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INDUCTION AND FLOW CYTOMETRY, GC-MS IDENTIFICATION OF TETRAPLOIDS THROUGH COLCHICINE TREATMENTS IN *DATURA STRAMONIUM* L.

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Abstract

A field experiment study was conducted during two seasons in spring 2017-2018 at the college of agriculture engineering sciences-university of Baghdad-Iraq.The induction of artificial polyploidy may prove useful in increasing the production of some important secondary metabolic medicinal compounds such as a tropan alkaloids. In this study we tested the effects of fore levels 0.125 ,0.25, 0.5 and 1% of colchicine as C_1, C_2, C_3 and C_4,respectively as the first factor and two incubation times of ploidy (T_1) 24 and (T_2) 48hours as the second factor in addition to the control C_0 treatment on jimsonweed plant (*Datura stramonium* L.) to determinate the ploidy levels may be accrued andobtaining tetraploid plants with the changes in their morphological and physiological traits such as of branches per plant, the height of plant, leaves area, total chlorophyll content, dry weight,, total alkaloids. Also, total chlorophyll content was determined by SPAD (single -pass albumin dialysis). The phytochemical content including Hyoscyamine and scopolamine levelswere measured by GC-MS and Polyploidy levels by Flow cytometry methods in the second generation plants. The Tetraploidy occurred by seeds treatment with 1% Colchicine for 48 h was improved the vegetative growth and increased the useful a tropan alkaloids percent in the Datura leaves. Several polyploidy plants of Datura were induced by colchicine's treatments were compared with their untreated plants on vegetative traits and phytochemical compounds, the results indicate a reduction in plant height, increases in plant wet and dry weight, leaves areaand total alkaloid ratio in the all polyploidy plants at the all Colchicine levels. The analysis results showed the presence of Polyploidy plants (4X =100%)when we treated Datura seeds with aqueous solution of colchicine 1% for 48h. The Tetraploidy 4X plant showed the significantly increased in total alkaloids content 7.25% compare with untreated plant 4.22% , with increasing in the active tropian alkaloids such as a Hyoscyamine and scopolamine levels up to 2.6 and 3.0 times respectively to controltimes the control, respectively. Also the phytochemical compounds were induced by 1% Colchicine for 48h seeds treatment in polyploidy plant, and new photochemical compounds were appeared in this treatment such as Palustrol, (S)-Citronellic acid and Widdrol or Increase the other oneswhich have a medicinal values .

Key words : *Datura stramonium* L, Flow cytometry, GC-MS analysis, Tetraploidy, Colchicine, Hyoscyamine, Scopolamine, Total alkaloids, Photochemical compounds.

Introduction

The *Datura stramonium* L. (Solanaceae) had contained different types of phytochemical compounds including Alkaloids such as hyoscyamine and scopolamine, saponins, tannins, steroids, phenols, glycosides and flavonoids (Waza *et al.*, 2015) with antiasthmatic, anticholinergic, antimicrobial, anticancer, antiinflamatory, antifungal, vibriocidal and analgesic activity (Soni *et al*., 2012). Among Solanaceae plants, *Datura stramonium* L. is highly interested by the researchers, since it has a great resource of tropane alkaloids. Botanical alkaloids are one of the important products and form the major part of medicinal compounds. The alkaloids found in *D. stramonium*, are organic esters used clinically as anticholinergic agents. Tropane alkaloids are the phytochemicals investigations of Datura demonstrated thatseeds and leaves especially were rich in alkaloids, including scopolamine hyoscyamine and tropane. The

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67thalkaloids were determinate inof *Datura stramonium* L. by GC/MS,Scopolamine and hyoscyamine seem as the major tropane alkaloids in the plant organs (El Bazaouia *et al*., 2012). These two tropane alkaloids are included in many official pharmacopoeias because of their anticholinergic activities. Chromosome duplication using colchicine has long been used successfully used in plant breeding to produce the superior crops and increase the content of useful main and secondary compounds. In most plants, artificial polyploidy is often accompanied by increase cell size, leading to larger vegetative organs (Ahmadi *et al*., 2008). Colchicine works by disrupting the polymerization of microtubules which disrupts spindle fibers development at mitosis stage. Amiri *et al*. (2010) reported that the effects that colchicine treatments showed that the stem of tetraploidy was shorter than diploids, the tetraploid plant was less in number of leaves per branch, butnumber of branches had increased, leaf dry weight and in total chlorophyll content.Polyploidy is generally viewed as an important driver of plant evolution in natural populations (Otto and Whitton, 2000). Moreover, polyploidy has played a crucial role in the domestication of crops, such as wheat, maize and cotton (Dubcovsky and Dvorak, 2007). Flow cytometry (FCM) would appear to offer advantage in plant breeding and taxonomy to screen ploidy levels and might be used especially are very rapid and easy marker for ploidy manipulation such as polyploidization by colchicine. Poly level assessed by flow cytometry of nuclei showed that the putative the measured fluorescence is correlated with the DNA content of the stained nuclei. Tetraploids had double the amount of DNA confirming autotetraploidy and an important advantage of (FCM) over other methods is the ability to identify mixoploids and greatly increase the efficiency of screening ploidy levels (Dolezel, 1991; Levin, 2002).

Materials and Methods

A factorial field experiment was carried out at College of Agriculture Engineering Sciences, University of Baghdad during two spring seasons of 2017 and 2018 to study the effect of fore levels 0.125, 0.25, 0.5 and 1% of colchicine (C) as the first factor symbolize to C_1, C_2, C_3 and C_4 in addition to the control C_0 and two incubation times of ploidy 24 and 48-hours (T) symbolize to T1 and T2 as the second factor in addition to the control C_0 treatment on Datura stramonium plant cultivated at the middle area of Iraq. A randomized complete block design by factorial arrangement was applied with three replicates to study the effect of incubation times with Colchicine different concentrations and ploidy inducers were studied. Datura seeds, obtained from the Medical and Aromatical Research Unit, College of Agricultural Engineering Sciences, Baghdad University. Seeds were treated with an aqueous solution of colchicine with different concentrations for different time intervals and identify the ploidy level by Flowcytometry Tech. (FCM), *Datura stramonium* seeds of the M_0 breeder will be mixed with the distilled water and changed for 24 hours and then placed in special dishes for three days at the temperature of the refrigerator to encourage germination. At the beginning of the emergence of the stalk length of 2 mm will be selected 250 seeds of each dish and soaked for 24 & 48 h for fore treatments in the fore levels of colchicine and in distilled water for Control. After the treatment time, the seeds of the first M_0 baby embryo are washed well with distilled water for later cultivation in special flint dishes. The strong seedlings are then selected for active growth to be transferred to the plastic house, these plants detected as the first generation (M_1) was planted at the field till the flowering stage to be under selfing process till capsules maturation at the end of June-2017, the seeds of these plants were taken and save in paper bags in room temperature until the next season, the seedlings were then transplanted at the field on 1st-5th of March 2018. Individual phenotypic selection on the first-generation plants at the beginning of the flowering the diagnose the differenced plants of each treatment in morphological traits such as the superior appearance in the strength of growth, number of leaves, leaves size, leaves dark green colored and branches numbers, free from diseases and insects was selected then selfing till maturity of the capsules, these plants detected the second generation (M_2) were determined by FCM and GC-MS methods.Plant leaves samples of each treatment were placing in oven at 50^0C for 3 days and then 50gm of dried leaves was taken to be extracted in Soxhlet extractor with 200ml methanol. The crude extract was concentrated in rotary evaporator and controlled temperature 50^0C. The extract was preserved for subsequent use to determine and analysis some traits as below under:

Vegetative & physical traits

At the begins of maturation stage when the capsule has been formed, The Plant height, number of branches per plant, leaves area, wet and dry weight chlorophyll content (SPAD) was measured. Alkaloids content was calculated and expressed as a percentage of weight of sample analyzed (Harborne, 1973; Ijarotimi *et al*., 2013).

Phytochemicals analysis

GC/MS is reliable and useful method for fast separation and determination of complex phytochemicals

of Datura leaves including tropane alkaloids (Witte *et al*., 1987). This analysis was carried out at Feed and Food regional center – Agriculture researches center-Egypt by using a GC-MS (Agilent Technologies 7890A). The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature (Santana, *et al*., 2013).

Flowcytometry analysis (FCM)

The flow cytometric analyses were carried out at Plant cytometry services (PCS). However a few studies exist which follow the flow cytometry technique for the polyploidy identification in Datura plant and its relationship with production of secondary metabolites such as the medicinal Alkaloids which investigated by GC-MS.For more information about the FCM developing method, contact with info@plantcytometry.nl.

Statistical analysis

We used the SAS (2012) Statistical program todetect the effect of difference factors (C) and (T) in study parameters. Duncan's test (ANOVA) was used to significant difference between the means at 0.05 level of probability (Steel *et al*., 1997).

Results and Discussion

Polyploidy identification

The ploidy level of control and the plants treated with colchicine were determined with flow cytometry analysis, Flow- cytometry Histogram detected diploids, tetraploid and mixaploid level. Flow cytometry (FCM) is a technique extensively used from 1980s on determination nuclear DNA content rapidly and has been already found very useful in plant taxonomy to screen ploidy levels and to determine genome size (Xing *et al*., 2011). The results inculcated to Datura plant is an endoploidy crop.The Flowcytometry results in table 1 from Datura leaves was showed that the DNA ratio ranged from 0.365 to 0.797 that's refers changes in level of ploidy,in the other words there was not any obvious relationship between the DNA ploidy level and endore duplication (table 1). Tetraploid levels were raised when seeds treated with different concentrations of colchicine solution for different time intervals, that's means these plants had a different levels of ploidy (Mixoploidy) in different percentages of colchicine accept the treatment C_4 T_2 (1% colchicine for 48 hours) got 100% tetraploidy as shown in table 2 and fig. 1B, compare with C_0 (untreated plants), which got

Table 1 : Real DNA ratio with Ophiopogon planiscapus niger as effected by Colchicine levels and incubation times.

Treatments	Incubation time	
Colchicine Conc.	T1	T2
C_0	0.387	0.389
C_1	0.380	0.387
C_2	0.375	0.377
C_3	0.377	0.380
C_4	0.365	**0.797**

Table 2 : The Polyploidy ratio in *Datura stramonium* plants as effect by Colchicine levels and incubation times

Colchicine levels	Incubation Time			
	T1		T2	
	4X	2X	4X	2X
C_0	5	95	5	95
C_1	10	90	15	85
C_2	15	85	20	80
C_3	35	65	35	65
C_4	40	60	**100**	**0**

X= % Nuclei

2X=95% and 4X = 5%. Fig. 1A which indicate that studied *Datura stramonium* was already endoploidy and not full diploidy (2X=100%) under the Iraqi environment due to genotype x environment interaction within many years ago of plant development. Flow cytometry histograms analysis reveal the nuclear DNA content corresponding to diploids, Mixoploids and Tetraploids respectively, that means 1% colchicine for 48-hour success rate in quadrature induction. Flow cytometry greatly facilitated the screening for ploidy levels after treatment with spindle inhibitor, as indicated (Dolezel *et al*., 2007; Ochatt *et al*., 2011). Weber *et al*. (2008) Indicated thatthe presence of the high fractions nuclei with doubled DNA content is most presumably due to endoreduplication. duplication of the plant genome without mitosisin this study we observed endoreduplication in Mixoploid and tetraploid plants.

Colchicine disrupts mitosis byinhibiting the formation of spindle microtubules and the polar migration of chromosomes which resulting in doubled chromosome number cells (Panda *et al*., 1995). Generally, the endopolyploidy term describing the multiplication of DNA within the nuclei of the cell without mitoses intervening. In plants, this takes place via manypath ways but mainly through the process of endoreduplication. The occurrence of this form of endopolyploidy is common in flowering plants (angiosperms). External and Internal factors participate to the basic mechanisms of endopolyploidy,

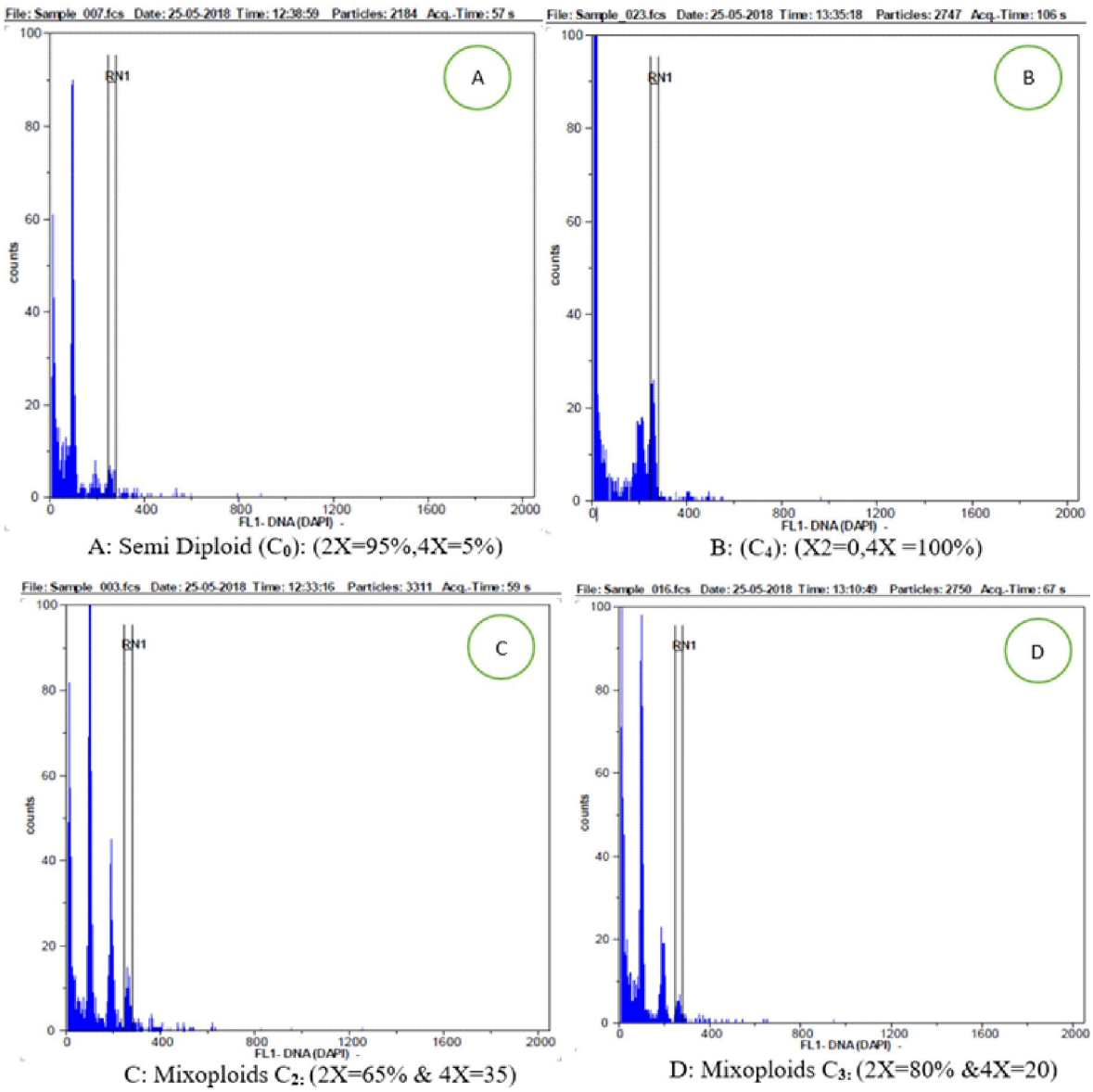

Fig. 1 : Histogram of the relative fluorescence intensity of nuclei isolated of *Datura stramonium* with different percentage of diploid and tetraploid levels.

which can be consider as a key of the developmental flexibility of plants. Recent searches have shown that endopolyploidy may also give an important role in the plant's response to environmental stress or /and increase some phytochemicals biosynthesis.

In fig. 1 C, D, all Colchicine treatments appeared the different endoreduplication levels with the differences in the fractions of the Polyploidy levels, i., e. full Tetraploidy (4X= 100%, 2X=0%) or Mixoploids such as (2X= 20%, 4X=80%) or (2X: 35% 4X =65%) for C_4, C_3, C_2 respectively as shown in fig. 1 (B, C, D) than control plant (A).

Flowcytometric histogram confirmed the production of tetraploids and mixoploids in Datura plants under study.The flowcytometry analysis results of the Datura leaves from semi diploid 2X=95% fig. 1(A) and Tetraploid 4X=100% (fig. 1B) showed in a single peak reflecting the main ploidy level of the corresponding plant. The table (1) results showed the presence of the high fractions of nuclei with doubled DNA content (0.797) is most presumably due to endoreplicated duplication of the genome without mitosis. In most plant species, Chromosomal doubling number may lead to larger the size of the plants cell and subsequently thicker and darker leaves with higher chlorophyll content, taller or shorter plant height or larger plant organs. The chromosome number can also have a positive effect on the content and composition of the constituents in plants. The production of bioactive secondary metabolites has

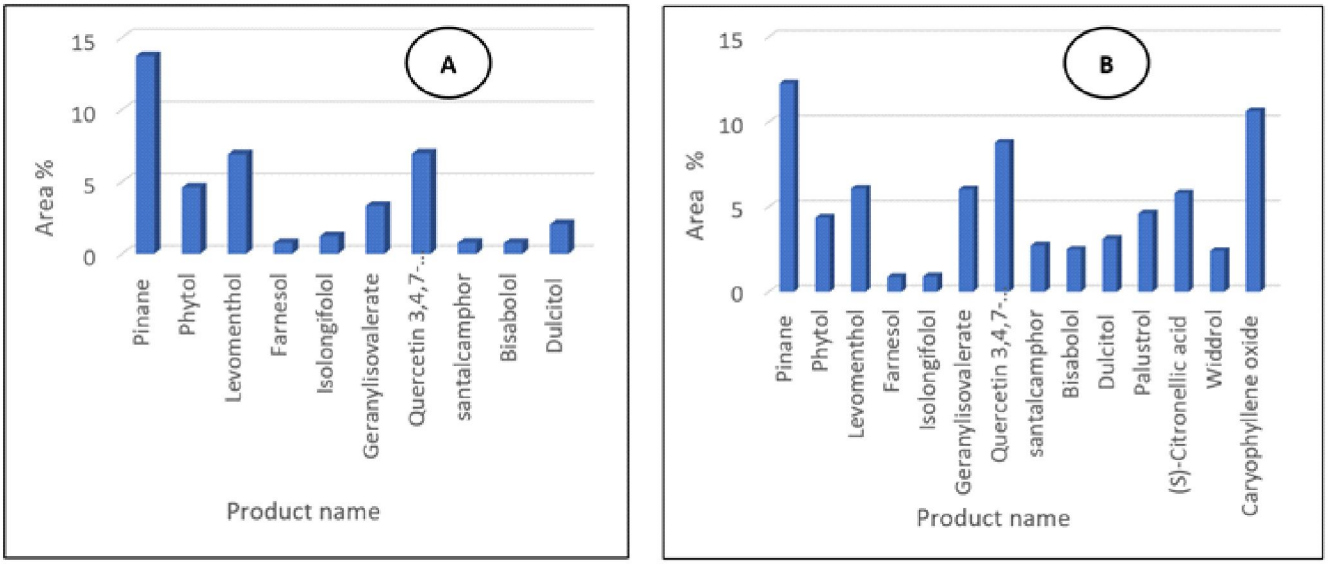

Fig. 3 : GC-MS Area % of Phytochemical products in control (A) and in Tetraploidy (B) treatment of *Datura stramonium* plant.

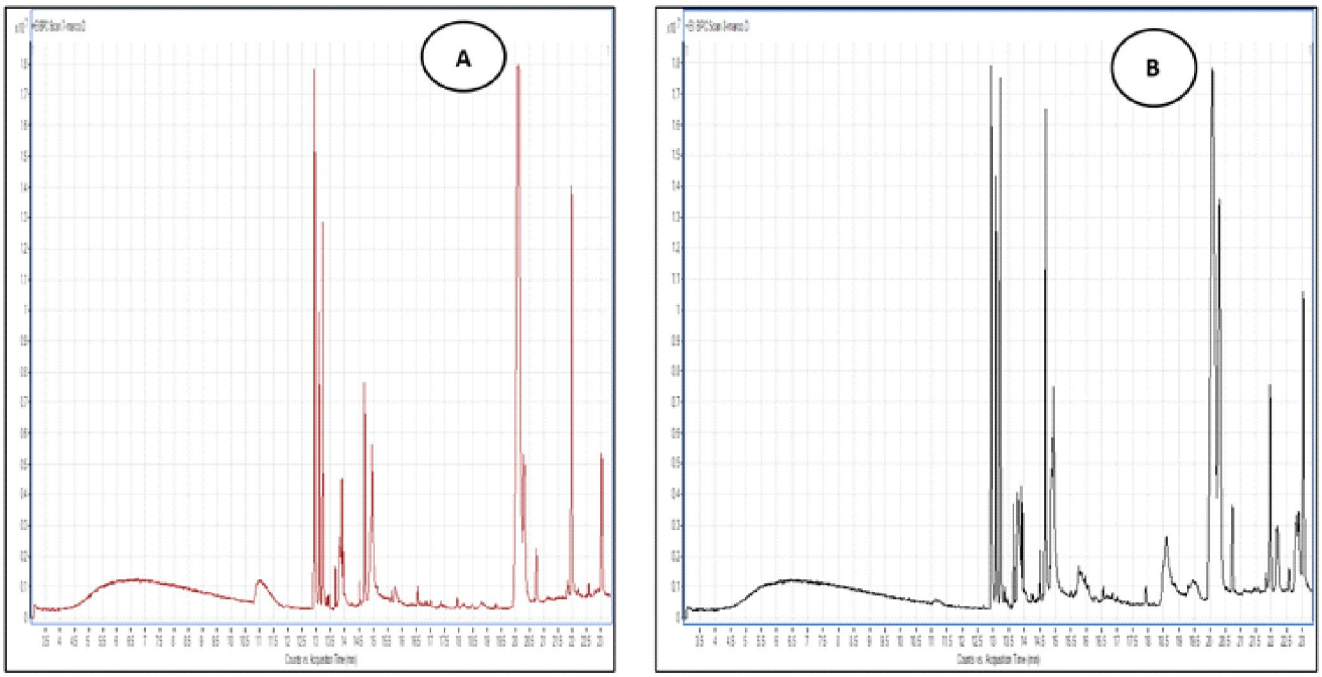

Fig. 4 : GC-MS Chromatogram of some Phytochemical compounds in control (A) and in Tetraploidy (B) treatments of *Datura stramonium* plant.

conspicuous changes (Shao *et al*., 2003). Rapid metabolic progress has been made in crops, meanwhile, the yields were doubled when the genomes were duplicated (Vandenhout *et al*., 1995; Pal *et al*., 1977). Polyploidy has also influence on gene expression either at the transcriptome level or at the proteome level (Hahn *et al*., 1990).

Vegetative traits

Plant height (cm) and Branching numbers

The results of table 3 indicated that the plant heightand Branching numbers was affected by different colchicine concentrations, which resulted in a decrease in the plants height with a greater concentration compared to the control C_0(89.5 cm). The duration times of soaking seeds with Colchicine aqueous solution T_1 and T_2did not have a significant effect in this trait.The Control treatment C_0 showed the highest plant height of 87, 92 cm in both incubation times compared to the lowest plant heightat treatment (C_4T_1) with no significant recorded from C_4T_2 (66.0, 67.67cm) respectively. Generally, the plant height decreased with increasing the incubation time and Colchicine levels. (Amiri *et al*., 2010). The lowering in plant height and produce the shorter Tetraploidy plants

Table 3 :. Effect of Colchicine levels and Incubation times on Plant Height (cm) and Branching No. of *Datura stramonium* plant.

Colchicine levels	Plant height		Mean	Branching No.		Mean
	T1	T2		T1	T2	
C_0	87.00 a	92.00 a	89.50 ± 6.83 A	4.33 bc	4.33 bc	4.33 ± 0.81 B
C_1	73.44 b	74.00 b	73.66 ± 4.96 B	3.67 c	3.33 c	3.50 ±0.54 B
C_2	73.00 b	74.00 b	73.50 ± 5.46 B	4.00 c	3.67 c	3.83 ± 0.41 B
C_3	74.00 b	69.00 b	71.50 ± 6.92 B	5.33 ab	5.33 ab	5.33 ± 0.51 A
C_4	66.00 b	67.67 b	66.83 ± 3.86 B	5.33 ab	6.0 a	5.67 ± 0.81 A
Mean	64.36 A	66.14 A	—	4.53 A	4.53 A	—

Means having with the different letters differed significantly. * (P<0.05).

Table 4 : Effect of Colchicine levels and Incubation timeson leaves dry and fresh weight (gm) of *Datura stramonium* plant.

Colchicine levels	Dry weight		Mean	Fresh weight		Mean
	T1	T2		T1	T2	
C_0	30.44 f	28.03 f	29.23 ± 3.98 D	125.10 ef	123.01 f	124.05 ± 9.23 E
C_1	36.92 e	45.44 cd	41.18 ± 5.13 C	145.33 e	174.65 d	159.99 ± 16.76 D
C_2	49.03 bcd	44.12 d	46.58 ± 3.75 B	194.18 cd	184.22 cd	189.20 ± 6.97 C
C_3	48.10 bcd	51.83 bc	49.97 ± 3.65 B	197.66 bc	217.94 b	207.80 ± 14.21 B
C_4	54.12 b	66.20 a	60.16 ± 8.06 A	259.80 a	261.95 a	260.87 ± 19.41 A
Mean	43.72 B	47.13 A	—	184.41 A	192.35 A	—

Means of each trait having with the different letters differed significantly. * (P<0.05).

Table 5 : Effect of Colchicine levels and Incubation times on leaves area (cm^2/plant) and Total Chlorophyll (SPAD) in *Datura stramonium* plant.

Colchicine levels	Leaves area (cm^2)		Mean	Chlorophyll (SPAD)		Mean
	T1	T2		T1	T2	
C_0	5295.67 ef	4848.00 f	5071.83 D	56.16 de	55.53 e	55.85 ± 4.66 C
C_1	5694.00 ef	6068.33 ef	5881.17 CD	62.03 cd	64.46 bc	63.25 ± 3.19 B
C_2	6153.00 ef	7134.00 de	6643.50 C	65.03 bc	68.13 abc	66.58 ± 1.93 AB
C_3	8520.00 d	14202.33 c	11361.17 B	70.11 ab	70.11 ab	70.11 ± 3.39 A
C_4	16367.00 b	26961.33 a	21664.17 A	68.50 abc	72.46 a	70.48 ± 3.30 A
Mean	8405.93 B	11842.80A	—	64.36 A	66.14 a	—

Means of each trait having with the different letters differed significantly. * (P<0.05).

due to shorter internode (Stebbins, 1971).

In the branches number, a significant increase was observed in C_3 and C_4 (5.67 and 5.33 branch) While the incubation times did not have a significant effect. Also, their Interaction C_4T_2 gave the highest number of branches with 6.00 branches compared with the lowest number (3.33 branches) at C_1T_2 which not significantly difference with control C_0 treatment. These results were not agreed with Lio *et al*. (2007), while it is agreed with Amiri *et al*. (2010), who reported that the number of branches were increased by colchicine treatment.Increase the ploidy level of a plant may cause higher vegetative growth. as well as secondary compounds increases with the increase level of ploidy. Morphologically, Leaf area, branching number, leaf darkness and also increase proportionately with increase in ploidy level.

Fresh and dry weight (gm)

The effect the maximum leaf dry weight (gm) were belong to the treatment Colchicine 1% for 48h and 24h. based on Duncan multi test (at level 5%), then the minimum dry weight was belonging to the control treatment. The significant differences were appearing between control and the other treatments and also between the colchicine treatments with different concentration as shown in table 5.

Leaf area (cm^2) & total chlorophyll (SPAD)

The results showed in table 6 indicate to a positive

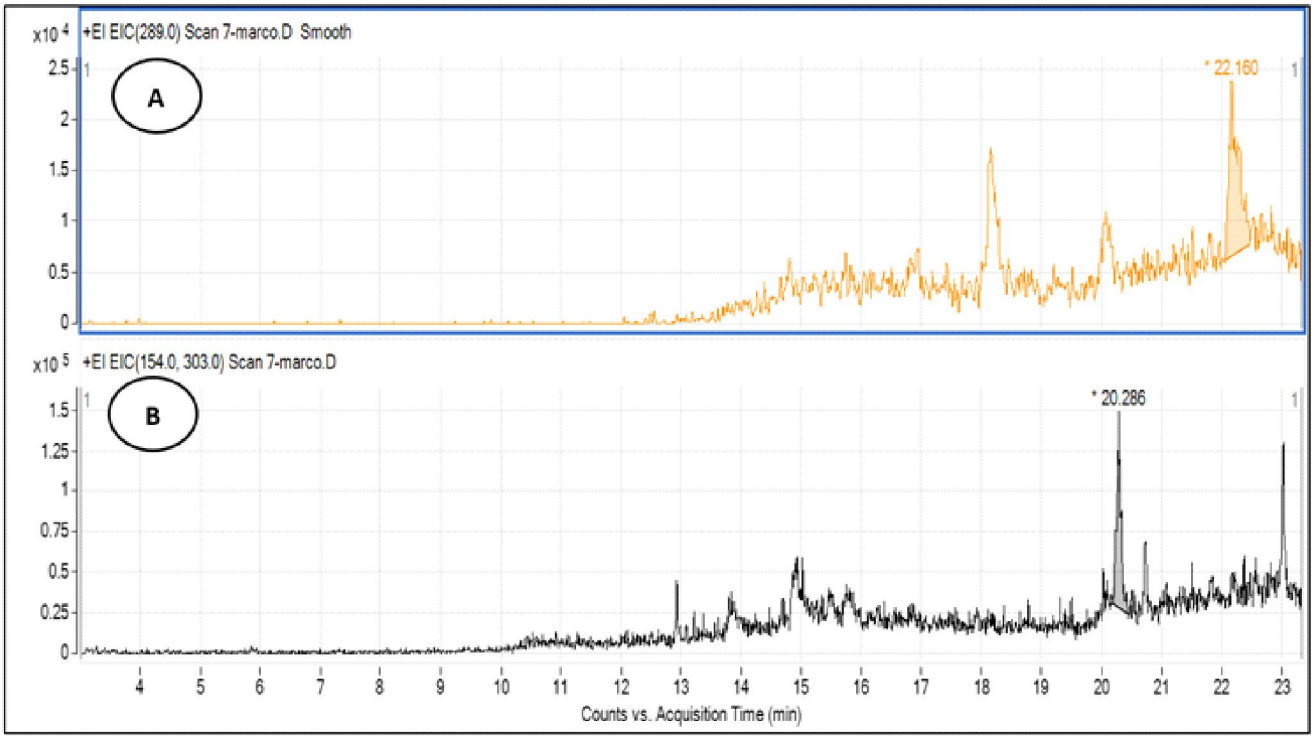

Fig. 5 : Tropain cartogramsof(C_0) treatment, (A) Hyoscyamine (2.4×10^4), (B): Scopolamine (1.5×10^5).

increasing in leaves area and chlorophyll content with increased of Colchicine concentration. The highest values were showed in C_4 and C_3 treatments (2166.17, 11361.17 Cm^2, 70.48, 70.11 (SPAD), respectively and significantly higher than the other treatments. The T_2 (48h) seeds incubation time was significantly higher in leaves area trait than T_1(24h) seeds (1184.8, 8405.93 cm^2) respectively, Interaction of C_4T_2 treatments reached the highest leaves area and chlorophyll content (269.33 Cm^2, 72.46 SPAD) compared to the lowest values of control treatment C_0T_2 (4848 cm, 55.53 SPAD). The observed results were agreed with Kehr and Woody (1996) and Amiri (2010).

The treatments means was comparing based on Duncan's multi range test at level 5%, so Colchicine 1% (C_1) and 0.5% (C_2) for 48 treatments observed the maximum significant in SPAD value. There were no significant differences between C_2T_2, C_3T_2 and C_4T_2. The minimum SPAD value was belonging to the control at the different incubation time.

Total alkaloids (%)

Datura stramonium crop has a great source of tropane alkaloids. This botanical alkaloid is one of the major parts of medicinal compounds. The researchers were indicated to percent of Alkaloids in Datura and this percent was different according to plant organs and growing time (Philipova and Berkovb, 2002). The results showed in table 7 that the total alkaloids percent were increasing when the colchicine levels increased. The percent of total alkaloid production included tropane one changed according to tetraploidylevel. The maximum significant value of total alkaloids will be reach under the effect of Colchicine1% for 48h in 100% tetraploidy nuclei cells, while the (4X) plant showed the significantly increased in total alkaloids content 7.25% compare with untreated plant 4.22%. In addition to the quantitative production of hyoscyamine and scopolamine and their patterns was quite different in tetraploid and diploid plants.

GC-MS Phytochemicals analysis

GC-MS is an accurate method for rapid identification of phytochemical compounds in all plant organs. The analysis results show that *Datura stramonium* leaves has been content more than thirty secondary compounds included such as Pinnae, phytol, Farnesol, Levomenthol, Quercetin and Caryophllene oxide as well as one of the major Phyto-sterols, terpenes, Phenols, figs. 3, 4 and a Tropane Alkaloids, fig. 5 with highly beneficial effects for medicinal uses suchas an anti-inflammatory, local anaesthetics, antioxidant and perhaps in cancer treatment.

In addition to the GC-MS above results, the C_4 treatment showed in fig. 4B that the effect of Colchicine on metabolic mechanism has been occurred by produce a new secondary phytochemical compounds in compare with control C_0 treatment, fig. 4(A) such as Palustrol,

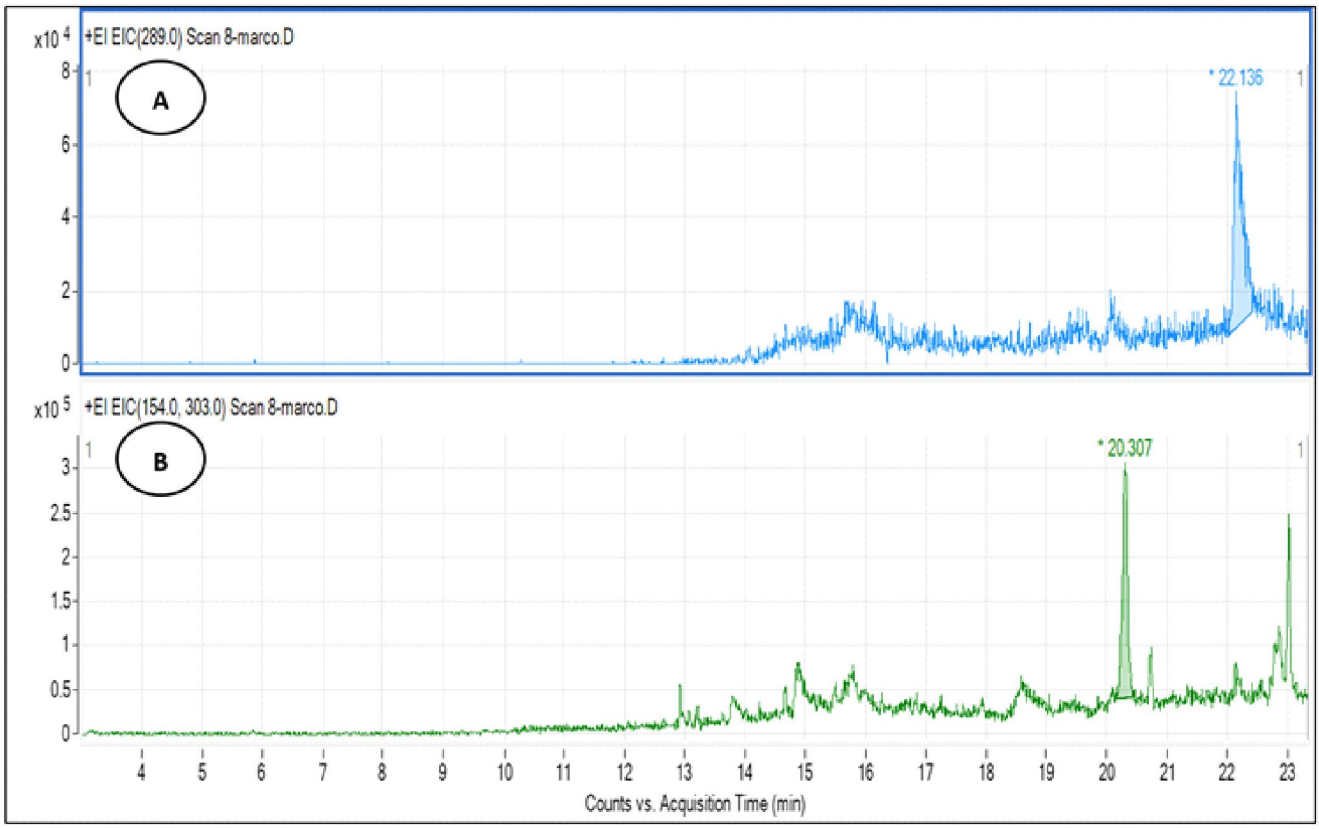

Fig. 6 : Tropain cartograms of (C_4) treatment, (A) Hyoscyamine (7.8×10^4), (B) Scopolamine (3.1×10^5).

Table 6 : Effect of Colchicine levels and incubation times of Total Alkaloids% in *Datura stramonium* plant.

Colchicine levels	Incubation Times		Mean
	T1	T2	
C_0	4.22 ± 0.59 g	4.22 ± 0.36 g	4.22 ± 0.44 E
C_1	4.70 ± 0.14 fg	4.92 ± 0.11 fg	4.81 ± 0.16 D
C_2	5.25 ± 0.52 ef	5.90 ± 0.31 de	5.57 ± 0.53 C
C_3	6.11 ± 0.27 cd	6.62 ± 0.47 bc	6.36 ± 0.44 B
C_4	6.94 ± 0.15 ab	7.57 ± 0.54 a	7.25 ± 0.49 A
Mean	5.44 ± 1.06 B	5.84 ± 1.27 A	—

Means of each trait having with the different letters differed significantly. * (P<0.05).

(S)-Citronellic acid and Widdrol or Increase the other ones such as Alkaloids *i.e.* Hyoscyamine and Scopolamine figs. 5 and 6 and some antioxidant compound i.e. Queretin3,4,7-trimethyl ether. Pinane (%), Levomenthol (%), Geranylisovlerate%, caryphyllene oxide% were the major constituents found in the alcoholic extract and they varied with the Colchicine levels Fig 4. Our results agreed with Senior *et al*. (2012) who indicate to the phytochemical analysis of the plant revealed that *D. stramonium* contained many compounds such as alkaloids, Phenols, saponins, tannins and glycosidesand has been a great pharmacological potential with a great utility in traditional medicine uses.

The GC-MS scan selected results appear the that the percent of two important tropane alkaloids Hyoscyamine and Scopolamine of Tetraploidy plant were increased 3.42 and 2.6 times than the control respectively figs. 5 and 6. These results were agreed with Lavania, (2005)who indicate toploidy manipulation role for enhanced production of Phyto-pharmaceuticals, that may be due to a greater ability of the polyploidy plants to express gene expression and enzymatic variation, with reduced respiration rate and higher photosynthesis rate.

These results were agreed with the another study Lavania (1986) showed 36% increase in total tropane alkaloid content in the autotetraploid plants of *H. muticus* and with Berkov *et al*. (2003), who reported increasing of scopolamine and hyoscyamine content in the leaves of *D. stramonium* L 2.63-(0.15–0.40% DW) and 1.41-fold (0.05–0.08% DW) respectively, but (Dehghan *et al*., 2012) reported the decrease in hyoscyamine content and increase in scopolamine content after induction of artificial tetraploidy by colchicine treatment. Philipova and Berkovb (2002) found twenty-nine alkaloids while, El Bazaoui *et al*. (2011) found sixty-seven tropane alkaloids, so the scopolamine and hyoscyamine as the major ones from the GC-MS detected in different organs of *Datura*

stramonium. In this study, the tetraploidy plants appear a different marker from Diploid plants (untreated plant) in many traits such as leaves area, more branches in addition to darker, thicker and largerleaves and shorter plants (Jaskani *et al*., 2005). These increases may due to a larger complement of chromosomes in that cells which grow larger to maintain a constant ratio between cytoplasmic to nuclear size and produce more quantitative or qualitative proteins with the presence of more different genes which lead to an increase in plant organs (Raufe *et al*., 2006).

Conclusion

It can be concluded that combination of 1% Colchicine and 48h seeds incubation time is an effective strategy to improve alkaloid production by introduce Tetraploidy plant (4X=100%) and improve the vegetative growth traits in *Datura stramonium* plant. So that, this treatment was induced the highest production of Scopolamine and Hyoscine up to 2.6 and 3 times respectively to control and a new phytochemicals biosynthesis elicitation including some medicinal ones such as phytosterol, Phenols or increased other onesby tetraploidy synergistic action.The results inculcated to Datura planted in Iraq is an endoploidy crop (2x=95%). In this study our results show that flow cytometry and GC-MS methods was for identification of ploidy levels and their phytochemicals content parallel to each other with positive relationship between ploidy level and phytochemicals production. The approach implemented in the present study displays itself as very promising for further steps of Tetraploidy genotypes of many other plants to increase or produce various important secondary metabolites. The results suggest that polyploidization may produce a shifting in genetic and phenotypic in plant systems that have the potential to produce in increased evolutionary diversification to new valuable genotype.

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