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EVALUATION OF SOME GROWTH CHARACTERS AND ALKALOID PRODUCTIVITY OF DIPLOIDY AND TETRAPLOIDY FORMS OF *Datura stramonium* AND *Datura innoxia* PLANTS

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ABSTRACT

Field experiment was conducted to study the diversity in growth parameters and alkaloid production between diploidy and tetraploidy levels of *Datura stramonium* and *Datura innoxia* plants. Increases in plant height, number of branches and fresh weight of different plant organs were observed at all growth stages, for tetraploid of both *Datura* spp, as compared with those of diploidy plants. Moreover, chromosome doubling led to increase fruit yield by 12% for *D. stramonium* and by 82% for *D. innoxia*. HPLC-analysis revealed that all plant organs of two *Datura* spp had relatively high concentrations of hyoscine and hyoscyamine. Hyoscine remained the principal alkaloid in roots, stem and leaves of diploidy and tetraploidy *D. stramonium* and *D. innoxia*, throughout the growing season. Fruits and leaves of both ploidy levels of two *Datura* spp were generally considered to be the organs with the higher amounts of alkaloids. Hyoscyamine was the main alkaloid in the fruits of diploidy and tetraploidy *D. stramonium*, whereas hyoscine was the main alkaloid in *D. innoxia* fruit. A great enhancement effect of chromosome doubling on concentration and amount of hyoscine and hyoscyamine in different plant parts was obtained by *D. innoxia*. Amount of alkaloids in tetraploid *D. innoxia* plant ranged between 177% and 406% relative to those occurred in diploid plant, at all growing stages. As for *D. stramonium* the consistent increase in hyoscine and hyoscyamine concentrations due to chromosome doubling was occurred only by roots. At harvest, tetraploidy *D. stramonium* plant had more alkaloids than those produced by diploid one. Positive correlations were observed between alkaloid formation in roots and stem of both ploidy levels of two *Datura* spp and its total N and soluble N contents.

Keywords: *Datura stramonium, Datura innoxia*, tropane alkaloids, hyoscine, hyoscyamine, diploid, tetraploid, polyploidy, Growth stages.

INTRODUCTION

Datura species belong to family Solanaceae, which contain variable tropane alkaloids. *Datura stramonium* and *Datura innoxia* are important medicinal plants for their contents of hyoscine and hyoscyamine alkaloids (Wallis, 1967). These compounds are anticholinergic agents used in the treatment of Parkinson's disease and organophosphate poisoning, that causes smooth muscle relaxation (Cordell, 1981). Levels of alkaloid in plants depended on the developmental stage and plant organ evaluated (Zarate, *et al.* 2001). Gendy and Rabie (2000) reported that the alkaloid content of *D. innoxia* seeds was higher than that of the other plant organs. As for *D. stramonium*, Miraldi *et al.* (2001) mentioned that maximum contents of alkaloids were found in the stem and leaves of young plants.

Studies of chromosome number in plants are of importance in several aspects. The chromosome sets are associated with many characters size, fertility and productivity, since the chromosomes carry the genes that condition these traits (Grove, 1982). Polyploidy in many plant species is often associated with increases in net CO_2 assimilation, expressed on a leaf area basis (Warner and Edwards, 1989), leaf anatomy (Syvertsen *et al*, 1995) and biochemical characteristics (Caemmerer and Farquhar, 1981). Recently, Berkov *et al.*, (2003) studied the variations in plant growth and alkaloid production of diploid (2n = 2x = 24) and tetraploid (2n = 4 x = 48) *D. stramonium* plants. Berkov and Philipov (2002) reported that in comparison to diploids of *D. stramonium,* the roots and leaves of tetraploids had a higher alkaloid content. Such enhancement effect of chromosome doubling on alkaloid production by seeds of *D. stramonium* and *D. innoxia* was also observed Berkov (2001). Thus polyploid effects could be confounded by interactions with environmental factors (Romero-Aranda *et al*, 1997). So this study was conducted under Egyptian conditions to evaluate the growth criteria and alkaloid production between diploidy and tetraploidy of *D. stramonium* and *D. innoxia* plants.

MATERIALS AND METHODS

Two field experiments were carried out in the Experimental Farm of the Cultivation and Production of Medicinal and Aromatic Plants Dept., National Research Centre, Giza, Egypt, during winter seasons of 2001/2002 and 2002/2003. Diploidy (2n = 2x=24) and tetraploidy (2n=4x=48) *Datura stramonium* and *Datura innoxia* seeds which originated from Bulgaria (provided by Prof. Dr. Liuba Evstatieva, Botany Institute, Bulgarian Academy of Sciences) were sown in the field on 1st November for two seasons. The experiment was arranged in a complete randomized design with four replicates. Plot area was 9 m^2 (3x3) with 5 rows, the distance between plants 60 cm. During soil preparation, 150 kg/fed. super phosphate (15.5 % P2O5) was applied. After transplanting, 200 kg ammonium nitrate (33.5 % N) and 100 kg potassium sulphate (48 %) per fed. were added. The mineral fertilization was conducted through two equal portions during the growing season, the first portion was added after one month of transplanting, while the second on was applied after two weeks of the first portion. Two monthes later after sowing, samples of both ploidy forms of either *D. stramonium* and *D. innoxia* were collected monthly as follows:

Date	*D. Stramonium*		*D. innoxia*	
	Diploid	Tetraploid	Diploid	Tetraploid
1st January	Vegetative	Vegetative	Vegetative	Vegetative
1st February	Flowering	Flowering	Vegetative	Vegetative
1st March	Early fruiting	Early fruiting	Vegetative	Vegetative
1st April	Fruiting	Fruiting	Flowering	Flowering
1st May	Fruiting	Fruiting	Fruiting	Fruiting
1st June	Fruiting	Fruiting	Fruiting	Fruiting

Samples of 5 plants were taken at randomly from the middle two rows of each plot. Plant height, number of branches and fresh weight of different plant organs were determined. Represented samples from the roots, stem, leaves and fruits of each ploidy form of *D. stramonium* and *D. innoxia* plants were dried and subjected to chemical analysis. Total N and soluble N (extracted with 10% trichloroacetic acid) were determined by modified Kjeldahl method as descried by A.O.A.C. (1980).

Analysis of hyoscine and hyoscyamine:

Hyoscine and hyoscyamine were analyzed by the method of Medina-Bolivar and Flores (1995). Ethanolic extract of dried samples was evaporated to dryness at 40°C by rotary evaporator and then 5ml of 0.5 M sulphuric acid and 20ml chloroform were added to the residue. The aqueous phase was adjusted to pH 10 with ammonia solution, chloroform layer was separated by separatory funnel, dried with sodium sulphate anhydrous and then evaporated to dryness. The residues were dissolved in methanol and filtered through a 0.2-µm filter. Extracts were subjected for separation using Shimadzu-HPLC with Nova-Pak C_{18} (Waters) steel column (3.9 x 150 mm) using a mobile phase of 12.5% acetonitrile and 87.5% aqueous phosphoric acid (0.3%) adjusted to pH 2.2 with triethylamine at a flow rate 0.8 ml/min. using UV-detector (260 nm). Identification and quantitification of hyoscine and hyoscyamine in the samples were done by reference to the retention time of authentic standards (Sigma).

Correlation coefficient between parameters was calculated according to Gomez and Gomez (1984).

RESULTS

(1) Growth parameters:

Data in Fig. (1) illustrated that duplication the chromosome number (from 2n = 2x = 24 to 2n = 4x = 48) increased all growth parameters of *D. stramonium* and *D. innoxia* plants, throughout the growing season. Tetraploid of both species showed plants with higher number of leaves and weights of different plant organs than those of diploidy forms. As for *D. stramonium*, increment in growth parameters due to chromosome doubling were obtained in the early stages of plant life. At harvest (1^{st} June) the fresh weight of either roots, stem, leaves or whole tetraploid plant increased with 9,14,11 and 12%, comparing with diploidy levels, respectively. As shown in Fig. (1), there was a predominant increases in all growth characters of 4X *D. innoxia* throughout the growing season. Such increases reached more than 24% of 2X plants, at the end of season. Fruit weight of both *Datura* species showed an enhancement effect as a result of chromosome doubling. At harvest, fruit yield were 327 and 160 g/plant for tetraploidy *D. stramonium* and *D.innoxia*, respectively, corresponded with 291 and 88 g/plant for diploidy forms, respectively.

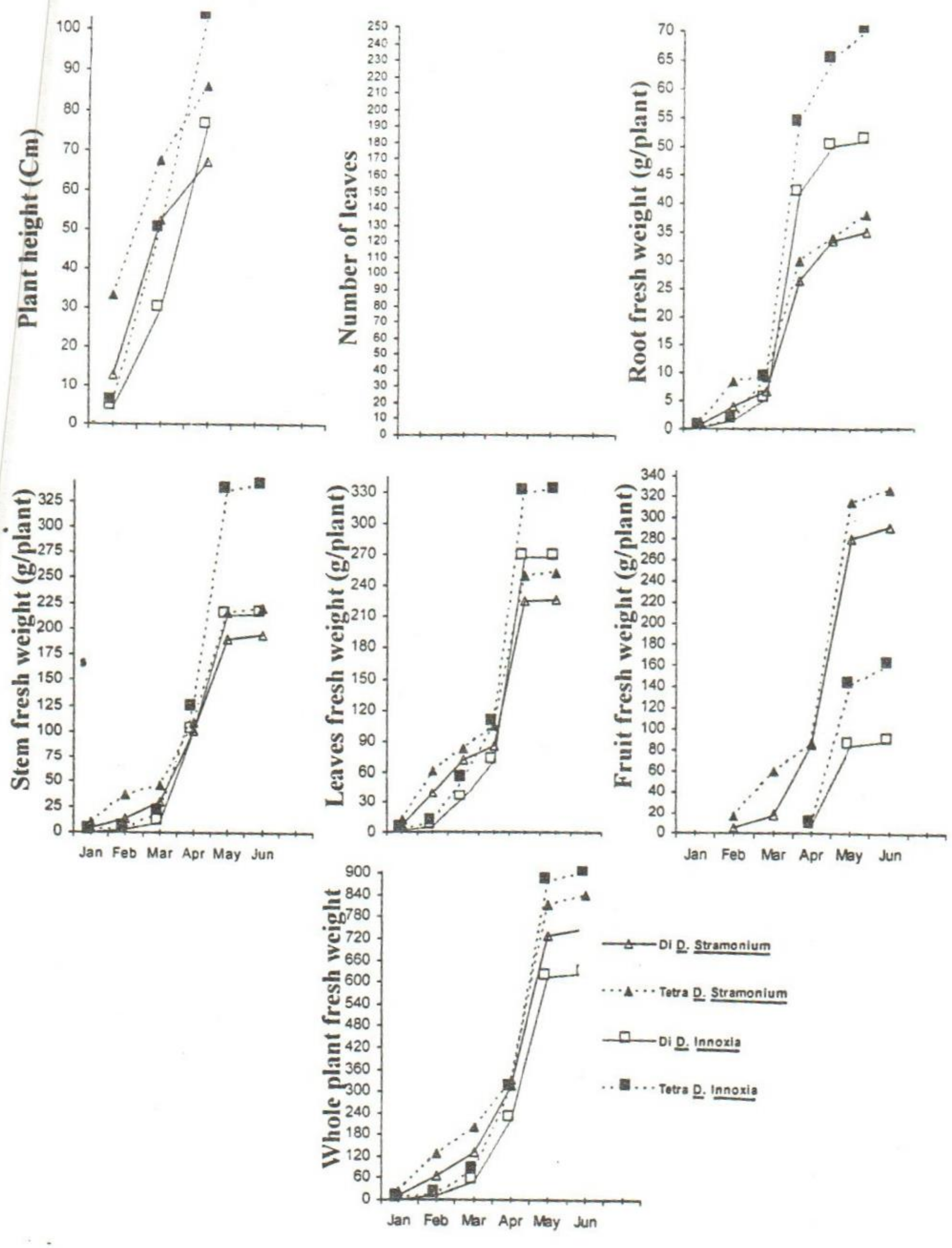

Fig. (1): Variation in growth characters between diploidy and tetraploidy *D. stramonium* and *D. innoxia* plants (average two seasons).

(2) Hyoscine and hyoscyamine concentration:

As shown in Table (1) hyoscine and hyoscyamine contents were found in different parts of *D. stramonium* and *D. innoxia* plants. Concentrations of two alkaloidal constituents depended on polyploidy, plant part and stage of plant growth. Hyoscine was found to be the main alkaloid in different parts of both ploidy levels for *D. innoxai* and in roots and stem of both ploidy *D. stramonium* plants. Whereas, leaves and fruits of *D. stramonium* tended to contain higher hyoscyamine concentrations that reached more than hyoscine, in some cases for leaves and in all cases for fruits.

The relatively high concentrations of hyoscine and hyoscyamine that occurred in roots of young *Datura* plants were dropped by the end of season. Roots of tetraploidy *D. stramonium* and *D. innoxia* plants contained higher alkaloid than those of diploidy forms. Chromosome doubling increased total hyoscine and hyoscyamine with more than 30% in *D. stramonium* roots, in most cases, corresponded by 5-95% increases for *D. innoxia* roots, throughout the growing season.

The decrement in stem alkaloids among the growing season were observed for both ploidy *D. stramonium* and *D. innoxia* plants. There were no clear effect for chromosome doubling was observed on stem alkaloids of *D. stramonium*. On the other hand, stem .of tetraploidy *D. innoxia* plants had more hyoscine and hyoscyamine concentrations than those of diploidy plants. Total alkaloid (hyoscine and hyoscyamine as mg/g dry weight) in stem of 4 x plant accounted between 158-258% relative to those of 2 x plant.

The enhancement effect of chromosome doubling on leaves alkaloids was observed for *D. stramonium* at harvest (more than 10%), but such enhancement effect was not observed at all examined times. While a predominant increases in hyoscine and hyoscyamine (more than 20%) was observed for leaves of tetraploidy *D. inoxia* among the growing season, as compared with diploid leaves.

Fruits of tetraploidy *D. stramonium* produced lower levels of hyoscine and hyoscyamine than diploid fruits. At harvest, concentration of total alkaloids reached 3.4 mg/g dry fruits for diploidy *D. stramonium*,

corresponded by only 2.31 mg/g for tetraploid fruits. On the contrary, the increment of total alkaloids in fruit of *D. innoxi* at Jun reached 27.48% due to chromosome doubling.

(3) Hyoscine and hyoscyamine content (mg/plant):

Data in Fig. (2), illustrated that amount of hyoscine and hyoscyamine in different organs of both ploidy *D. stramonium* and *D. innoxia* plants were varied according to variations in plant part, plant age and polyploidy forms. High amount of alkaloids were found in leaves and fruits, of either *D. stramonium* or *D. innoxia* plants. As shown in Fig. (2) quantities of hyoscine and hyoscyamine were gradually increased during growing season and maximized in May or June for all plant parts. Hyoscine tended to be the major alkaloid in all plant parts, except fruits of *D. stramonium*, at all plant stages for two *Datura* spp. Hyosciamine was considered the main alkaloid in fruits of *D. stramonium*.

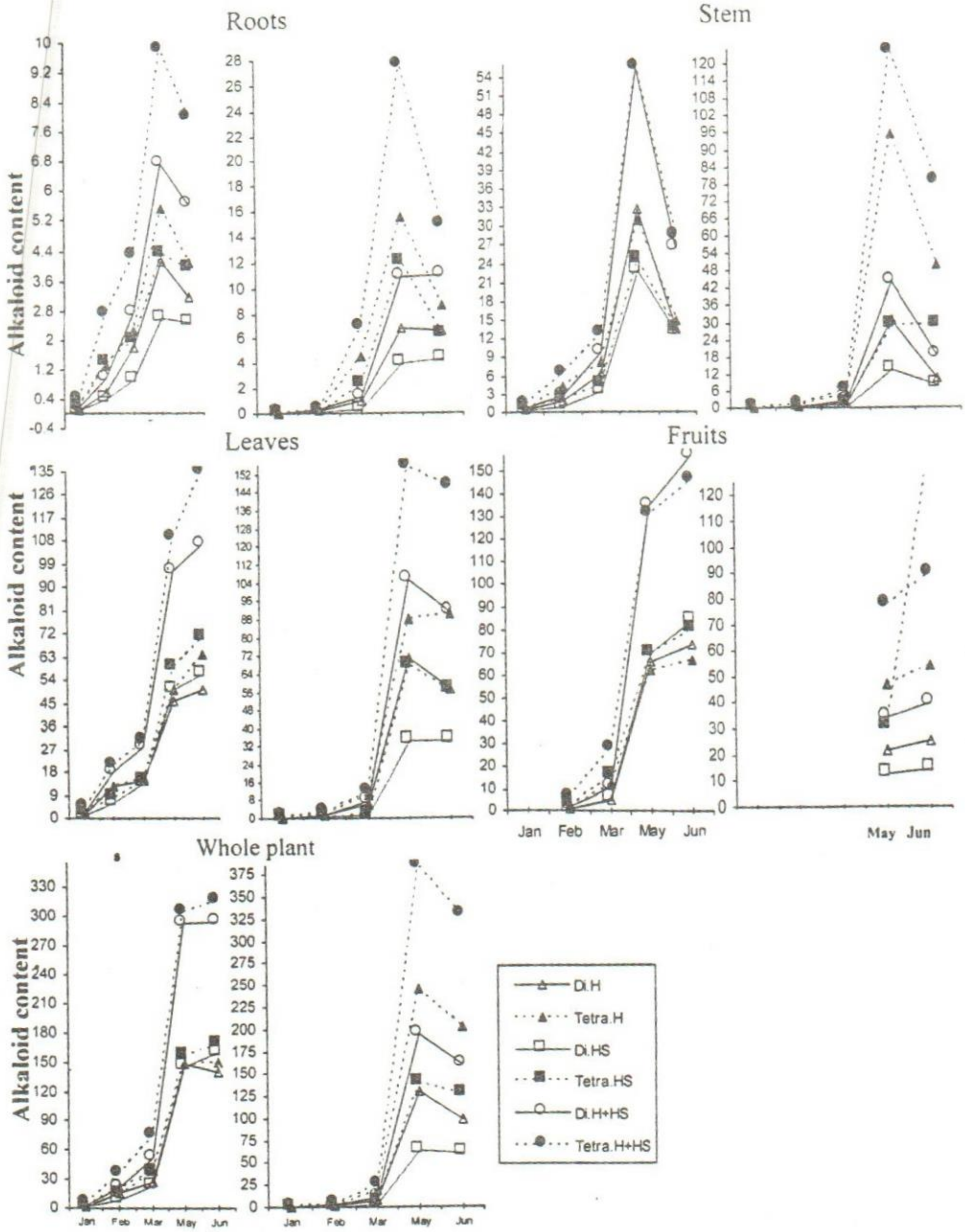

Fig. (2): Hyoscine (H) and hyoscyamine (HS) accumulation in different organs of diploidy and tetraploidy *D. stramonium* and *D. innoxia* plants (average two seasons).

Table (1): Concentration (mg/g dry matter) of hyoscine and hoscyamine in different organs of diploid and tetraploid *D. stramonium* and *D. innoxia* plants, throughout the growing season.

Species	*D. stramonium*						*D. innoxia*					
Alkaloid	Hyoscine		Hyoscyamine		Total		Hyoscine		Hoscyamine		Total	
Ploid	Di.	Tetra.	Di.	Tetra.	Di.	Tetra	Di.	Tetra	Di.	Tetra	Di.	Tetra.
Roots												
January	1.107	1.289	0.851	1.581	1.958	2.870	1.388	1.489	0.715	1.130	2.103	2.619
February	1.200	1.270	0.814	1.350	2.014	2.620	1.394	1.402	0.704	1.000	2.098	2.402
March	1.388	1.211	0.715	1.08	2.103	2.291	1.578	2.220	0.538	1.127	2.116	3.347
May	0.661	0.854	0.409	0.665	1.070	1.519	0.971	1.713	0.579	1.317	1.550	3.030
June	0.495	0.627	0.388	0.603	0.883	1.230	0.938	0.939	0.611	0.686	1.549	1.625
Stem												
January	1.934	1.071	0.986	1.700	2.920	2.771	1.617	3.208	0.694	0.678	2.311	3.886
February	1.210	1.660	0.598	0.788	1.808	2.448	1.470	3.200	0.578	0.670	2.048	3.870
March	1.317	1.315	0.694	0.723	2.011	2.038	1.448	2.418	0.591	0.860	2.079	3.278
May	1.170	0.943	0.800	0.760	1.970	1.703	1.320	2.578	0.563	0.785	1.883	3.363
June	0.448	0.460	0.435	0.417	0.883	0.877	0.427	1.244	0.333	0.714	0.760	1.958
Leaves												
January	1.647	1.560	1.500	1.876	3.147	3.436	1.204	1.932	0.644	0.534	1.848	2.466
February	2.170	1.675	1.018	1.081	3.188	2.756	1.668	2.360	0.447	0.562	2.115	2.922
March	1.514	1.284	1.390	1.213	2.904	2.497	1.667	1.784	0.451	0.390	2.118	2.174
May	1.463	1.439	1.588	1.679	3.051	3.118	2.091	2.104	1.008	1.608	3.099	3.712
June	1.527	1.735	1.726	1.908	3.253	3.643	1.620	2.139	0.977	1.339	2.597	3.478
Fruits												
February	1.330	1.088	1.371	1.350	2.701	2.438	-	-	-	-	-	-
March	1.340	1.092	1.583	1.414	2.923	2.506	-	-	-	-	-	-
May	1.300	1.091	1.372	1.226	3.147	2.131	1.947	2.501	1.099	1.620	3.046	4.121
June	1.330	1.090	1.515	1.331	3.400	2.310	1.941	2.468	1.093	1.615	3.034	4.083

Accumulation of hyoscine and hyoscyamine in different plant parts of two *Datura* spp was affected by diploid and tetraploid forms. Chromosome doubling of *D. stramonium* enhanced amount of alkaloids in roots, stem and leaves throughout the growing season (Fig, 2). Such enhancement effect on total alkaloids ranged from 25% to 42% for roots and from 7% to 105% for leaves among the growing season. At harvest, a slight decrease in alkaloids was observed in fruits as a result of chromosome doubling. It could be observed that tetraploid of *D. stramonium* plant accumulated more alkaloids than diploidy plants. At harvest time, 4x plants contained 149.02, 167.85 and 316.87 mg/plant from hyoscine, hyoscyamine and total alkaloids, compared with 139.99, 158.87 and 298.6 mg/plant for diploid ones, respectively. Also, the comparison between diploidy and tetraploidy *D. Innoxia* plants revealed the superiority of tetraploidy one. There was a great acceleration effect of alkaloids in different plant parts at all growing stages due to chromosome doubling (Fig., 2). More than twice of the amount of alkaloids was found in different organs of tetraploid *D. innoxia* plant, as compared with those of diploid forms. Amount of hyoscine, hyoscyamine and total alkaloids in 4 x plant reached 200.7, 126.64 and 327.34 mg/plant at harvest, corresponded with only 98.84, 60.74 and 159.58 mg/plant for 2x *D. innoxia* plant, respectively.

(4) Total and soluble nitrogen contents:

Total and soluble N in different parts of both ploidy *D. stramonium* and *D.innoxia* plants were determined, data represented Fig. (3). The results revealed that leaves of both *Datura* spp had higher N percentages than those occurred in other plant parts. It could be observed that levels of total and soluble N in different parts of 4x *D. stramonium* and *D. innoxia* plants tended to change, among the season, in the same manner to changes occurred by 2x plants. So, levels of total and soluble N in different plant parts tended to decrease dramatically at June. Roots and stem of young tetraploidy *D. stramonium* plants tended to contain higher percentages of total and soluble N, as compared with those of diploid forms. No other clear and stable effect on total and soluble N, due to chromosome doubling.

5- The correlation between alkaloidal N and other N forms.

Correlation between two nitrogen forms (soluble and total) and alkaloidal N in different parts of both ploidy levels of *D. stramonium* and *D. innoxia* were statistically calculated during the growing season, **r** values represented in Table (2). Positive correlations were observed between alkaloidal N and either total N or soluble N for roots and stem of both ploidy levels of two *Datura* spp, the correlation coefficient generally being more than 0.51. But, alkaloidal N in leaves did not appear any significant correlation with soluble N, while it correlated negatively with total N.

Table (2): Correlation between alkaloidal nitrogen and either total N or Soluble N in different parts of diploidy and tetraploidy *D. stramonium* and *D. innoxia* during the season. (r* values)

Specie	*D. Stramonium*						*D. inoxia*					
Plyploid forms	Roots		Stem		Leaves		Roots		Stem		Leaves	
	Total N	Soluble N	Total N	Soluble N	Total N	Soluble N	Total N	Soluble N	Total N	Soluble N	Total N	Soluble N
Diploid	0.51	0.82	0.84	0.84	-0.63	0.01	0.66	0.39	0.86	0.98	0.30	0.35
Tetraploid	0.86	0.63	0.97	0.94	-0.55	0.36	0.39	0.09	0.96	0.89	-0.61	0.17

*** r = Correlation coefficient**

DISCUSSION

Obtained results revealed that chromosomal duplication increased all growth characters of both *Datura sp.*, during the growing season. These results are in agreement with those obtained by Molin *et al* (1982) and Romero-Aranda *et al.* (1997). In the same direction, Berkov (2001) mentioned that tetraploid seeds of *D. innoxia*, *D. stramonium* and *Hyoscyamus niger* were larger and heavier than those of diploid. Such growth enhancement may be attributed to the increases in net CO_2 assimilation (Warner and Edwards, 1989), biochemical characteristics (Caemmerer and Farquhar, 1981) or to the increase in levels of photosynthetic enzymes and pigments per cell (Joseph *et al*, 1981).

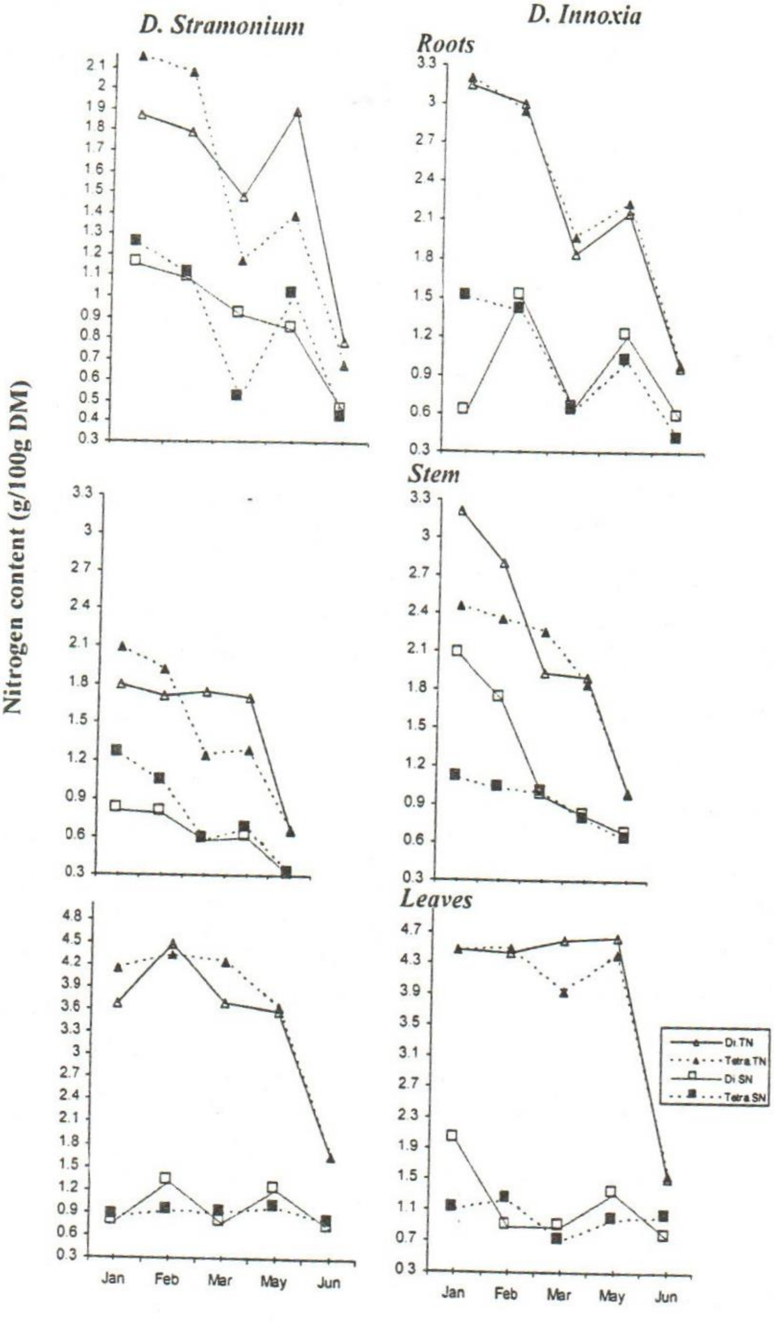

Fig. (3): Total N (TN) and soluble N (SN) contents in different organs of diploidy and tetraploidy *D. stramonium* and *D. innoxia* plants (average two seasons).

It is clear that the concentration and amount of hyoscine and hyoscyamine alkaloids in *Datura* plants depended on plant species, stage of plant growth, plant organs and ploidy form. Obtained results revealed that different plant parts of both ploidy *D. stramonium* and *D. innoxia* contained relatively high hyoscine and hyoscyamine concentrations. There was a drastic fall in alkaloid contents in roots and stem of both ploidy levels of two *Datura* spp at the end of fruiting stage. Presence of hyoscine and hyoscyamine in different parts of *D. sramonium* was observed by Miraldi *et al* (2001). They mentioned that the content of tropan alkaloids was higher in young plants than in adult plants.

This study confirmed that hyoscine was the major alkaloid in different plant parts of both ploidy of *D. stramonium* and *D. innoxia*, except fruits of *D. stramonium*, throughout the growing season. These results are in line with those obtained by Demeyer *et al* (1989) and Berkov and Phillpov (2002). But, Miraldi *et al.* (2001) mentioned that hyoscyamine only was the main alkaloid in *D. stramonium*, in contrast with some previous reports. Obtained results showed that hyoscyamine was the major alkaloid in the fruits of both ploidy of *D. stramonium* and this constituent was presented with high levels in the leaves, reached higher than hyoscine, in some cases. Leaves and fruits of both *Datura* spp were generally considerd to be organs with the higher content of tropane alkaloids, since at harvest, about 89% of plant alkaloids was found in leaves and fruits of both ploidy *D. stramonium* plants, corresponded with 81.7% and 71.6% for diploidy and tetraploidy *D. innoxia* plants, respectively. These results are in the same direction with those obtained by Gendy and Rabie (2000) and Miraldi *et al* (2001).

D. innoxia alkaloids showed more enhancement effect by chromosome doubling than those of *D. stramonium*. For synergistic effect of chromosome doubling on alkaloid concentrations and plant biomass, amount of alkaloids in tetraploidy *D. innoxia* plant ranged between 177% and 406% relative to those accumulated in diploidy plants at all growing stages. On the other hand, increase in alkaloid concentrations by tetraploidy *D. stramonium* was obtained by roots only. These increments in the amount of alkaloids/plant were attributed mainly to the enhancement effect of chromosome doubling on plant biomass. The favourable effect of chromosome doubling on *Datura* alkaloids was observed by Berkov (2001), Berkov & Philipov (2002) and Berkov *et al.* (2003). In this conection, Leitch (2000) mentioned that it is generally assumed that polyploidization occurs to amplify desirable gene necessary for the synthesis of products in a given tissue.

Total N and soluble N in diploidy forms of two *Datura* spp followed the same trend of these compounds occurred in corresponded tetraploidy plants, among the growing season. There was not any consistent clear effect of chromosome doubling on total and soluble N.

The previous results revealed that, in contrast to leaves, the presence of alkaloid in roots and stem of datura plant depended mainly on nitrogen availability. The presence of a relatively trace amounts of leaves nitrogen in alkaloid form and the presence of most leaves soluble N in photosynthetic enzymes (Huffaker, 1982) might explaine the unexpected relation between alkaloids and other nitrogen forms in *Datura* leaves.

In conclusion, tetraploid forms of both *Datura spp* showed superiority of all growth parameters at all growth stages as compared with diploid forms. Moreover, hyoscine and hyoscyamine contents in tetraploidy plants of both species are much higher than diploidy forms. So tetraploidy of Datura plants were considered as a better source for alkaloid production than the corresponded diploidy plants.

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تقييم صفات النمو وإنتاج القلويدات للنباتات ثنائية الكروموسوم ورباعية الكروموسوم للداتوره استرامونيوم والداتوره أنوكسيا

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**قسم زراعة وإنتاج النباتات الطبية والعطرية-المركز القومى للبحوث-الدقى- القاهرة

أجريت تجربة حقلية لدراسة الاختلافات فى صفات النمو وتكوين القلويدات بين النباتات ثنائية الكروموسوم ورباعية الكروموسوم لنوعين من الداتوره استرامونيوم والداتوره أنوكسيا وعلاقة ذلك بالمحتوى النيتروجينى فى أجزاء النبات المختلفة. وقد أظهرت النباتات رباعية الكروموسوم زيادة فى طول النبات وعدد الأفرع وأوزان الأجزاء المختلفة من النبات مقارنة بالنباتات ثنائية الكروموسوم طوال عمر النبات. وقد زاد محصول الثمار بحوالى ١٢% بالنسبة لنباتات الداتوره استرامونيوم مقابل زيادة قدرها ٨٢% فى محصول ثمار الداتوره أنوكسيا. وقد تم تقدير القلويدات فى أجزاء النبات المختلفة شهريا طوال موسم النمو باستخدام جهاز كروماتوجرافى السائل ذو الاداء العالى (HPLC) وتبين وجود نسبة عالية من الهيوسين والهيوسيامين فى الأعضاء النباتية لنوعى الداتوره سواء ثنائية أو رباعية الكروموسوم. كذلك تبين وجود الهيوسين كمركب رئيسى فى جذور وسيقان وأوراق نباتات الداتوره للنوعين تحت الدراسة. وقد احتوت الثمار والأوراق على معظم القلويدات التى يكونها نبات الداتوره سواء ثنائى أو رباعى الكروموسوم. يوجد الهيوسيامين فى ثمار الداتوره استرامونيوم بنسبة أعلى من الهيوسين طوال فترة نضج الثمار وعلى العكس من ذلك تحتوى ثمار الداتوره أنوكسيا على نسبة أقل من الهيوسين. وقد نتج عن أزدواج الكروموسومات زيادة كبيرة فى نسبة وكمية القلويدات فى أعضاء نبات الداتوره أنوكسيا طوال موسم النمو حيث تراوحت كمية القلويدات التى يكونها النبات رباعى الكروموسوم ما بين ١٧٧%-٤٠٦% مقارنة بالكمية التى يكونها النبات ثنائى الكروموسوم طوال موسم النمو. لم يؤدى ازدواج الكروموسومات الى زيادة واضحة وثابتة فى نسبة القلويدات فى أجزاء نبات الداتوره استرامونيوم إلا فى منطقة الجذور. وقد زاد محصول نبات الداتوره استرامونيوم رباعى الكروموسوم من القلويدات عند الحصاد عند مقارنته بالنبات ثنائى الكروموسوم. وقد وجدت علاقة إيجابية قوية بين محتوى الجذور والسيقان لنباتات الداتوره