INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–2–210–213
http://www.ijab.org

Physiological Response of Periwinkle Plants (*Catharanthus roseus* L.) to Tryptophan and Putrescine

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ABSTRACT

A pot experiment was conducted in the screen of the National Research Centre during two successive seasons to study the response of periwinkle plants to foliar spray with tryptophan or putrescine at the concentrations 10^{-5} M, 10^{-4} M or 10^{-3} M. The obtained data indicated that exogenous application of tryptophan or putrescine on periwinkle transplants considerably increased plant growth at successive developmental stages. The effect was more pronounced with 10^{-3} M tryptophan or putrescine. Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids), soluble and total insoluble sugars, total proteins and total alkaloids in the leaves of periwinkle plants were increased as a result of application of tryptophan or putrescine, more so with 10^{-3} M each. Tryptophan at 10^{-4} or 10^{-3} M increased gibberellic acid (GA_3), IAA and ABA contents, while low concentration (10^{-5} M) of tryptophan decreased their endogenous levels. Tryptophan at 10^{-5} M or 10^{-4} M decreased cytokinins, while application of tryptophan at 10^{-3} M increase the endogenous cytokinins. Foliar application of putrescine at used concentrations caused obvious increases in the quantitative amounts of endogenous GA_3, IAA, cytokinins and ABA.

Key Words: *Catharanthus roseus* L.; Tryptophan; Putrescine

INTRODUCTION

Periwinkle *(Catharanthus roseus),* Family Apocynaceae, is a perennial herbaceous subshrub with creeping stems that root at the nodes and with short ascending flowering shoots. The opposite, short-stalked leaves are evergreen, leathery and elliptic. The flowering stems are used medicinally, containing several alkaloids, tannins, saponins, pectin and organic pigments. These substances give the plant tonic, astringent, hypotensive, vasodilating and diuretic properties (Stodola & Volák, 1992). It is used in some proprietary preparations for cardiovascular disorders and in herbalism for treating bleeding from the nose and gums, for diarrhea, coughing spasms and stomatitis, and in gynaecology (Stodola & Volák, 1992). It has also been found that its two alkaloids vincristine and vinblastine inhibit the growth of certain cancer-forming cells (Stodola & Volák, 1992).

The role of the amino acid tryptophan in stimulating the growth of several plant species were studied by Phillips (1971) who indicated that several alternative routes of IAA synthesis exist in plants all starting from amino acids. Russell (1982) reported that the increase in growth as a result of tryptophan application may be due to its conversion into IAA. Attoa *et al.* (2002) reported that spraying *Iberis amara* L. plants with the amino acid tryptophan increased plant growth.

Polyamines are small ubiquitous molecules that have been involved in nearly all developmental processes, including the stress response (Perez-Adamor *et al*., 2002). They are cationic molecules, positively charged under intracellular pH, which are essential for plant growth and differentiation, and thus are involved in various physiological processes (Flores & Galston, 1982; Friedman *et al*., 1989). They regulate growth, probably by binding to negatively charged macromolecules (Smith, 1985; Altman & Levin, 1993; Messiaen *et al.,* 1997). Rowland *et al.* (1988) reported that the most common polyamines studied in plants are the diamine putrescine (Put), the triamine spermidine (Spd) and the tetramine spermine (Spm). They also report that polyamines are part of the overall metabolism of nitrogenous compounds.

The first unequivocally established function of polyamines at the molecular level is the donation of a 4-aminobutyl moiety by spermidine to the eukaryotic initiation factor 5A (eIF-5A) precursor of protein to form the amino acid hypusine (Park *et al.,* 1993). Polyamines can influence the transcriptional and translational stages of protein synthesis (Pegg, 1986), stabilize membranes (Schuber, 1989) and alter intracellular free calcium levels (Khan *et al*., 1993). Smith *et al*. (1985) work on peas (*Pisum sativum*) suggested that polyamines might be important in the portion of the GA response that results from cell division, but not in the portion resulting from cell elongation.

The aim of this work was to study the effect of the amino acid tryptophan and the diamine putrescine on the growth, some chemical constituents and endogenous hormones, as well as their effect on improving total alkaloids of periwinkle plants.

MATERIALS AND METHODS

Plant materials and growth conditions. A pot experiment was carried out during two successive seasons of (2001-2003) at the screen of National Research Centre, Dokki,

Giza, Egypt. Seeds of *Catharanthus roseus* were obtained from Horticulture Research Institute, Agricultural Research Centre, Ministry of Agriculture, A.R.E. The seeds were sown in the nursery on 21st February, 2001 and 2002, respectively. Forty five days later, the seedlings were transferred into clay pots 30 cm in diameter. Each pot contained 8 kg loamy clay soil. Thirty days later, transplants were sprayed with different concentrations of tryptophan and putrescine (10^{-5}, 10^{-4} and 10^{-3} M).

Treatments were distributed in complete randomized block design with five replications comprized with five pots for each replicate. Fifteen days after transplanting, the seedlings were thinned to the most three uniform plants in each pot and fertilized with two grams of potassium sulphate. Each pot received equal and adequate amounts of water (one Liter water twice every week). Each pot was fertilized with 4 g phosphorous as calcium superphosphate mixed with the soil before transplanting. Three grams of nitrogen as ammonium sulphate in three applications (one g for each) with two weeks intervals started 30 days after transplanting.

Measurement of growth parameters. Plant height (cm), number of branches per plant, fresh and dry weights of leaves and stems (g/plant) were determined at different growth stages (vegetative, flowering and fruiting stages, respectively).

Chemical analysis. Three plant samples were taken during the growing season, at vegetative growth stage, flowering stage and fruiting stage. Total soluble and insoluble sugars contents of the leaves were determined according to Yemm and Willis (1954) and Herbert *et al.* (1971). Total protein was determined using the method of Bradford (1976). Photosynthetic pigments were determined according to the method described by von Wettstein (1957).

Alkaloids mainly (perivine, vinblastine and ajmalicine) were extracted and determined as total alkaloids using the methods described in the U.S. Pharmacopoeia (1970), Merck Index (1976) and modified by Trease and Evans (1978). Standard curve was firstly established for the authentic of the alkaloids as ajmalicine was extracted according to Masoud *et al.* (1968). Extraction and estimation of gibberellins, auxins and cytokinins was carried out as follows: The plant samples were taken at the vegetative growth stage, weighed, frozen with liquid nitrogen and stored in deep freezer at -20ºC until use. The frozen plant materials (100 g fresh samples after 10 days from the second spray) were homogenized with absolute ice cold methanol in blender and stored at 3ºC for 24 h. The methanol was filtered and two subsequent extractions were made with 80% ice cold methanol. The alcoholic extracts were evaporated to aqueous phase at a temperature not exceeding 30ºC under reduced pressure (Badr *et al*., 1971). The aqueous phase was adjusted to pH 8.2 with 1 N KOH and partitioned three times against equal volumes of petroleum ether according to (Van Bragt, 1969), the petroleum ether phase was discarded. Separation of gibberellins, auxins and cytokinins was carried out according to Hiraja *et al.* (1972). Extracts were analyzed using a Waters 54100 High performance liquid chromatograph (HPLC). Samples were separated isocratically on a M Bonda Pak C_{18}- (3.9 x 300 mm) column was used for determination of endogenous hormones.

Statistical analysis. Data obtained were subjected to standard analysis of variance procedure. The values of LSD were obtained whenever F values were significant at 5% level as reported by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Growth measurements. Data presented in Table I show that foliar application of tryptophan significantly increased plant height, number of branches, fresh and dry weights of leaves and branches in the vegetative growth stage, flowering stage and fruiting stage, more so with 10^{-3} M tryptophan. These results are in agreement with previous reports (Russell, 1982; Harridy 1986; Attoa *et al*., 2002). The increase in the growth as a result of tryptophan application may be due to its conversion into IAA (Russell, 1982).

Putrescine significantly promoted growth at successive developmental stages (Table I). The effect was more pronounced with 10^{-3} M. Other studies report that

Table I. Effect of tryptophan and putrescine on different growth parameters of periwinkle plants. The data are of two seasons

Treatment	Plant height (cm)	No. of branches	Fresh wt. of leaves (g/plant)	Dry wt. of leaves (g/plant)	Fresh wt. of stems (g/plant)	Dry wt. of stems (g/plant)
Vegetative growth stage						
Control	18.00	5.67	8.20	1.22	2.20	0.16
T 10^{-5} M	25.00	8.00	10.43	1.37	3.35	0.36
T 10^{-4} M	27.00	8.33	11.24	1.53	3.52	0.43
T 10^{-3} M	34.00	9.00	15.33	2.09	5.15	0.57
P 10^{-5} M	28.00	6.33	10.61	1.57	3.73	0.49
P 10^{-4} M	35.33	6.33	11.85	1.63	6.72	0.61
P 10^{-3} M	40.00	9.00	19.85	2.71	8.51	1.26
LSD (5%)	2.16	1.17	1.82	0.46	0.77	0.21
Flowering stage						
Control	41.00	9.00	34.37	5.56	25.77	3.98
T 10^{-5} M	52.00	14.67	48.91	7.97	36.44	6.49
T 10^{-4} M	54.67	18.67	53.16	8.85	43.70	8.01
T 10^{-3} M	73.67	19.67	54.87	9.59	46.77	8.17
P 10^{-5} M	54.67	14.33	46.81	8.96	38.27	9.21
P 10^{-4} M	76.00	14.67	59.02	9.00	47.33	12.54
P 10^{-3} M	82.00	19.67	67.40	10.72	59.33	13.73
LSD (5%)	1.90	1.77	1.78	0.99	2.43	1.09
Fruiting stage						
Control	64.00	11.67	57.85	11.76	41.70	9.50
T 10^{-5} M	64.67	12.67	73.07	12.42	45.43	9.52
T 10^{-4} M	76.00	15.00	77.59	13.82	67.90	12.86
T 10^{-3} M	82.67	15.33	83.05	14.03	72.46	14.17
P 10^{-5} M	83.67	12.33	58.54	12.94	69.08	17.12
P 10^{-4} M	95.33	17.00	76.79	17.33	81.89	21.07
P 10^{-3} M	102.33	19.00	99.08	19.87	84.51	22.27
LSD (5%)	2.38	2.10	2.89	2.08	2.85	1.58

T = Tryptophan , P = Putrescine

polyamines influence plant growth in various species (Smith, 1982; Cohen, 1998; Egea-Cortines & Mizrahi, 1991). Polyamines exhibit their effects on growth through enhancing cell division and expansion (Cohen, 1998). Polyamines can also act as source of nitrogen, which stimulates growth (Smith, 1982).

Chemical constituents. Data presented in Table II indicate that foliar application of tryptophan slightly increased the photosynthetic pigments (i.e. chlorophyll a, chlorophyll b and carotenoids), total soluble and insoluble sugars and alkaloidal contents in the leaves of periwinkle plants at the flowering and fruiting stages. The effect was more pronounced as the tryptophan concentration was increased. Similar results have been reported in other plant species (Milad, 1998; Shoala, 2000; Attoa *et al.,* 2002).

Foliar application of putrescine to periwinkle plants significantly increased chlorophyll a, chlorophyll b contents, total carotenoids, as well as total protein and alkaloids at the two growth stages (Table II). The putrescine induced effects on the previous parameters increase with concentration (Table II). Polyamines have been found to affect protein synthesis and nitrogenous compounds metabolism (Rowland *et al.,* 1988; Serafini-Fracassini, 1991). Polyamines effects may be attributed to their binding to negatively charged macromolecules (Smith, 1985; Altman & Levin, 1993; Messiaen *et al.,* 1997).

Endogenous hormones. Table III shows that spraying periwinkle plants with different concentrations of tryptophan and putrescine caused differences in the content of endogenous growth promoters (IAA, GA_3 and cyrokinins) and inhibitor (abscissic acid, ABA). High concentrations of tryptophan (10^{-4} M or 10^{-3} M) increased IAA GA_3 and ABA, while low concentration decreased their contents.

Table III. Effect of tryptophan and putrescine on the endogenous hormones of periwinkle plants at the vegetative growth stage (calculated as µg/g fresh weight)

Treatment	GA_3	Cytokinins	IAA	ABA
Control	8.471	305.784	128.903	100.290
T 10^{-5} M	7.691	189.838	117.989	114.300
T 10^{-4} M	15.156	241.654	240.512	252.758
T10^{-3} M	14.138	417.003	246.004	215.732
P 10^{-5} M	18.529	326.766	240.925	218.659
P 10^{-4} M	13.528	839.772	301.969	203.051
P 10^{-3} M	16.551	483.068	343.759	279.560

T = Tryptophan , P = Putrescine

Application of tryptophan at the rates of 10^{-5} M or 10^{-4} M decreased cytokinins, while 10^{-3} M increased the endogenous cytokinins (Table III).

Several investigators have suggested that polyamines are part of the signal response pathway for various plant hormones. Bagni (1966) demonstrated that polyamines could help break dormancy and stimulate cell proliferation in tubers of *Helianthus tuberosus*, normally a hormone-dependent process. Since then, polyamines have been implicated in the response of plants to ethylene, to gibberellic acid and to cytokinins. They may be also regulated by phytochrome.

Our results are in agreement with the findings of various studies. Harridy (1986) demonstrated that tryptophan applied to *Catharanthus roseus* plants increased IAA concentration. The increase of indoles may be attributed to the conversion of tryptophan to IAA as stated by Phillips (1971). In addition, Nag *et al*. (2001) reported that using putrescine at the concentration of 10^{-4} M on mung bean cuttings (*Vigna radiata* cv. 105) increased the level of IAA and decreased the level of IAA-oxidase activity

Table II. Effect of tryptophan and putrescine on chemical constituents of periwinkle plants. The data are of one season (2001/2002)

Treatment	Chl a (mg/g F.wt.)	Chl b (mg/g F.wt.)	Carotenoids (mg/g F.wt.)	Total Sol. Sugars (mg/100 g DW)	Total Insoluble Sugars (mg/100g DW)	Total protein (µg/g FW)	Total Alkaloids (mg/g DW)
Flowering stage							
Control	6.36	1.73	3.44	7991.06	10250.81	1439.10	0.67
T 10^{-5} M	6.83	2.25	3.74	10167.05	17542.90	1483.58	1.12
T 10^{-4} M	7.96	2.84	4.88	11017.39	18753.36	1620.07	1.49
T 10^{-3} M	8.59	4.62	5.94	14231.68	20693.41	1632.71	1.59
P 10^{-5} M	8.37	5.94	3.96	10915.35	12303.10	1437.36	1.13
P 10^{-4} M	8.98	6.71	3.54	13139.90	14951.06	1461.78	1.47
P 10^{-3} M	9.79	9.79	4.06	13588.82	21889.84	1552.48	1.56
LSD (5%)	1.64	0.49	0.70	710.47	820.12	18.60	0.12
Fruiting Stage							
Control	4.69	1.63	4.26	11588.82	12300.55	1173.56	0.83
T 10^{-5} M	7.24	2.38	4.76	12323.51	12578.61	2217.01	1.13
T 10^{-4} M	8.18	2.82	4.06	12741.88	12846.98	2241.43	1.18
T 10^{-3} M	9.24	3.15	3.43	12752.09	14496.98	2319.05	1.48
P 10^{-5} M	5.98	2.00	3.44	13874.53	12558.21	1461.78	0.98
P 10^{-4} M	6.14	2.01	3.23	14180.66	14435.76	2130.67	1.32
P 10^{-3} M	7.24	2.38	3.86	14588.82	19206.16	2298.99	1.90
LSD (5%)	1.50	0.65	0.49	688.47	636.94	23.10	0.48

T = Tryptophan , P = Putrescine

compared with untreated cuttings. In this respect, Zaghlool (2002) found that application of spermidine to *Vigna radiata* L. increased endogenous IAA and decreased the ABA content during seedling stage. Recently, Bais and Ravishnakar (2003) indicated that treating hairy roots of *Cichorium intybus* L. with putrescine at 1.5 mM concentration showed maximum endogenous IAA content as compared to the control.

In conclusion, treatment periwinkle plants with tryptophan or putrescine had beneficial influence for increasing plant growth and alkaloids content.

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(Received 10 December 2004; Accepted 07 February 2005)