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IAA-oxidase in *Impatiens balsamina* Affected by GA_3 and Tannic Acid

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ABSTRACT

The activity of IAA-oxidase increased in the leaves of *Impatiens balsamina* plants receiving inductive photoperiodic cycles and in plants receiving treatments with gibberellic acid (GA_3) and/or tannic acid (TA), even under non-inductive photoperiods; the activity also increased in the stem receiving inductive photoperiodic cycles (8 h). Treatment with GA_3 and TA mimics the effect of SD cycles in the development of some isoenzymes of IAA-oxidase. Thus a new isoenzyme at R_f 0·48 developed in the leaves and one at R_f 0·82 developed in both the stem and the leaves of all plants receiving inductive treatments – photoperiodic or chemical – but not in water-treated controls under non-inductive photoperiods. Another isoenzyme at R_f 0·68 developed only in the stems.

Key words: Flowering, gibberellic acid, IAA oxidase, *Impatiens*, phenols, photoperiod.

INTRODUCTION

Phenolic compounds have been reported to act as analogues of growth hormones (Vendrig and Buffel, 1961; Wain and Taylor, 1965), and they also affect germination (White, Hillman and Phillips, 1972), growth (Henderson and Nitsch, 1962) and other physiological functions (Tomaszewski, 1964). Gibberellic acid as well as tannic acid induce floral buds under strictly non-inductive photoperiods and together they synergistically increase the number of floral buds in the qualitative short-day plant *Impatiens balsamina* (Nanda *et al.*, 1969: Kumar, Sharma and Nanda, 1978; Kanwar and Nanda, 1985).

Phenolics are reported to influence the effect of phytohormones on growth and development by affecting the activity of IAA-oxidase and thereby IAA metabolism (Basu, 1972; Sheen, 1973; Haissig, 1974), while monophenols and m-diphenols and polyphenols are considered to act as inhibitors of IAA-oxidase (Pilet, 1966). It was considered of interest to study the effect of GA_3 and tannic acid (TA) on changes in the activities and electrophoretic patterns of IAA-oxidase enzymes in *I. balsamina* to check if they were in any way related to floral induction.

MATERIALS AND METHODS

Pure-line seeds of *Impatiens balsamina* L. cv. Rose were obtained from experimental plots of the Punjab University Botany Department, Chandigarh, India. The seeds were sown in earthenware pots (20 cm diameter) containing a 3:1 mixture of sand and garden soil. The plants were raised under continuous illumination until used for experimentation. For

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continuous illumination, the plants received natural daylight from 0900 to 1700 h and were then transferred to illuminated cabinets in an air-conditioned room maintained at 28 ± 1 °C to receive artificial illumination from 1700 to 0900 h. The source of artificial illumination was cool white fluorescent tubes (Philips, 80 W each) with incandescent lamps (Philips, 120 W each) in between, fitted on light banks that were maintained at a height to provide a light intensity of 3000 lux. For 8 h photoperiods, the natural daylight was curtailed by screening the plants with a thick canvas sheet daily from 1700 to 0900 h.

When the plants had grown to $9{\cdot}5 \pm 0{\cdot}1$ cm and had $8{\cdot}7 \pm 0{\cdot}2$ unfolded leaves, 565 plants were selected for uniformity of size and divided into two equal lots to be exposed to 24 h and 8 h photoperiods respectively. Application of $2{\cdot}9 \times 10^{-6}$ mol m^{-3} GA_3, $5{\cdot}9 \times 10^{-7}$ mol m^{-3} TA and $2{\cdot}9 \times 10^{-6}$ mol m^{-3} $GA_3 + 5{\cdot}9 \times 10^{-7}$ mol m^{-3} TA along with photoperiodic cycles was given as shown in figures. The chemical treatments were given to the apices of plants wrapped with small cotton wads on alternate days by dropping 30 μg of the test solution each time. Ten plants in each group were set apart for observations on the days taken to floral-bud initiation and the total number of floral buds produced per plant. The remaining plants were used for collecting samples initially and after 1, 2, 6, 16 and 50 d.

IAA-oxidase activity

Fully mature young leaves on main axis and correspondingly the stem portion of each sample were homogenized at 4 °C. The crude enzyme was extracted in 0·067 mol dm^{-3} sodium phosphate buffer, pH 7. The supernatant was collected after centrifugation at 23000 g and stored at 0 °C. The IAA-oxidase activity was determined by the method of Tang and Bonner (1948). The activity was expressed as IAA oxidized per mg protein per hour.

Electrophoretic pattern of IAA-oxidase

Isoenzymes were separated by disc electrophoresis on polyacrylamide gels at 4 °C following the method of Ornstein (1964) and Davis (1964). Lithium hydroxide boric acid buffer 0·025 M (pH 9) was used in the electrode vessels. An aliquot of extract equivalent to 1500 μg protein was loaded above the gels and was overlayered by 10 per cent urea solution. Protein content was determined by the method described by Lowry *et al.* (1951) using folin reagent. The gels after their removal were stained by the method described by Endo (1968) in a mixture consisting of 0·8 mg potassium indole acetate, 0·08 mg 2,3,6-trichlorophenol (TCP) and 2 mg fast blue BB salt per ml of phosphate buffer at pH 6·0 and was incubated at 30 °C for 24 h to obtain a dark staining. The chemicals used were from Sigma (USA), BDH (England) and Sarabhi (India).

RESULTS

Floral data

Table 1 shows that water-treated plants did not flower under 24 h photoperiods; floral buds were produced on plants treated with either GA_3 or TA or GA_3+TA. Floral bud initiation occurred earlier and the number of buds was higher in plants treated with the combination GA_3+TA than in plants treated with each alone under both photoperiods.

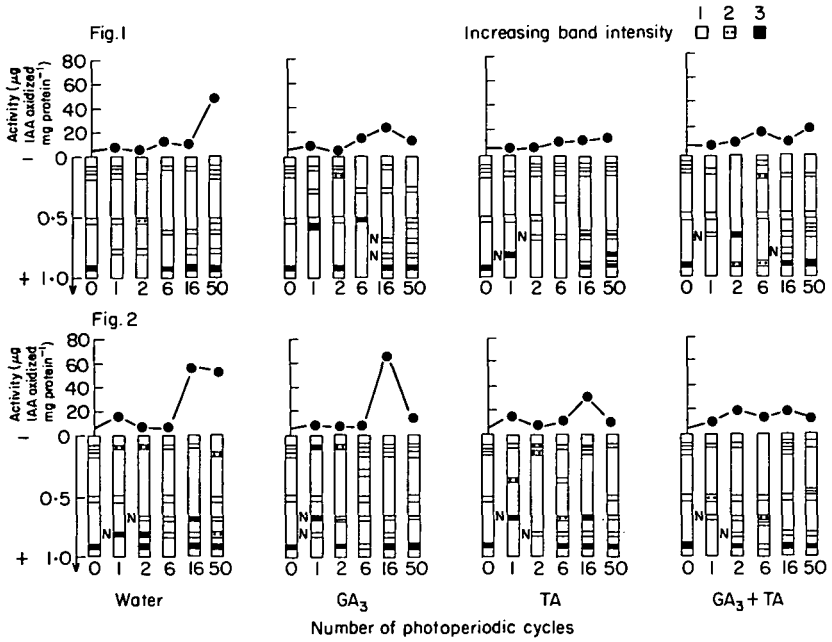
IAA-oxidase

Stem: total activity. Figure 1 shows that IAA-oxidase activity of the stem of water- as well as chemical-treated plants did not change much throughout the period of

TABLE 1. *Effect of GA_3 and tannic acid, alone and in combination with each other on days of floral bud initiation and total number of floral buds produced in* Impatiens balsamina *exposed to 24 h and 8 h photoperiods*

Treatment	Days to floral bud initiation		Total number of floral buds	
	24 h	8 h	24 h	8 h
Control	0	13·2±0·2	0	19·5±0·3
GA_3	19·7±1·2	11·5±0·3	57·3±4·4	37·3±2·3
TA	37·8±1·9	15·0±0·6	14·8±1·8	34·2±1·4
GA_3+TA	16·2±1·2	11·80±0·5	65·6±3·3	47·5±3·0

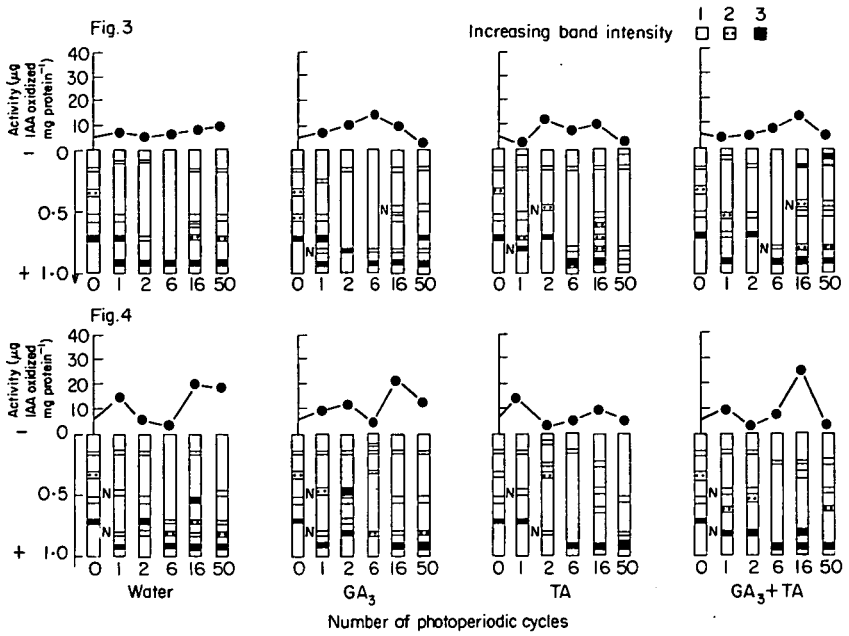
Values are mean ± s.e. at 95 per cent level of significance.



FIGS 1–2. Changes in the activity and electrophoretic pattern of IAA-oxidase in the stem of *Impatiens balsamina* receiving treatments with water, $2{\cdot}9 \times 10^{-6}$ mol m^{-3} GA_3, and $5{\cdot}9 \times 10^{-7}$ mol m^{-3} TA, singly and together accompanying varying numbers of long day (Fig. 1) and short day (Fig. 2) cycles. Numbers of abscissa represent number of photoperiodic cycles indicated. N stands for a new isoenzyme band.

experimentation except in control plants, where it increased at the end of the experimental period.

In water-treated controls and plants receiving GA_3 and TA treatments, the IAA-oxidase activity of the stem increased appreciably with eight treatments accompanying 16 short-day (SD) cycles, but decreased with 25 treatments accompanying 50 SD cycles. In plants receiving GA_3+TA a slight increase in activity occurred with a single treatment accompanying two SD cycles but there was little change with a further increase in the number of treatments and SD cycles (Fig. 2).



FIGS 3–4. Changes in the activity and electrophoretic pattern of IAA-oxidase in the leaves of *Impatiens balsamina* receiving treatments with $2{\cdot}9 \times 10^{-6}$ mol m^{-3} GA_3 and $5{\cdot}9 \times 10^{-7}$ mol m^{-3} TA, singly and together accompanying varying numbers of long day (Fig. 3) and short day (Fig. 4) cycles. Numbers of abscissa represent number of photoperiodic cycles indicated. N stands for a new isoenzyme band.

Electrophoretic patterns

Initially four bands could be observed in the stem. Two new bands at R_fs 0·68 and 0·82 developed in GA_3, TA and GA_3+TA but not in water-treated plants (Fig. 1). The band at R_f 0·68 appeared in GA_3+TA-treated plants receiving a single long-day (LD) cycle and also in plants receiving a single treatment with TA accompanying two LD cycles or eight treatments with GA_3 accompanying 16 LD cycles. The second band at R_f 0·82 appeared with eight treatments with GA_3 or GA_3+TA accompanying 16 LD cycles and also in plants receiving 1 and 25 treatments with TA accompanying 1 and 50 LD cycles respectively.

Both these bands developed even in water-treated plants exposed to short-day cycles (Fig. 2). The band at R_f 0·68 appeared in the stems of plants receiving a single treatment with GA_3 or TA or GA_3+TA accompanying a single SD cycle but in water-treated controls with two SD cycles. While it persisted in plants receiving an increasing number of treatments, with GA_3 or TA accompanying the increasing number of SD cycles, it disappeared in plants receiving eight treatments with the combination GA_3+TA accompanying 16 SD cycles. The band at Rf 0·82 also appeared in water-treated controls and plants receiving a single treatment with GA_3 when accompanied by a single SD cycle and a single treatment with TA or GA_3+TA when accompanied by two SD cycles.

Leaf: total activity. Figure 3 shows that IAA-oxidase activity of the leaves of water-treated controls did not change significantly with the number of 24 h photoperiodic cycles (Fig. 3). In plants receiving GA_3 and GA_3+TA, the activity increased with the increasing number of treatments accompanying LD cycles, the highest value being reached with

three and eight treatments accompanying 6 and 16 LD cycles, respectively, to decrease with further increase in the number of treatments accompanying LDs. In plants receiving TA, the activity decreased with a single treatment accompanying one LD cycle but increased when it was accompanied by two LD cycles.

The trends of changes in IAA-oxidase activity of the leaves of water-treated controls and of plants receiving treatments with GA_3, TA and GA_3 + TA accompanying SD cycles were more or less similar. Thus in all cases the activity increased in plants receiving a single treatment accompanying a single SD cycle, to decrease subsequently. The activity again increased in all cases with eight treatments accompanying 16 SD cycles, to decrease with 25 treatments accompanying 50 SD cycles (Fig. 4).

Electrophoretic patterns

Four bands could be observed in the leaves initially. Two bands at R_fs 0·48 and 0·82 appeared in the plants receiving GA_3, TA and GA_3 + TA treatments under 24-h photoperiods but not in water-treated controls under this photoperiod (Fig. 3). The band at R_f 0·82 developed in plants receiving a single treatment with either GA_3 or TA accompanying a single LD cycle but three treatments with the combination GA_3 + TA accompanying six LD cycles. Another band at R_f 0·48 appeared in plants receiving eight treatments with GA_3 + TA accompanying 16 LD cycles but a single treatment with TA accompanying two LD cycles or eight treatments with GA_3 accompanying 16 LD cycles. These two bands developed even in the leaves of water-treated control plants under 8 h photoperiods (Fig. 4). The band at R_f 0·48 appeared with a single treatment accompanying one SD cycle in all cases. Another band at R_f 0·82 appeared in water-treated controls and in plants receiving a single treatment with GA_3 or GA_3 + TA accompanying one SD cycle and also in those receiving a single treatment with TA accompanying two SD cycles.

DISCUSSION

The results presented in this paper confirm the earlier findings that both GA_3 and tannic acid cause floral induction under non-inductive conditions, while in combination with each other they accelerate floral bud initiation and increase the production of floral buds in this plant (Nanda *et al.*, 1969; Kumar, Sharma and Nanda, 1978; Kanwar and Nanda, 1985).

The increase in activity of IAA-oxidase in the leaves of plants receiving inductive photoperiodic cycles or treatments with GA_3 or TA or GA_3 + TA even under non-inductive photoperiods suggests that floral induction may be related to a lowering of the level of IAA. This is in accord with the results reported earlier from this laboratory that while exogenous application of IAA delays flowering, application of tri-iodobenzoic acid induces flowering in *Impatients balsamina* (Toky, Sawhney and Nanda, 1969; Sawhney, Toky and Nanda, 1970, 1971). The inductive effect of TIBA on this plant has been ascribed to the lowering in the level of endogenous auxin, attributable in turn to an increase in the activity of IAA-oxidase. A number of workers consider that phenolics act as analogues of plant growth hormones and affect flowering through their effect on IAA metabolism (Andreae, 1952; Henderson and Nitsch, 1962; Tomaszewski and Thimann, 1966; Sheen, 1973). An increase in IAA-oxidase activity with GA_3 treatment of pea buds has also been reported by Ockerse, Waber and Mescher (1970) and in *Nicotiana* by Zucker, Nitsch and Nitsch (1965).

Another point that emerges from this investigation is the appearance of the isoenzymes of IAA oxidase at R_f 0·48 in the leaves and at R_f 0·82 in both stem and leaves of plants receiving inductive treatments – either photoperiodic or chemical – and their absence in water-treated controls under non-inductive photoperiods. This points towards their

involvement in floral-bud initiation and flower development. But the appearance of the isoenzyme at R_f 0·68 in the stem only in plants receiving inductive treatments could indicate that it is concerned in the extension growth concomitant with floral induction. Ockerse *et al.* (1970) showed that oxidation of IAA by GA_3-promoted IAA-oxidizing isoenzymes, might lead to the formation of some intermediate products which are more active than IAA in stimulating growth and development.

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