± (0.86)	5.60 a ± (1.21)
T6	0.0 control	24	15.65 de ± (1.66)	0.87 a ± (0.08)	0.33 def ± (0.05)	35.34 d ± (2.56)	4.47 e ± (0.41)	2.13 d ± (0.31)
T7	500		23.99 abc ± (3.36)	0.867 a ± (0.17)	0.37 bcdef ± (0.07)	43.01 bcd ± (11.71)	4.77 cde ± (0.57)	2.33 cd ± (0.81)
T8	1000		20.01 bcde ± (2.62)	0.90 a ± (0.06)	0.34 cdef ± (0.04)	62.13 a ± (7.46)	5.63 abcde ± (o,61)	4.33 ab ± (1.11)
T9	1500		25.67 ab ± (4.42)	0.85 a ± (0.06)	0.42 abcde ± (0.05)	52.49 ab ± (7.62)	5.92 abcd ± (0.61)	3.47 bcd ± (1.30)
T10	2000		19.47 cde ± (3.70)	0.88 a ± (0.04)	0.32 f ± (0.03)	44.99 bcd ± (8.11)	5.04 bcde ± (0.65)	3.67 bcd ± (0.81)
T11	0.0 control	48	16.04 de ± (1.70)	0.84a ± (0.06)	0.30 f ± (0.03)	34.45 d ± (2.73)	4.58 de ± (0.46)	2.40 cd ± (0.53)
T12	500		19.86 bcde ± (2.77)	0.87 a ± (0.07)	0.451 abc ± (0.07)	49.920 abc ± (6.59)	5.765 abcde ± (0.79)	4.000 abcd ± (1.40)
T13	1000		23.03 bc ± (3.46)	0.92 a ± (0.07)	0.430 abcd ± (0.06)	42.560 bcd ± (7.52)	4.477 e ± (0.64)	2.867 bcd ± (0.52)
T14	1500		18.26 cde ± (4.64)	0.94 a ± (0.09)	0.349 bcdef ± (0.10)	37.993 cd ± (5.62)	4.633 de ± (0.71)	2.600 bcd ± (0.42)
T15	2000		22.95 bc ± (2.78)	0.85 a ± (0.05)	0.459 ab ± (0.06)	58.587 a ± (6.48)	6.104 ab ± (0.94)	3.233 bcd ± (1.10)
P-value			0.0112	0.5450	0.0208	0.0037	0.0660	0.0655

Note: Means ± standard deviations of 3 independent replications in the same column followed by the same letters do not differ significantly (p<0.05) as determined by Duncan's multiple range test.

Red indicates the highest mean of seed germination and seedling morphological characters

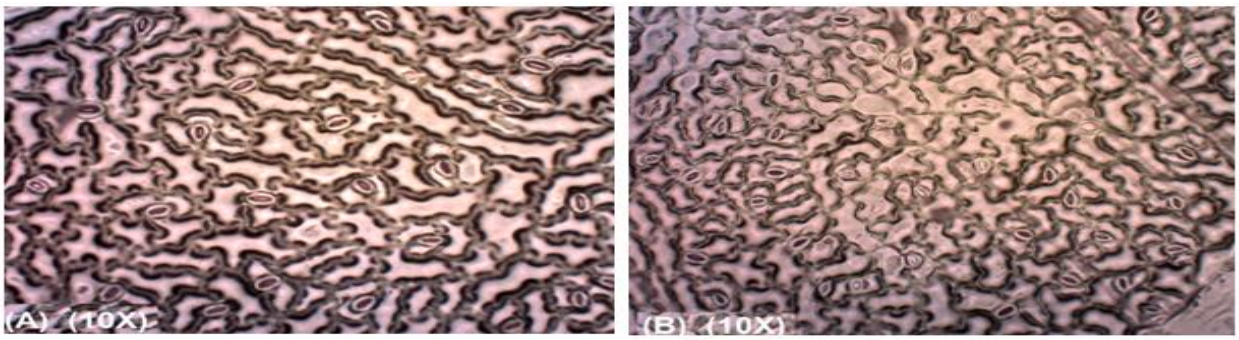

Figure 4. Stomata size and density for (A) Polyploid plant, (B) Diploid plant in *Cercis siliquastrum* L.

The results demonstrated of induced tetraploid treatment T13 (0.10% of colchicine and socked for 48h) was most efficiency and recorded high value on stomata length (21.62 ± 2.17µm) and stomata width (10.30 ± 0.99µm) which were significant from control and other colchicine treatments at (P<0.0001) followed it T15 (0.2% for 48 h.), while the few number or lower density of stomata observed high colchicine concertation solution (0.2% for 48h) treatment with means and standard deviation (68.0 ± 4.60 stomata/mm^2). Whereas the small stomata and highly density recorded in control treatment in T1, T6 and T11), the averages of stomata characteristics of diploid plants were (14.90 µm) length, (7.35 µm) width and stomata density of diploid plants was (133.3cells/mm^2).

The stomata length and width dramatic increased while reduction in the stomata density of colchicineinduced tetraploid plants have also been reported in other ploidy induction studies (Aina *et al.*, 2012; Majdi *et al.*, 2010; Toma 2015; Xu *et al*. 2016; Talebi *et al.*, 2017; and Yang, 2018). The higher photosynthetic efficiency of the triploid plants may be able to explain their significant faster growth in plant height and ground diameter (Liao *et al.*, 2016).

Table 4. The effect of different colchicine treatments on seedling traits of *C. siliquastrum*

No. of Treatments	Colchicine mgl^{-1}	Duration (h)	Chlorophyll (a) Content (mg/g)	Chlorophyll (b) Content (mg/g)	Chlorophyll (b) Content (mg/g)	Stomata Length (mm)	Stomata Width (mm)	No. of stomata / mm^2
T1	0.0 control	12	10.45 fg ± (0.42)	1.21 cde ± (0.19)	11.66 f ± (0.59)	14.46 e ± (0.35)	7.35 e ± (0.16)	132.67 a ± (3.79)
T2	500		11.74 def ± (0.81)	2.14 abc ± (0.25)	13.88 de ± (0.96)	17.13 cd ± (0.69)	8.02 de ± (0.39)	118.66 b ± (13.87)
T3	1000		15.56 a ± (1.01)	3.18 a ± (0.98)	18.74 a ± (1.69)	17.29 cd ± (0.91)	8.09 cde ± (0.26)	101.67 cd ± (12.58)
T4	1500		14.60 ab ± (0.88)	2.04 bcd ± (0.21)	16.64 bc ± (1.06)	21.33 a ± (1.84)	9.75 ab ± (1.19)	96.67 cd ± (7.64)
T5	2000		12.07 de ± (0.53)	2.15 abc ± (0.43)	14.22 d ± (0.84)	16.13 de ± (1.02)	8.04 de ± (0.75)	109.667 bc ± (6.90)
T6	0.0 control	24	9.28 g ± (0.70)	1.16 cde ± (0.18)	10.44 f ± (0.88)	14.64 e ± (0.41)	7.347 e ± (0.28)	134.00 a ± (4.58)
T7	500		11.49 def ± (0.31)	0.74 e ± (0.28)	12.23 ef ± (0.27)	18.29 cd ± (0.78)	9.22 abcd ± (0.71)	94.67 d ± (9.50)
T8	1000		11.56 def ± (0.79)	3.20 a ± (0.54)	14.76 cd ± (1.11)	16.47 de ± (1.51)	8.62 bcd ± (0.85)	117.50 b ± (12.50)
T9	1500		12.26 cd ± (0.97)	3.08 ab ± (0.24)	15.34 bcd ± (0.74)	17.67 cd ± (0.87)	8.75 bcd ± (0.75)	96.67 cd ± (8.95)
T10	2000		14.37 ab ± (1.23)	2.80 ab ± (0.45)	17.17 ab ± (1.33)	17.61 cd ± (0.87)	8.70 bcd ± (0.40)	96.67 cd ± (7.63)
T11	0.0 control	48	10.32 fg ± (0.38)	1.02 de ± (0.16)	11.34 f ± (0.24)	14.90 e ± (0.19)	7.32 e ± (0.26)	133.33 a ± (8.51)
T12	500		12.52 cd ± (0.67)	2.90 ab ± (0.94)	15.42 bcd ± (1.37)	17.35 cd ± (1.14)	7.97 de ± (0.46)	100.00 cd ± (7.04)
T13	1000		11.03 ef ± (0.21)	2.59 ab ± (1.15)	13.62 de ± (1.23)	21.62 a ± (2.17)	10.30 a ± (0.99)	93.33 d ± (6.29)
T14	1500		11.74 def ± (1.06)	2.57 ab ± (0.74)	14.31 d ± (1.74)	18.92 bc ± (1.82)	9.12 abcd ± (0.73)	71.34 e ± (9.86)
T15	2000		13.58 bc ± (0.62)	3.28 a ± (0.51)	16.86 ab ± (1.08)	20.74 ab ± (1.46)	9.33 abc ± (0.56)	68.00 e ± (4.60)
P-value					<.0001	0.0037	<.0001	<.0001

Note: Means ± standard deviations of 3 independent replications in the same column followed by the same letters do not differ significantly ($p<0.05$) as determined by Duncan's multiple range test.

Red indicates the highest mean of seed germination and seedling morphological characters

Wood shoot anatomical characters

The overall mean values and varies of fiber length, fiber diameter, double cell wall thickness, vessel length and vessel width are observed to be 0.72 (0.52-0.88) mm, 13.33 (9.10-17.21), 5.99(4.78-7.46), 71.87 (44.74-91.56) and 11.19 (7.82-19.26) µm respectively (table 5). On comparing the fiber and vessel dimensions achieved the fiber and vessel of tetraploid plants was longer and wider than diploid plants (Table 5). Test of means using Duncan multi indicates the length fiber and vessel were observed in T14 (0.15% of colchicine soaked for 48 h.) an overall mean of 0.83 mm and 88.91 µm, while the dwarf fiber and vessels were founded in diploid plants (T1) with the overall mean is of 0.57mm 54.59 µm. The maximum values of both fiber diameter and double cell wall thickness were also observed to be 15.69 and 7.03 µm receptively in 0.15% of colchicine solution for 24h., while the minimum value of fiber diameter and double cell wall thickness is found to be 12.07 and 5.26 µm receptively in control or diploid plants. In the end the width vessel was recorded in 0.05% followed it 0.25 of colchicine concentration, while thinner was observed in control treatment plants. If these differences between the wood of polyploid and diploid individuals investigated persisted until the plants attained commercial timber size, a breeding program to increase the fiber length by selecting polyploid Judas tree can be easily justified by cholchploidy induction.

According to the results the increased in dimensions and area were probably due to the fact that cells with a larger complement of chromosomes grow larger to maintain a constant ratio of cytoplasmic to nuclear volume, and express more proteins with the presence of more genes (Amiri *et al*., 2010). In study by Griffin *et al*., (2014) revealed the tetraploid plants produced length, wider and greater cell wall thickness in libiform fiber and fiber tracheid, in addition to the longer and diameter of vessel, compared to the diploid plants which were less than treated seedlings. In the other hand, Nelson (2000) detected the amount of primary and secondary fiber was greater in tetraploid plants comparing to diploid plants, and the change of ploidy caused changes on economically valuable characteristics of wood.

Table 5. The effect of different colchicine treatments on seedling traits of *C. siliquastrum*

No. of Treatments	Colchicine mgl^{-1}	Duration (h)	Fiber Length (mm)	Fiber Diameter (µm)	Double Cell Wall Thickness of Fiber (µm)	Vessel Length (µm)	Vessel Diameter (µm)
T1	0.0 control	12	0.57 h ± (0.042)	12.07 cde ± (0.64)	5.26 f ± (0.59)	54.595 f ± (5.01)	9.78 cde ± (0.79)
T2	500		0.66 def ± (0.039)	10.91 e ± (0.79)	5.62 cdef ± (0.53)	69.60 de ± (4.33)	11.20 c ± (1.04)
T3	1000		0.66 efg ± (0.028)	13.45 bcd ± (0.48)	5.99 bcdef ± (0.20)	83.49 ab ± (6.51)	9.99 cde ± (0.89)
T4	1500		0.77 abc ± (0.042)	14.25 b ± (0.96)	6.38 abc ± (0.43)	69.15 de ± (5.00)	9.26 ed ± (0.45)
T5	2000		0.787 ab ± (0.04)	12.81 bcd ± (0.57)	6.16 bcde ± (0.18)	69.49 de ± (5.66)	10.52 cde ± (0.70)
T6	0.0 control	24	0.58 gh ± (0.034)	11.99 ed ± (0.78)	5.49 def ± (0.36)	63.17 ef ± (5.31)	9.09 e ± (0.25)
T7	500		0.67 def ± (0.043)	13.12 bcd ± (0.65)	5.78 cdef ± (0.31)	70.58 cde ± (5.28)	10.73 cd ± (0.62)
T8	1000		0.70 cde ± (0.039)	14.28 b ± (0.67)	6.14 bcde ± (0.29)	75.14 bcd ± (5.03)	10.27 cde ± (0.61)
T9	1500		0.76 abc ± (0.050)	15.69 a ± (0.54)	7.03 a ± (0.29)	76.27 bcd ± (2.41)	10.35 cde ± (0.99)
T10	2000		0.739 abc ± (0.064)	13.62 bc ± (1.29)	6.21 bcd ± (0.19)	80.12 abc ± (7.62)	14.46 b ± (1.34)
T11	0.0 control	48	0.61 fgh ± (0.028)	12.13 cde ± (0.39)	5.38 ef ± (0.59)	60.78 ef ± (3.47)	9.83 cde ± (0.31)
T12	500		0.70 bcde ± (0.047)	13.19 bcd ± (0.88)	6.23 bcd ± (0.33)	67.00 de ± (4.58)	16.52 a ± (1.62)
T13	1000		0.68 def ± (0.065)	13.48 bcd ± (0.92)	6.12 bcde ± (0.41)	80.73 ab ± (5.20)	14.52 b ± (1.31)
T14	1500		0.83 a ± (0.038)	13.57 bc ± (0.85)	6.32 abc ± (0.36)	88.91 a ± (2.70)	13.45 b ± (1.12)
T15	2000		0.82 a ± (0.041)	14.10 b ± (0.91)	6.73 ab ± (0.63)	75.45 bcd ± (7.23)	16.02 a ± (1.19)
P-value			0.5910	0.0359	0.3571	0.0048	<.0001

Note: Means ± standard deviations of 3 independent replications in the same column followed by the same letters do not differ significantly ($p<0.05$) as determined by Duncan's multiple range test.

Red indicates the highest mean of seed germination and seedling morphological characters

CONCLUSION

Morphological and physiological characteristics can be used as useful parameters for preliminary screening of putative polyploids in this Judas tree. Results showed that treated seed with colchicine at different concentrations in various periods significantly affect the germination rate of seed, seedling performance, and morphological, physiological and anatomical characters. Many superior traits in tetraploids as compared to control seedlings depending on the interaction were achieved. Also there were high variations in characteristics studies results of this interaction. These changes in seedling characters suggested ploidy manipulation as a rapid, effective method for enhancing genetic diversity and metabolite production and to use in breeding program.

REFERENCES

Amiri, S.; S.K. SKazemitabaar; G. Ranjbar; M. Azadbakht (2010). The Effect of Trifluralin and Colchicine Treatments on Morphological Characteristics of Jimsonweed (*Datura stramonium* L.) Trakia Journal of Sciences. 8 (4): 47-61.

Amer, J.; N. Jaradat; S. Hattab; S. Al-hihi and R. Juma'a (2019). Traditional Palestinian medicinal plant *Cercis siliquastrum* (Judas tree) inhibits the DNA cell cycle of breast cancer – Antimicrobial and antioxidant characteristics. *European Journal of Integrative Medicine 27 (2019) 90–96*

Aina, O; K. Quesenberry and M. Gallo (2012). In vitro induction of tetraploids in *Arachis paraguariensis*. Plant Cell Tissue Organ Cult (PCTOC) 111(2):231–238

Ariyanto, S.E. and P. Supriyadi (2011). Pengaruh kolkisin terhadap fenotipe dan jumlah kromosom Jahe (*Zingiber officinale* rosc.). University Muria Kudus. Available at: http:// eprints.umk. ac.id / 123/ 1/ PENGARU H _ KOLKISIN _ TERHADAP _ FENOTIPE.pdf

Barwary, V. H. A (2015). A Comparative systematic study of Caesalpiniaceae and Mimosaceae in Kurdistan region Iraq. M.Sc. Thesis submitted to the council of the Collage of Agriculture/University of Duhok.

Beest, M.; J. J. Le Roux; D. M. Richardson; A. K. Brysting; J. Suda; M. Kubesˇova and P. Pysˇek (2012). The More the Better? The Role of Polyploidy in Facilitating Plant Invasions. Annals of Botany. 109: 19-45.

Cejka, J. and S. Al-Aamiry (1981). Trees and shrubs for landscapes use in Jordan and neighboring countries. Selden and Tamm, Jordan.

David, J.R., and J.W. Dennis (2016). Genome size and ploidy levels of *Cercis* (Redbud) species, cultivars, and botanical varieties. HORTSCIENCE 51(4):330–333.

Davis, C.C.; P.W. Fritsch; J. Li and M.J. Donoghue (2002). Phylogeny and biogeography of *Cercis* (Fabaceae): Evidence from nuclear ribosomal ITS and chloroplast ndhF sequence data. Syst. Bot. 27:289–302.

Fritsch, P.W.; K.W. Larson and A.M. Schiller (2009). Taxonomic implications of morphological variation in *Cercis canadensis* (Fabaceae) from Mexico and adjacent parts of Texas. Syst. Bot. 34:510–520.

Franklin, G. L. (1945). Preparation of Thin Sections of Synthetic Resins and Wood-Resin Composites, and a New Macerating Method for Wood. Nature. 155: 51.

Gebre, G.H. and N.S Karam (2004). Germination of *Cercis siliquastrum* seeds in response to gibberellic acid and stratification. Seed Sci. Technol. 32(1):255-260.

Gomes, S.S.L.; C,W. Saldanha; C.S. Neves; M. Trevizani; N.R.B.Raposo; M.M. Notini; M. de Oliveira Santos; J.M.S. Campos; W.C. Otoni and L.F. Viccini (2014). Karyotype, genome size, and in vitro chromosome doubling of *Pfaffia glomerata* (Spreng.) Pedersen. Plant Cell, Tissue and Organ Cult (PCTOC) 118(1):45–56.

Grouh, M.S.H.; H. Meftahizade; N. Lotfi; V. Rahimi and B. Baniasadi (2011). Doubling the chromosome number of *Salvia hians* using colchicine: Evaluation of morphological traits of recovered plants. J. Med. Plant. Res. 5(19), 4892-4898.

Griffin, A. R.; H. Twayi; R. Braunstein; G.M. Downes; D.H. Sona and C.E. Harwood (2014). A comparison of fiber and pulp properties of diploid and tetraploid *Acacia mangium* grown in Vietnam. Peer Reviewed. 67(1).

Jazirei, M. H (2001). Aforestation in dryland. Tehran University Publication 447 p.

Liao, T.; S. Cheng; X. Zhu; Y. Min and X. Kang (2016). Effects of triploid status on growth, photosynthesis, and leaf area in *Populus*. Trees-Struct. Funct. 30, 1137–1147.

Li, P. Z. Cheng; B. Ma; J.A. Palta; H. Kong; F. Mo; J. Wang; Y. Zhu; G. Lv; A. Batool; X. Bai; F. Li and Y. Xiong (2014). Dry land wheat domestication changed the development of aboveground architecture for a well-structured canopy. Plos One. 9 (9).

Liu G.; Z. Li and M. Bao (2007). Colchicine-induced chromosome doubling in *Platanus acerifolia* and its effect on plant morphology. Euphytica, 157: 145–154.

Knudsen, L. L.; T.W. Tibbitts and G.E. Edward (1977). Measurement of ozone injury by determination of chlorophyll concentration. Plant Physiol. Vol. 60: 606-608.

Han, D.S.; Y. Niimi and M. Nakamo (1999). Production of doubled haploid plants through colchicine treatment of anther-drived haploid calli in *Asiatic hybrid lilly*. - J. Jap. Soc. Hort. Sci., 68: 979-983.

Handayani, Rd. S.; M. Yusuf and A. Akma (2018). Potential changes in watermelon (*Citrullus lannatus*) ploidy treated by colchicine. J. of Tropical Horticulture 1(1),10-14.

Hannweg, K.; G. Visser; K. de Jager and I. Bertling (2016). In vitro-induced polyploidy and its effect on horticultural characteristics, essential oil composition and bioactivity of *Tetradenia riparia*. S Afr J Bot 106:186–191.

Haroni, N. N.; M. Tabari and D. Dey (2015). Effect of halopriming treatment on seed germination and seedling emergence of Judas tree (*Cercis siliquastrum* L., Caesalpiniaceae) from Zanjan, Iran. Afr. J. Agric. Res.10(23) 2355-2362.

Majdi, M.; G. Karimzadeh; M.A. Malboobi; R. Omidbaigi and G. Mirzaghaderi, (2010). Induction of tetraploidy to feverfew (*Tanacetum parthenium* Schulz-Bip.) morphological, physiological, cytological, and phytochemical changes. HortScience 45(1):16–21

Murali, K.M.; V. Jeevanandam; J. Shuye and R. Srinivasan (2013). Impact of colchicine treatment on *Sorghum bicolour* BT× 623, Mol. Plant Breed. 4(15):128–135.

Nelson, R. A. (2000). Hemp husbandry. Botany & Breeding. Available at: http: // www. rexresearch. com /hhusb/hh4bot.htm

Noori1, S. A. S.; M. Norouzi; G. Karimzadeh; K. Shirkool1 and M. Niazian1 (2017). Effect of colchicine-induced polyploidy on morphological characteristics and essential oil composition of ajowan (*Trachyspermum ammi* L.) Plant Cell Tissue Organ Cult DOI 10.1007/s11240-017-1245-0

Omidbaigi R.; S. Yavari; M.E. Hassani and S. Yavari (2010). Induction of autotetraploidy in dragonhead (*Dracocephalum moldavica* L.) by colchicine treatment. J Fruit Ornam. Plant Res. 18(1), 23-35.

Omar, O.M. (2008). Induction of chromosomal polyploidy and early evaluation of *Ceratonia siliqua* L. and *Robinia pseudoacacia* L. transplants. M.Sc. thesis, forest science, collages of agriculture and forestry, Mosul University.

Papanastasis V.P.; P.D. Platis and O. Dini-Papanastasi (1997). Productivity of deciduous woody and fodder species in relation to air temperature and precipitation in a Mediterranean environment. Agroforest. Syst. 37:187-198.

Pereira R.C.; M.T.M. Ferreira; L.C. Davide; M. Pasqual; A. Mittelmann and V.H. Techio (2014). Chromosome duplication in *Lolium multiflorum* Lam. Crop Breed. Appl. Biot. 14(4), 251-255.

Pijut, P.M. (2008). *Carpinus*. In: Bonner FT and Karrfalt RP (eds.). Woody plant seed manual, Agriculture Handbook 727, USDA Forest Service, Washington, DC. pp. 328-332.

Pirkoohi M.H.; M. Keyvanloo and M. Hassanpur (2011). Colchicine induced polyploidy in mint by seed treatment. Int. J. Agric. Crop. Sci. 3-4, 102-104.

Rao, E. M. S. (1996). Improvement of mulberry through polyploidy breeding. Ph.D. Thesis, Bangalore University, Bangalore, India.

Romero-Aranda, R.; B.R. Bondada; J.P. Syvertsen and J.W. Grosser (1997). Leaf characteristics and net gas exchange of diploid and autotetraploid citrus. Ann. Bot. 79:153–160.

Sadhukhan, R.; A. Ganguly; P.K. Singh and H.K. Sarkar (2014). Study of induced polyploidy in african marigold (*Tagetes crecta* L.). Environment and Ecology. 32(4).1219-1222.

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

Schneider, C. A.; W.S. Rasband and K.W. Eliceiri (2012). NIH Image to ImageJ 25 years of image analysis, Nature methods, 9(7):671-675.

Shabaz, S. E. (2010). A field guide to the trees and shrubs of Kurdistan Region of Iraq. UoD. Iraq.

Sourour, A.; B. Ameni and C. Mejda (2014). Efficient production of tetraploid barley (*Hordeum vulgare* L.) by colchicine treatment of diploid barley. J. Exp. Biol. 2(1S), 113-119.

Talebi, S.F.; M.J. Saharkhiz M.J. Kermani; Y. Sharifi and F.F. Raouf (2017). Effect of different antimitotic agents on polyploid induction of Anise Hyssop (*Agastache Foeniculum* L.). Caryologia, 70(2): 184-193.

Tojyo, I. (1966). Studies on polyploidy mulberry tree I. Breeding of artificial autotetraploids. Bull. Seri. Expt. Stn., 20(3): 187 – 207.

Toma, R., H. (2015). Induction of chromosomal polyploidy and early evaluation of valonia oak *(Quercus aegilops* L.) Transplants. M.Sc. thesis, forest science, collages of agriculture, Duhok University.

Tulay, E. and M. Unal (2010). Production of colchicine induced tetraploids in *Vicia villosa* roth. Caryologia 63(3), 292-303.

Unal, H.; M. Zencirkiran and Z. Tümsavaş (2009). Some engineering properties of *Cercis siliquastrum* L. seed as a function of stratification and acid treatment durations. Afr. J. Agric. Res. 4(3):247-258.

Xu, C.; Z. Huang; T. Liao; Y. Li and X. Kang (2016). In vitro tetraploid plants regeneration from leaf explants of multiple genotypes in *Populus*. Plant Cell Tissue Organ Cult (PCTOC), 125(1):1–9

Yang, J.; J. Wang; Z. Liu; T. Xiong; J. Lan; Q. Han; Y. Li and X. Kang (2018). Megaspore chromosome doubling in *Eucalyptus urophylla* S.T. Blake induced by colchicine treatment to produce triploids. Forests 9, 728.

Yaşar Ü.; I.I. Özyiğit and M. Serin (2010). Judas tree (*Cercis siliquastrum* L. subsp. *siliquastrum*) as a possible biomonitor for Cr, Fe and Ni in Istanbul (Turkey). University of Bucharest, Romanian Biotechnological Letters 15(1): 4979–4989.

Yildiz, M. (2013). Plant responses at different ploidy levels. current progress in biological research. Available a http: // www .intechopen. com / books /current-progress-in-biological

Zlesak, D.C.; C.A. Thill and N.O. Anderson (2005). Trifluralin-Mediated Polyploidization of *Rosa chinensis* minima (Sims) Voss Seedlings. Euphytica. Vol. 141: 281-290.

Zinan Luo; J. Brian; Iaffaldano; and C. Katrina (2018). Colchicine-induced polyploidy has the potential to improve rubber yield in *Taraxacum kok-saghyz* Industrial Crops & Products 112, 75–81.

Zhang, Y.; B. Wang; S. Qi; M. Dong; Z. Wang; Y. Li; S. Chen; B. Li and J. Zhang (2018). Ploidy and hybridity effects on leaf size, cell size and related genes expression in triploids, diploids and their parents in *Populus.* Planta, 1–12.

تأثير معاملة الكولشيسين على النموو الصفات المظهرية و الفسيولوجبة و التشريحية لشتلات الأرغوان

هشيار حازم سليمان[1] و هاجر سعيد أسكندر[2]

قسم الغابات- كلية علوم الهندسة الزراعية - جامعة دهوك hishyar.suliman@uod.ac

قسم المحاصيل الحقلية - كلية علوم الهندسة الزراعية - جامعة دهوك hajar.askandar@uod.ac

أجريت هذه الدراسة لتقييم تأثير أستحداث التضاعف الكرموسومي على خصائص مختلفة لشتلات الأرغوان(*Cercis siliquastrum*) وقد أستخدمت طريقة القطعات العشوائية البلوكات (RCBD) مع ١٥ معاملات و ٣ مكررات. تمت معاملة البذور باستخدام محلول كولشيسين ٠.٠ ، ٠.٥ ، ١.٠ ، ١.٥ و ٢.٠ ٪ في درجة حرارة الغرفة لأوقات غمر مختلفة ١٢ و ٢٤ و ٤٨ ساعة. تم تسجيل الملاحظات على الاختلافات المورفولوجية على كل نبات في كل معاملة. لوحظة أختلافات في نسبة إنبات البذور ، الخصائص المورفولوجية والنمو بسبب تأثير التدخلات بين تركيز الكولشيسين وفترات تعرض البذور. بزيادة تركيز الكولشيسين انخفض معدل إنبات البذور بشكل كبير من ٨٠ ٪ إلى ٥٠ ٪ ، وكانت الآثار على أنبات البذور أكثر وضوحا في تركيزات الكولشيسين (١.٥ إلى ٢.٠ ٪). أطول شتلة ، وأكبر قطر لسيقان وجذور، وعدد أكبر من الأوراق ، والفروع والجذور لكل شتلة ، وأوراق ذات مساحة سطح و سمك كبيرة وأخضر داكن اللون مقارنةً بالشتلات غير معالجة ، وتم تحقيق تباين في صفات التشريحية لساق الشتلات وتحسينها مقارنةً بالنباتات الغير معاملة. وتم زيادة طول وعرض الثغور (٢١.٦ و ١٠.٣ مليماكرون) على التوالي حيث أزداد بشكل كبير في نسبة ٠.١ ٪ من الكولشيسين لمدة ٤٨ ساعة ، في حين انخفضت كثافة الثغور إلى ٦٨ لكل مساحة في ٠.٢٪ من الكولشيسين لمدة ٤٨ ساعة. على العكس من النباتات ثنائي المجموعة الكروموسومية الأولية. ومع ذلك ، فإن تراكيز الكولشيسين بنسبة ١.٥ ٪ و ٠.٢ ٪ في أوقات الغمر المختلفة كانت أكثر فعالية في أستحداث التضاعف و أنتاج التباين بين النباتات. قد تكون هذه النباتات المفترضة مفيدة لمزيد من التطوير والتحسين لهذه الشجرة.

كلمات الدال .*Cercis siliquastrum* L **:**التباين، الكولشيسين ، تربية بالتضاعف الكروموسومي , خصائص الثغور