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# Influence of Gibberellic Acid on Growth, Flowering, and Alkaloidal Content of *Atropa belladonna* L. Grown in Egypt

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**Abstract** □ Gibberellic acid seed treatment and dripping at the fourth to fifth leaf stage of *Atropa belladonna* L. increased the stem length, number of leaves, and number of branches. Gibberellic acid had a differential effect on the area of the leaf at different internodes. The dry weight of shoots of treated plants was increased, especially at the full-flowering stage. A pronounced increase in the alkaloidal content was obtained in the shoots of treated plants at 50 mg./l. gibberellic acid, especially at flowering phases.

**Keyphrases** □ Gibberellic acid—effect on growth, flowering, and alkaloidal content of *Atropa belladonna* L. □ *Atropa belladonna* L.—effect of gibberellic acid on growth, flowering, and alkaloidal content □ Plant growth regulators—effect of gibberellic acid on *Atropa belladonna* L.

Gibberellic acid has been reported to influence the vegetative growth, flowering process, and major metabolic pathways in plants (1–5). In addition, its influence on the biosynthesis of some active constituents of medicinal plants has been studied, especially on *Datura stramonium* L., *Catharanthus roseus* L., *Hyoscyamus niger* L., and *Atropa belladonna* L. (6–12).

This work was carried out to study the effect of soaking the seeds in gibberellic acid and then dripping gibberellic acid on the same plants raised from the treated seeds on the growth, development, and alkaloidal pattern of *A. belladonna* L. at different physiological stages.

## EXPERIMENTAL

**Growing the Plant**—Seeds of *A. belladonna* L.[1] were germinated in culture flats. After 1 month, the uniform young seedlings (two to three leaves) were transplanted into small pots and were kept there for an additional month. The plants were then transferred to larger pots (30 cm. in diameter), containing 12 kg. of soil[2], until the end of the experiment. At 1-month intervals, plants within each pot were fertilized with 3 g. of a mixture containing calcium superphosphate, potassium sulfate, and calcium nitrate.

**Treatment**—The seeds were soaked for 24 hr. in different concentrations of a freshly prepared aqueous solution of gibberellic acid[3] (25, 50, and 100 mg./l.). Furthermore, 2 ml. of the same concentrations of gibberellic acid was dripped on the terminal buds and the young unfolded leaves of the plants raised from the treated seeds. Dripping of gibberellic acid was carried out, using a graduated pipet, at the fifth to sixth leaf stage. Dripping was repeated four times at 4-day intervals.

Samples of the shoots and roots from each treatment were drawn at random for dry weight determination before flowering, at flower budding, at full flowering, and at fruiting stages. The samples were dried to constant weights in a circulating hot air oven at 70°, reduced to powder (40 mesh), and stored in airtight containers for chemical analysis. The leaf area per plant (after 10 days from the end of gibberellic acid dripping) was determined using a planimeter.

**Growth and Flowering Studies**—The length of the main stem and each of the successive 10 internodes, as well as the number of leaves and branches, was recorded at 10-day intervals. Flowering and fruiting dates were also recorded. The flowering date was calculated as the days from germination to the appearance of the first visible flowerbud for each treatment. The numbers of flowers and fruits were recorded daily. In all cases, the mean of 10 plants for each treatment was calculated.

**Alkaloidal Content Determination**—Extraction of the alkaloids from the plant samples was carried out according to the procedure of Allport and Wilson (13). The spectrophotometric determination of the alkaloids was performed using the method of Durick *et al.* (14). The total alkaloidal content was calculated as milligrams hyoscyamine per gram dry weight as well as per dry weight of every plant organ.

**Statistical Analysis**—Available data were subjected to analysis of variance to calculate the *F* test and the least significant difference at 0.01 according to the design of complete randomized plots with interaction (15). The least significant difference was calculated between

[1] The seeds of *A. belladonna* L. were supplied through the courtesy of Prof. Dr. R. T. Voigt, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60680.

[2] Loamy clay soil having the following mechanical analysis: sand, 24%; silt, 47%; and clay, 29%.
[3] Gibberellic acid was supplied by Merck Sharp & Dohme, Rahway, N. J.

Table I—Effect of Gibberellic Acid on Vegetative Growth of *A. belladonna* L.

| Plant Characteristic | Concentration of Gibberellic Acid, mg./l. | Plant Age, Days 175 | 182 | 190 | 197 | 205 | 215 | 224 | 234 | 241 | Mean (Treatment) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Stem length, cm./plant | 00 | 3.2[a] | 4.5 | 9.6 | 16.0 | 30.0 | 34.0 | 54.0 | 62.0 | 63.0 | 30.7 |
| | 25 | 11.5 | 12.3 | 20.0 | 22.5 | 37.5 | 46.8 | 55.2 | 63.2 | 64.3 | 35.6 |
| | 50 | 12.0 | 12.5 | 22.5 | 27.5 | 44.3 | 47.3 | 58.2 | 58.2 | 62.0 | 67.3 |
| | 100 | 14.1 | 14.5 | 28.2 | 30.5 | 45.2 | 60.2 | 63.0 | 66.0 | 70.2 | 67.9 |
| | Mean | 10.2 | 10.6 | 20.1 | 24.0 | 39.2 | 47.1 | 57.6 | 54.1 | 67.9 | — |
| Diameter of stem, cm./plant | 00 | — | — | — | 6.1 | 7.3 | 7.7 | 7.7 | 7.9 | 8.3 | 7.5 |
| | 25 | — | — | — | 6.0 | 6.7 | 6.9 | 7.2 | 7.3 | 7.5 | 6.9 |
| | 50 | — | — | — | 6.2 | 6.6 | 7.2 | 7.4 | 7.7 | 7.8 | 7.1 |
| | 100 | — | — | — | 6.5 | 6.7 | 6.9 | 7.0 | 7.3 | 7.6 | 7.0 |
| | Mean | — | — | — | 6.2 | 6.8 | 7.2 | 7.3 | 7.5 | 7.8 | — |
| Number of leaves per plant | 00 | 9.1 | 9.6 | 12.2 | 15.0 | 17.0 | 20.5 | 25.2 | 27.5 | 30.0 | 17.8 |
| | 25 | 10.2 | 11.3 | 16.2 | 17.0 | 21.0 | 23.2 | 26.2 | 31.5 | 33.0 | 21.4 |
| | 50 | 11.2 | 12.3 | 17.5 | 18.5 | 22.5 | 25.2 | 28.5 | 32.0 | 34.5 | 20.5 |
| | 100 | 10.5 | 12.2 | 18.4 | 19.5 | 24.0 | 26.2 | 31.5 | 32.0 | 36.0 | 21.1 |
| | Mean | 10.2 | 11.3 | 16.1 | 17.5 | 21.1 | 23.8 | 27.8 | 30.8 | 33.4 | — |
| Number of branches per plant | 00 | — | — | — | 1.5 | 2.0 | 2.4 | 2.6 | 2.8 | 3.0 | 2.4 |
| | 25 | — | — | — | 1.6 | 1.9 | 3.2 | 3.2 | 3.6 | 3.6 | 2.9 |
| | 50 | — | — | — | 3.0 | 3.1 | 3.1 | 3.1 | 3.4 | 3.4 | 3.2 |
| | 100 | — | — | — | 3.0 | 3.0 | 3.1 | 3.2 | 3.8 | 3.8 | 3.3 |
| | Mean | — | — | — | 2.3 | 2.5 | 2.9 | 3.0 | 3.4 | 3.5 | — |

| Least significant difference <0.05 | Treatment | Age |
|---|---|---|
| Stem length | 3.9 | 5.9 |
| Diameter of stem | 0.4 | 0.4 |
| Number of leaves | 0.8 | 4.4 |
| Number of branches | 0.6 | 0.7 |

[a] Average of 10 plants per treatment.

means of treatments within the same stage of growth, between means of stages within the same treatment, and that of the interaction to compare the means in all directions.

## RESULTS AND DISCUSSION

**Vegetative Growth**—The treated plants demonstrated characteristic gibberellic responses. Stem length, number of leaves, and number of branches were significantly increased by treatment with gibberellic acid, especially at 100 p.p.m. (Table I). However, the diameter of the main stem of treated plants was significantly decreased. This stimulatory effect of gibberellic acid was pronounced at the early stage of growth and continued at a decreasing rate until the end of growth. This might be due to the combined effect of soaking the seeds and dripping gibberellic acid on division and elongation of the cells. The increase of stem length occurred mainly in the third to the seventh internode of treated plants (Table II). This promotion of growth by stem elongation as a result of gibberellic acid treatment was observed previously for several plants, including Atropa (7, 8, 11).

Although there were no significant increases in the dry weights of the roots, the dry weights of shoots were significantly above the controls, especially at full flowering stage (Table III). This result clearly indicates that the physiological stage may play an important role in obtaining a beneficial response from gibberellic acid treatment. The increase in dry weight of shoots of Atropa as a result of gibberellic acid treatment was confirmed by Brummett and Sciuchetti (8). The gain in dry weight of tops often was not accompanied by a corresponding increase in root weight (16, 17). The areas of the first and second leaves were increased by the treatment with 100 mg./l. gibberellic acid (Fig. 1). However, the areas of the fourth to the seventh leaves of treated plants were markedly decreased.

Table II—Influence of Gibberellic Acid on the Length of Different Internodes (Centimeters per Plant) of *A. belladonna* L.

| Plant Age, Days | Gibberellic Acid Concentration, mg./l. | Internode Arrangement 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | 10th |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 205 | 00 | 2.5[a] | 1.7 | 2.2 | 2.2 | 1.9 | 1.6 | 1.5 | 1.5 | 1.4 | 1.8 |
| | 25 | 2.6 | 2.3 | 2.3 | 2.4 | 2.2 | 2.0 | 2.2 | 1.8 | 2.1 | 2.0 |
| | 50 | 2.9 | 2.7 | 2.6 | 2.8 | 2.9 | 2.4 | 2.6 | 2.0 | 2.1 | 2.2 |
| | 100 | 3.8 | 2.9 | 3.1 | 3.1 | 3.4 | 3.0 | 3.0 | 2.9 | 3.0 | 2.8 |
| 215 | 00 | 2.6 | 2.3 | 2.3 | 2.4 | 1.9 | 1.7 | 1.6 | 1.7 | 1.5 | 2.2 |
| | 25 | 2.7 | 2.6 | 2.7 | 2.6 | 2.3 | 2.2 | 2.4 | 2.1 | 2.5 | 2.5 |
| | 50 | 3.2 | 3.1 | 2.9 | 3.2 | 3.0 | 2.8 | 2.8 | 2.4 | 2.3 | 2.6 |
| | 100 | 2.8 | 3.2 | 3.2 | 3.4 | 3.5 | 3.2 | 3.3 | 3.4 | 3.2 | 3.6 |
| 224 | 00 | 3.1 | 2.9 | 2.4 | 2.6 | 2.4 | 2.2 | 1.8 | 1.9 | 1.8 | 2.5 |
| | 25 | 3.2 | 3.3 | 2.9 | 2.9 | 2.7 | 2.4 | 2.7 | 2.3 | 2.6 | 2.9 |
| | 50 | 3.4 | 3.5 | 3.1 | 3.4 | 3.2 | 3.0 | 2.9 | 2.6 | 2.7 | 3.0 |
| | 100 | 3.9 | 3.6 | 3.8 | 3.7 | 3.7 | 3.4 | 3.7 | 3.9 | 3.3 | 3.7 |
| 234 | 00 | 3.2 | 3.0 | 2.6 | 2.8 | 2.6 | 2.6 | 2.1 | 2.3 | 2.2 | 2.6 |
| | 25 | 3.3 | 3.3 | 3.2 | 3.2 | 2.8 | 2.6 | 2.8 | 2.6 | 3.0 | 3.1 |
| | 50 | 3.4 | 3.6 | 3.4 | 3.6 | 3.3 | 3.3 | 3.0 | 2.9 | 3.1 | 3.4 |
| | 100 | 3.9 | 4.0 | 4.0 | 3.9 | 3.4 | 3.6 | 3.8 | 3.9 | 3.6 | 3.8 |

[a] Average of 10 plants per treatment.

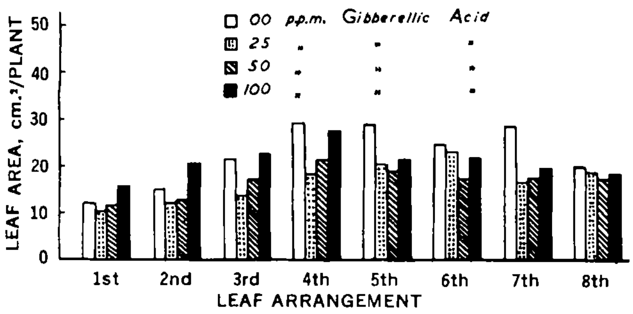


**Figure 1**—*Effect of gibberellic acid on leaf area.*

Therefore, the increase in the dry weight of the shoots of the treated plants might be explained by the increased number of branches and leaves. In this respect, the response was attributed to the increase in carbon fixation of gibberellic acid-treated plants (1) and to the redistribution of materials from the roots to the shoots (16).

**Flowering and Fruiting Responses**—It is obvious from Table IV that treatment with gibberellic acid significantly enhanced both the processes of flowering and of fruiting compared with the controls. The plants treated with 100 mg./l. gibberellic acid yielded flowers 14 days earlier and fruits 12 days earlier than the controls. Early flowering after gibberellic acid treatment was reported previously for other plants (18–21).

The flowering rate was increased for all treatments during the first period of the flowering season, reaching the highest values at 100 mg./l. gibberellic acid (Fig. 2). However, as the season of flowering progressed, the flowering rate declined for plants treated with gibberellic acid at concentrations of 50 and 100 mg./l. These results indicate that gibberellic acid plays an indirect and important role in the stimulation and promotion of the flowering process during its first period.

It was found that gibberellic acid significantly increased the number of fruits, especially for plants treated with 50 mg./l. (Table IV). This result shows that treatment with 50 mg./l. of gibberellic acid might have a more favorable influence on the retention of flowers and the development of fruits of Atropa. The promotion of fruit setting and the development of tomatoes treated with gibberellic acid were reported by Wittwer *et al.* (22). In addition, the retention of flowers was observed for cotton plants treated with gibberellic acid (23, 24).

**Alkaloidal Analysis**—The alkaloidal content (milligrams hyoscyamine per gram dry weight) of shoots and roots of treated plants was significantly increased at different stages of growth (Table V).

A pronounced increase in alkaloidal content of shoots was recorded for plants treated with 50 mg./l. gibberellic acid, especially at flowering stages. This response was true whether the alkaloidal

**Table V**—Effect of Gibberellic Acid on Total Alkaloidal Content (as Milligrams Hyoscyamine per Gram Dry Weight)

| Plant Organ | Gibberellic Acid Concentration, mg./l. | Physiological Stages: Before Flowering | At Flower Budding | At Full Flowering | Mean |
|---|---|---|---|---|---|
| Shoot | 00 | 7.22[a] | 4.11 | 3.71 | 5.01 |
| | 25 | 6.84 | 5.40 | 4.52 | 5.59 |
| | 50 | 7.41 | 6.54 | 5.53 | 6.49 |
| | 100 | 7.62 | 4.90 | 4.60 | 5.71 |
| | Mean | 7.27 | 5.24 | 4.78 | — |
| Least significant difference (0.01): | | | | | |
| Gibberellic acid treatments | | 1.38 | | | |
| Stages | | 1.21 | | | |
| Interaction | | Not significant | | | |
| Root | 00 | 3.20 | 2.73 | 5.12 | 3.68 |
| | 25 | 4.60 | 3.32 | 5.21 | 4.38 |
| | 50 | 4.34 | 3.52 | 5.50 | 4.45 |
| | 100 | 4.30 | 3.10 | 5.20 | 4.20 |
| | Mean | 4.11 | 3.17 | 5.28 | — |
| Least significant difference (0.01): | | | | | |
| Gibberellic acid treatments | | 0.32 | | | |
| Stages | | 0.28 | | | |
| Interaction | | 0.56 | | | |

[a] Average based on three replicates per treatment.

**Table III**—Influence of Gibberellic Acid on Dry Weight of Shoots and Roots of *A. belladonna* L.

| Stage of Growth | Shoot: 00 | 25[a] | 50 | 100 | Root: 00 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|---|---|
| Before flowering | 0.36[b] | 0.43 | 0.50 | 0.43 | 0.04 | 0.06 | 0.10 | 0.08 |
| At flower budding | 4.86 | 4.93 | 5.90 | 7.10 | 0.71 | 0.48 | 0.74 | 0.83 |
| At full flowering | 12.83 | 12.16 | 15.20 | 16.66 | 2.40 | 2.17 | 2.03 | 2.20 |
| At fruiting | 16.76 | 16.80 | 17.83 | 19.86 | 5.70 | 6.00 | 6.87 | 6.57 |
| Least significant difference: | | | | | | | | |
| Gibberellic acid treatments | | | | 0.94 | | Not significant | | |
| Stages | | | | 0.94 | | 0.49 | | |
| Interaction | | | | 1.95 | | Not significant | | |

[a] Concentration of gibberellic acid as milligrams per liter. [b] Average in grams of three replicates per plant per treatment.

**Table IV**—Flowering and Fruiting Times and Number of Fruits as Influenced by Gibberellic Acid Treatments

| Gibberellic Acid Concentration, mg./l. | Flowering[a] Time | Fruiting[a] Time | Number of Fruits per Plant: 233[b] | 238 | 243 | 248 | 253 | Mean |
|---|---|---|---|---|---|---|---|---|
| 00 | 210.5[c] | 223.0 | 16.8 | 19.2 | 21.8 | 23.9 | 24.6 | 21.3 |
| 25 | 201.7 | 224.0 | 17.5 | 24.1 | 22.0 | 26.2 | 28.2 | 23.6 |
| 50 | 196.3 | 210.9 | 16.0 | 25.9 | 28.3 | 29.7 | 30.7 | 26.0 |
| 100 | 196.0 | 211.4 | 18.0 | 19.7 | 21.9 | 25.7 | 28.8 | 22.8 |
| Mean | — | — | 18.2 | 19.4 | 23.0 | 26.9 | 29.7 | — |
| Least significant difference (0.01): | | | | | | | | |
| Flowering | | 2.4 | | | | | | |
| Fruiting | | 4.1 | | | | | | |
| Gibberellic acid treatments | | 4.98 | | | | | | |
| Stages | | 5.57 | | | | | | |

[a] Days from germination to the first visible flowerbud or fruit. [b] Plant age in days from germination. [c] Average per plant based on 20 plants per treatment.

**Table VI**—Effect of Gibberellic Acid on Total Alkaloidal Content (as Milligrams per Total Dry Weight)

| Stage of Growth | Shoot 00 | Shoot 25[a] | Shoot 50 | Shoot 100 | Root 00 | Root 25 | Root 50 | Root 100 |
|---|---|---|---|---|---|---|---|---|
| Before flowering | 2.59[b] | 2.94 | 3.71 | 3.28 | 0.13 | 0.28 | 0.43 | 0.34 |
| At flower budding | 19.97 | 26.62 | 38.59 | 34.79 | 1.94 | 1.59 | 2.61 | 2.57 |
| At full flowering | 47.59 | 54.96 | 84.05 | 76.63 | 12.29 | 11.31 | 11.16 | 11.44 |

[a] Concentration of gibberellic acid as milligrams per liter. [b] Average based on three replicates per treatment.

content of shoots was calculated per unit or per total dry weight (Tables V and VI). However, the alkaloidal content of roots did not show any significant changes on the basis of the total weight of roots (Table VI). An increase in alkaloidal content due to gibberellic acid treatment might be the result of an indirect influence of the stimulation of the vegetative growth, flowering process, and metabolic activity of the plants. Yunusov (25) explained the increased alkaloidal content of different plants by the intensive metabolism of the substances in which the alkaloids serve as biochemical catalysts. Furthermore, Brummett and Sciuchetti (8) reported that due to increased growth, the older plants of Atropa displayed about a 32% increase in total alkaloid production following gibberellic acid treatment.

## SUMMARY


Plants treated with gibberellic acid showed significant increases in the following characteristics: stem length (mainly at the third to the seventh internodes), number of leaves, number of branches, leaf area (first and second leaves), and dry weight of shoots. However, the dry weight of roots did not show any significant increase. The stem diameter was significantly decreased.

Gibberellic acid treatment enhanced and stimulated the flowering process of Atropa, especially at the first period.

An increased alkaloidal content of shoots was obtained for plants treated with 50 mg./l. gibberellic acid at the full flowering stage.


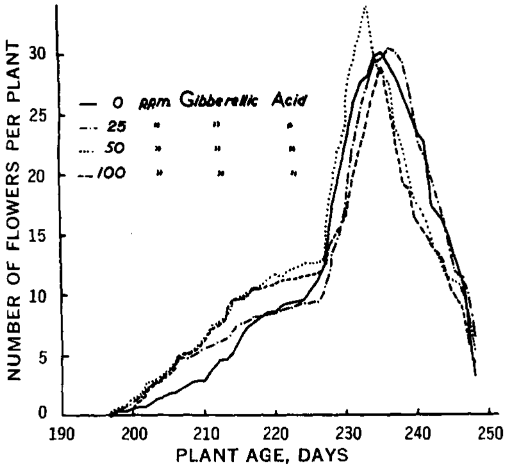


**Figure 2**—*Effect of gibberellic acid on the flowering rate.*

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