Response of Datura Innoxia Mill Plants to Jasmonic Acid Application

A.A. Gendy[1] and Kawthar A.E. Rabie[2]

1) Agricultural Botany Dept., Faculty of Agriculture
Menofiya University, Shibin El-Kom, Egypt.
2) Agricultural Botany Dept., Faculty of Agriculture
Ain-Shamis University, Cairo, Egypt.

Abstract

Two experiments were carried out during the seasons of 1994/1995 and 1995/1996 to study the effect of Jasmonic acid* at three concentrations; i.e., 200, 400 and 800 ppm, besides the distilled water as a control on the growth, chlorophyll content, the levels of endogenous hormones and alkaloid contents as well as yield of Datura innoxia Mill plants. The obtained results indicated that JA application at different concentrations significantly decreased plant heights and increased the number of both leaves and branches as well as dry weights of the different organs of Datura plants. Plants treated with JA showed a decrease in transpiration rate and total Chl a + b, with an increment of the JA concentration.

Results also showed that alkaloid content of the seeds was higher than that the different organs of Datura innoxia. Seeds yield and alkaloids as well as oil content in the seeds were significantly increased by JA treatments. The effect was more pronounced by JA at 800 ppm during the two seasons. On the other hand, JA treatments decreased growth promoters and increased growth inhibitors with raising of JA concentration.

Introduction

Datura innoxia Mill is one of the most important solanaceous plants, which is considered as a main source for producing tropane alkaloids needed for the pharmaceutical industries. In the last few years, great attention was drawn to improve the yield and its quality of Datura. Application of wide variety of both naturally occurring and synthetic chemical growth regulators have been extensively used in order to ascertain their beneficial effects upon the growth and development of plants. A number of synthetic growth retardants have been discovered and proved to be of considerable importance in agriculture (Yamane *et al.*, 1990; Yamane *et al.*, 1981; Sembdner and Klose, 1985; Vick and Zimmermann, 1986).

Jasmonic acid (JA) and its methyl ester (JA-Me) are endogenous physiologically active compounds with a phytohormone-like action and they are widely distributed in higher plants (Ueda *et al.*, 1981; Sembdner and Klose, 1985 and Parthier, 1990). They cause growth inhibition (Miersch *et al.*, 1986 and Popova *et al.*, 1988).

According to these contradictory opinions, we have carried out this investigation in order to determine the optimum JA levels, which may realize an increment in the obtained drug and alkaloid yield from Datura innoxia Mill plants, under our experimental and environmental conditions.

Materials and Methods

Two field experiments were carried out at the Experimental Farm of the Faculty of Agriculture at Shibin El-Kom, Minufiya University during two successive seasons of 1994/1995 and 1995/1996 for studying the effect of Jasmonic acid (JA) on the growth, phytosynthetic pigments, photohormone concentrations drug yield, and alkaloids content of the different plant organs of Datura innoxia Mill plants.

The seeds from the local plants were planted in seed pans at the 1st of October 1994 and 1995 in the nursery. The seedlings of 2-3 pair of leaves were transplanted at the mid of December in the seasons of 1994/1995 and 1995/1996 in plots of 2 x 4 m at 40 cms. apart between the plants. During the soil preparation, chemical fertilizers as calcium superphosphate (15% P_2O_5) and potassium sulphate (50% K_2O) were added to the soil at rates of 130 and 85 Kg/fed. respectively. Concerning, the nitrogen fertilizer, the plants received urea (46% N) at rate of 85 Kg/fed. in three equal side dressings on 10^{th} of January, February and March in the two seasons. All other cultural practices were performed as usual. The study was conducted in a randomized complete block design with three replicates.

Jasmonic acid (JA) was applied as a foliar application at the rates of 200, 400 and 800 ppm. Distilled water was used as a control plants. Plants were sprayed twice, at 35 days from transplanting and 7 days later by means of a hand atomizer until run-off. Tween 20 was used a wetting agent at a concentration of 0.5%.

At the full flowering stage, plant height, number of both leaves and branches and dry weights of leaves, stems, flowers and roots were measured. The different plant organs were dried at 70°C and grinded as a preparation of chemical analysis. The chlorophyll, and transpiration rate were taken, at the full flowering stage.

1) Chlorophyll a, Chl b and carotenoids were determined spectrophoto-metrically as described by Wettstein (1957).
2) The transpiration rate (g water/cm²/h) was determined according to Kreeb (1990).
3) The total alkaloid percentage in the dried different plant organs was determined according to the method described by Karawya *et al.* (1975).

Determination of endogenous growth hormones:

Extraction of endogenous growth hormones was carried out according to method of Shindy and Smith (1975), 30 grams fresh weight of the leaves at full flowering stage were used for the determination of auxin and their inhibitors, gibberellins and cytokinins. Plant material was extracted three times with 80% cold methanol. The combined alcohol extracts were evaporated under reduced pressure and the aqueous residue was partially purified by partition with ethyl acetate. The acidic ethyl acetate fraction was then collected and dried under vacuum at 37°C to dryness to determine auxin and their inhibitors and gibberellins whereas, the alkaline fraction was used to determine cytokinins. Separation was carried out by paper chromatography using a solvent composed of isopropanol : ammonia : water (10:1:1). Bioassay techniques were followed using wheat coleoptile straight assay (Bently and Housley, 1954) for auxins and their inhibitors, lettuce hypocotyl assay (Frankland and Wareing, 1960) for gibberellins and cucumb cotyledons assay (Fletcher *et al.*, 1982) for cytokinins. The results of phytohormones were statistically analyzed according to Tukey (1953).

At harvest, the seed yield, oil and alkaloid yield were recorded. The oil percentage in Datura seed was determined using Soxhlet continuous extraction apparatus according to A.O.A.C (1980). All data were subjected to statistical analysis of variance (Snedecor and Cochran, 1969).

Results and Discussion

1 - Growth analysis:

Data in Table (1) indicated clearly that, plant heights significantly decreased with increasing Jasmonic acid concentrations. the heighest value in this respect was obtained by 800 ppm at which plant heights were decreased by about 13% and 15% in the first and second seasons, respectively, as compared with untreated plants. It was clear from the same Table that, all levels of JA caused a significant increase in the number of both leaves and branches per plant, drug weights of different parts of Datura innoxia Mill plants (Table 2). The highest value in this respect was obtained by 800 ppm of JA. These results were true in both 1994/1995 and 1995/1996 seasons.

The dwarfing effect of Jasmonic acid may be due to the influences of JA on preventing cell elongation and/or stopping cell division, act as antigibberellin (Fig. 2). In this regard, several investigators reported that JA shortened the heights of many plants species (Dathe *et al.*, 1981; Sembdner and Gross, 1986; Parthier 1990; Gendy and Schilling, 1990; Gendy and Selim, 1994)

Table (1): Effect of Jasmonic acid on growth parameters of Datura plants during 1994/95 and 1995/96 seasons.

JA levels (ppm)	Plant height cm/plant	No. of leaves/plant	No. of branches/plant
1994/95 season			
Control	90.2	89.2	15.2
200	87.4	95.4	17.6
400	80.6	96.8	18.3
800	78.4	80.2	15.0
L.S.D 5%	5.2	18.1	4.2
1995/96 season			
Control	86.4	84.8	14.2
200	82.1	89.9	16.3
400	80.2	92.4	19.4
800	73.4	81.4	14.8
L.S.D 5%	4.8	16.4	3.4

Table (2): Effect of Jasmonic acid on the drug yield in gms/plant of Datura innoxia Mill plants during the seasons of 1994/95 and 1995/96.

JA levels (ppm)	Leaves	Stems	Flowers	Total herb	Roots
1994/85 season					
Control	80.2	45.5	9.4	135.1	15.8
200	82.4	48.2	9.8	140.4	18.2
400	86.9	50.4	10.2	147.5	19.6
800	88.1	52.8	10.3	151.2	19.8
L.S.D 5%	4.2	4.2	0.8	7.8	2.4
1995/96 season					
Control	79.2	46.2	9.2	134.6	14.2
200	80.4	49.4	9.6	139.4	15.4
400	83.2	50.8	10.0	144.0	18.4
800	84.6	53.7	10.2	148.5	19.6
L.S.D 5%	3.1	4.6	0.6	6.6	2.6

The increase in drug weights of treated plants (Table 2) might be attributed to the positive effect of JA on number of both leaves and branches and dry matter deposition. This increase also might be due to the enhancement at JA on CO2 fixation and/or the increase in the anabolic metabolism (Sembdner and Parthier, 1993).

2 - Transpiration rate:

Data recorded in Table (3) showed clearly that all levels of JA caused a significant decrease in the transpiration rate. The most effective treatments to decrease the transpiration rate was at the highest levels of JA. This decrease may be attributed to the stomata closure by JA (Satler and Thimann, 1981; Horton, 1991; Gendy and Selim, 1994). Jasmonic acid activities seem to be similar to abscisic acid on this character (Sembdner and Parthier, 1993).

3 - Photosynthetic pigments:

Data in Table (3) indicated clearly that all JA levels decreased Chl a, Chl b and total Chl (a+b) as well as carotenoids in both seasons. On the contrary, the carotenoids significantly increased with raising the JA levels. The lowest value of total Chl a+b resulted from plants sprayed at the highest levels of JA.

The decrease in photosynthetic pigments as a result of JA treatments was reported in other investigations by Parthier *et al.* (1987a), Ueda and Kato (1982), Parthier (1990), Gendy and Schilling (1990), Gendy and Selim (1994). The role of JA to inhibition the chlorophyll content was attributed to the capability of JA to reduce of chloroplast development during leaf growth and to promote the rate of leaf senescence as well as to stimulate the degradation of chlorophyll (Sembdner and Parthier, 1993). The reduction in Chl content may be due to several factors: 1) inhibition of endogenous hormonal activity, 2) suppression of rRNA incorporation into plastid nucleic acid and its synthesis, 3) inhibition of GA-dependent DNA biosynthesis which decrease protein content necessary for Chl biosynthesis, and/or 4) increasing chlorophyllase synthesis (Parthier, 1991).

4 - Phytohormones concentration:

Data presented in Table (4) exhibit a marked decrease in IAA, GA and Cytokinin concentration of treated leaves at all JA levels as compared with untreated leaves. On the contrary, the ABA concentration was unsignificantly increased. Similar findings were reported by Ueda *et al.* (1981), Ueda and Kato (1982), who found that the application of JA decreased the cytokinin concentration. In this connection, Gendy and Selim (1994) reported that the gibberellin concentration in faba bean was decreased after leaf treatment with JA.

Table (3): Effect of Jasmonic acid on chlorophyll concentration and transpiration rate in leaves of Datura plants (1994/1995 and 1995/1996 season).

JA levels	Chlorophyll concentration (mg/g.f.wt)			Transpiration rate	
(ppm)	Chl a (mg/cm2/h)	Chl b	Chl a + b	Carotenoid	
1994/1995 season					
Control	6.32	2.18	8.50	2.77	5.8
200	6.12	2.12	8.24	2.80	5.0
400	5.80	2.08	7.88	2.90	4.2
800	5.42	2.00	7.42	2.98	3.2
L.S.D 5%	0.85	0.12	0.89	0.21	1.0
1995/1996 season					
Control	5.12	2.00	7.12	2.30	4.9
200	5.00	1.90	6.90	2.45	4.6
400	4.82	1.82	6.64	2.54	3.9
800	4.34	1.68	6.02	2.62	3.2
L.S.D 5%	0.67	0.16	0.94	0.32	1.2

Table (4): Effect of JA on endogenus phytohormones concentration (ng/g/fwt) of datura leaves.

JA levels (ppm)	IAA	GA	Cytokin	ABA
1994/95 season				
Control	58.0	25.0	95.0	72.0
200	50.0	20.0	90.0	72.5
400	48.0	17.0	84.0	72.8
800	45.0	14.0	80.0	73.0
L.S.D 5%	6.2	4.5	4.8	NS
1995/96 season				
Control	59.0	28.0	96.0	74.0
200	51.0	22.0	91.0	74.6
400	49.0	19.0	83.0	74.8
800	46.0	15.0	80.0	75.0
L.S.D 5%	7.1	5.6	4.9	NS

5 - Total alkaloids:

a) alkaloid percentage:

The reported data in Table (5) show clearly that JA application on Datura in-noxia Mill plants caused a slightly decrease in the alkaloid concentration in the dried leaves, stems, and roots than the untreated plants, whereas, the total alkaloid per-centage in the flowers showed an increase tendency than untreated plants in both seasons.

The decrease in the alkaloid percentages as a result of JA application in the leaves, stems and roots of Datura innoxia Mill plants could be due to the dilution effect of alkaloid concentrations in previous mentioned organs as a result of the increment of drug yield. Similar results were obtained by Mostafa *et al.* (1984) on Datura plants.

b) alkaloid content:

The presented data in Table (5) indicate that JA application on Datura innoxia Mill plants at its different concentrations increased the alkaloid yield/plant in flowers than the control plants. The best results in this respect was obtained by spraying the plants with 400 and 800 ppm of JA in the two experimental seasons.

It seems from these results (Tables 3 and 5) that JA application was more effective in increasing alkaloids yield of Datura flowers as compared to untreated plants. These results are in agreement with the findings of Parthier (1991).

The beneficial effects of growth regulators on the biosynthesis of alkaloids and other secondary metabolites in many medicinal plants were also reported by Moskova-Simenova *et al.* (1987); Moftah and Attia (1992). The increase in N uptake and amino nitrogen content resulted from these growth regulators and reported by Abdou (1987) might be reasons for stimulating alkaloid biosynthesis.

6 - The yield:

As shown in Table (6) seeds yield was affected positively and significantly by Jasmonic acid application. The highest seed yield was obtained at the moderate rate of JA. The highest increase in seed yield over the control reached about 63% and 56% in the first and second seasons, respectively.

It can be also noticed from Table (6) that foliar spray of JA to Datura plants leads generally to an increase in oil and alkaloid percentages and yields in the seeds as compared with untreated plants. Seed content of alkaloid reached the maximum value when plants sprayed at 400 ppm of JA.

From the aforementioned discussion it could be concluded that JA application modify Datura plants growth. The modifications are characterized by significantly shorter plants. Moreover, the seed yield and alkaloid yield were increased.

Table (5): Effect of Jasmonic acid on the alkaloids content of the different Datura plant organs during the seasons of 1994/95 and 1995/96.

JA levels (ppm)	Leaves		Stems		Flowers		Total herb		Roots	
	%	g/plant	%	g/plant	%	g/plant	%	g/plant	%	g/plant
1994/95 season										
Control	0.86	0.69	0.56	0.25	0.89	0.08	0.79	0.59	0.40	0.06
200	0.74	0.61	0.52	0.25	0.96	0.09	0.70	0.56	0.31	0.06
400	0.72	0.63	0.45	0.23	1.20	0.12	0.62	0.57	0.24	0.05
800	0.70	0.62	0.36	0.19	1.42	0.15	0.60	0.42	0.20	0.04
L.S.D5%	-	0.06	-	0.02	-	0.02	-	0.08	-	0.02
1995/96 season										
Control	0.58	0.54	0.48	0.22	0.72	0.07	0.61	0.43	0.31	0.04
200	0.60	0.48	0.40	0.20	0.84	0.08	0.58	0.49	0.28	0.04
400	0.52	0.43	0.36	0.18	0.99	0.10	0.50	0.47	0.21	0.04
800	0.48	0.41	0.30	0.16	1.20	0.12	0.48	0.33	0.18	0.04
L.S.D5%	-	0.09	-	0.04	-	0.04	-	0.09	-	N.S.

Table (6): Effect of Jasmonic acid on seed yield, seed oil and seed alkaloids yields of Datura plants.

JA levels (ppm)	Seed yield		Seed oil		Seed alkaloid
	g/plant	%	g/plant	%	g/plant
1994/95 season					
Control	50.1	14.6	7.31	1.40	0.70
200	55.3	15.2	8.41	2.10	1.16
400	64.2	16.8	10.79	3.30	2.12
800	60.4	16.0	9.66	2.80	1.69
L.S.D 5%	2.5	1.2	1.45	1.12	0.62
1995/96 season					
Control	48.2	14.2	6.84	1.30	0.63
200	52.4	15.0	7.86	2.20	1.15
400	66.8	16.2	10.82	3.28	2.19
800	61.6	15.8	9.73	2.60	1.60
L.S.D 5%	2.9	1.4	1.66	1.20	0.75

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