ENZYME ACTIVITY AND ELECTROPHORETIC PATTERN OF ISOENZYMES OF AMYLASE, CATALASE AND PEROXIDASE IN PHOTO- AND GIBBERELLIN-INDUCED PLANTS OF IMPATIENS BALSAMINA L. VAR. ROSE

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Summary

Floral buds were induced either by 8 h photoperiods or by the application of GA$_3$ and GA$_{13}$ in Impatiens balsamina. Total enzyme activity was not directly related to floral morphogenesis although some interesting qualitative correlations existed. Thus, the presence of a new isoenzyme of amylase ($R_p$ 0.05) may be related to flower formation since it was present only in the induced plants and not in the vegetative ones, regardless of whether the flowering was caused by inductive photoperiods or by gibberellin treatment. Catalase activity, as well as its isoenzyme forms, could not be detected in the stem and the isoenzyme profile in leaves did not alter with photoperiodic or gibberellin treatment. Treatment with gibberellins induced the synthesis of new isoenzymes of peroxidase but inductive photoperiods did not. The enzyme profiles in relation to photoperiod and gibberellin application are discussed.

Introduction


Impatiens balsamina is a qualitative short-day plant (Nanda and Krishnamoorthy, 1967) in which gibberellins A$_3$ and A$_{13}$ induce the formation of floral buds under non-inductive conditions (Nanda et al., 1969). However, the two gibberellins differ in their effect on extension growth and enzyme activity. GA$_3$ enhances extension growth and also increases the activity of amylase and catalase but GA$_{13}$ does not affect extension growth and decreases enzyme activity slightly (Sawhney, Sawhney and Nanda, 1972). The present investigation was carried out to study the effect of GA$_3$ and GA$_{13}$ on enzyme activity and on the electrophoretic pattern of amylase, catalase and peroxidase in plants exposed to 24 and 8 h photoperiods.

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Material and Methods

Seedlings of Impatiens balsamina L. var. Rose were raised under continuous illumination until they were 3.5 ± 0.41 cm tall and had 8.0 ± 0.32 unfolded leaves. The plants were divided into two lots, to be exposed to either 24 h or 8 h photoperiods. Each lot was further divided into three groups to be treated with water or 10 mg 1⁻¹ of GA₃ or GA₁₃. Gibberellins (0.3 ml per plant) were given to the apices wrapped with tiny cotton wads, on alternate days. Observations on floral induction were recorded daily; height and number of leaves were measured after 20 days.

Samples for the estimation of the enzyme activity were taken at the beginning and after 20 days. For this purpose the plants of each treatment were separated into four batches of stem and leaves. The fresh plant material was washed and homogenized, the crude enzyme was extracted in 0.067 M phosphate buffer and the slurry centrifuged at 16000 rev min⁻¹ for 20 min. All operations were carried out at 4 °C. The resultant cell-free extracts were equalized with respect to their total protein content, using Folin's reagent (Lowry et al., 1951) before subjecting them to analysis.

Measurement of enzyme activities

Amylase activity was determined colorimetrically using soluble starch and I⁻KI following method of Sawhney, Toky and Nanda (1970). Catalase activity was determined by the method of Sawhney, Sawhney and Nanda (1972); the activity bears an inverse relationship to the time taken by an enzyme extract-dipped filter-paper disc to rise in diluted H₂O₂. Peroxidase activity was determined colorimetrically using benzidine-H₂O₂ as substrate and is expressed as units of activity per mg protein. The method was that described by Mitra, Jagannath and Bhatia (1970).

Electrophoretic separation of isoenzymes

Determination of different isoenzymes was made by disc electrophoresis (running buffer, pH 8.1, with lithium hydroxide–boric acid in electrode compartments and with 10% polyacrylamide gel at 4 °C). The method was essentially similar to that of Davis (1964). For amylase isoenzymes, gels (with 0.5% starch) were incubated for 1 h at 40 °C in acetate buffer (pH 5.6) and stained afterwards with 0.6% I⁻₂KI for 3 min. For catalase isoenzymes, gels (with 1% starch) were dipped in 0.5% H₂O₂ for 1 min, rinsed with water and placed in acidified 0.5% KI solution for 3 to 5 min. Colourless bands in both cases indicated the position of isoenzymes. Peroxidase isoenzymes were determined by staining the gels with equal volumes of 1% benzidine in 25% acetic acid and 1% H₂O₂, incubated at 30 °C for 2 min.

Results

Vegetative growth

Table 1 shows that water-treated plants grew taller under 8 h than under 24 h photoperiods. Of the two gibberellins, GA₃ and GA₁₃, only the former enhanced extension growth, the effect being more pronounced under 24 h than under 8 h photoperiods. More leaves were produced on plants under 24 h than under 8 h photoperiods. Treatment with GA₃ increased the number of leaves under both the photoperiods; treatment with GA₁₃ had no effect.
**Floral induction**

Floral buds were initiated on all plants under 8 h photoperiods. In 24 h photoperiods only gibberellin-treated plants formed floral buds and their formation was delayed.

### Table 1. Vegetative growth and floral induction of Impatiens balsamina plants treated with GA₃ or GA₁₃ under 24 and 8 h photoperiods

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Height (cm) after 20 days under the photoperiod</th>
<th>Leaf number after 20 days under the photoperiod</th>
<th>Days to floral induction under the photoperiod</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>8 h</td>
<td>24 h</td>
</tr>
<tr>
<td>Water</td>
<td>6.5 ± 0.12</td>
<td>21.0 ± 0.31</td>
<td>22.2 ± 0.34</td>
</tr>
<tr>
<td>GA₃</td>
<td>11.5 ± 0.18</td>
<td>26.0 ± 0.17</td>
<td>23.5 ± 0.20</td>
</tr>
<tr>
<td>GA₁₃</td>
<td>7.0 ± 0.29</td>
<td>21.1 ± 0.24</td>
<td>22.1 ± 0.43</td>
</tr>
</tbody>
</table>

**Total amylase activity**

In stem tissue, amylase activity was more or less the same with or without gibberellin treatment under both the photoperiods. However, in leaves, the activity decreased in water- and GA₁₃-treated plants exposed to 24 h photoperiods.

**Pattern of amylase isoenzymes**

In stem tissue only one band with $R_F$ 0·10 could be observed initially. Two new bands ($R_F$ 0·05 and 0·20) appeared in GA₃-treated plants both under 24 and 8 h photoperiods. While one of these bands ($R_F$ 0·05) appeared in GA₁₃-treated plants as well, the other one ($R_F$ 0·20) did not appear at all. It may be noted that the band at $R_F$ 0·05 appeared in water-treated plants also but only when subjected to 8 h photoperiods.

As in the stem, in leaves also, only one band with $R_F$ 0·10 was present initially. Another band ($R_F$ 0·05) identical to that in stem tissue was observed only in gibberellin-treated plants under 24 h photoperiods and in both water as well as gibberellin-treated plants under 8 h photoperiods. Another isoenzyme ($R_F$ 0·20) appeared in water as well as gibberellin-treated plants both under 8 and 24 h photoperiods (Fig. 1). It may be noted that all the isoenzymes in both the stem and the leaves were of low electrophoretic mobility.

**Total catalase activity**

Catalase activity was not detected at all in stem tissue regardless of the gibberellin treatment and the photoperiod to which the plants were exposed. Catalase activity of leaves was low initially but increased with time, the increase being maximal in GA₃-treated plants under 24 h photoperiods. In water-treated plants the maximum activity occurred under 8 h photoperiods.

**Pattern of catalase isoenzymes**

In stem tissue, no isoenzymes were detected. In leaves, six isoenzymes of catalase could be detected initially – four with low electrophoretic mobility and two ($R_F$ 0·63) and ($R_F$ 0·80) with higher mobility. No new band appeared in leaves, irrespective of gibberellin or photoperiodic treatment.
Fig. 1. Total enzyme activity and isoenzyme profiles in stem tissue and leaves of gibberellin-treated plants under 24 and 8 h photoperiods after 20 days. W, Water. Shading indicates increasing density: □ → □ → □.
**Isoenzymes in Impatiens**

**Total peroxidase activity**

Peroxidase activity which was high initially, decreased considerably in stems of water as well as gibberellin-treated plants under 24 h photoperiods. Under 8 h photoperiods, the decrease was checked by treatment with either of the gibberellins.

The peroxidase activity in leaves was initially lower than that in the stem. As in the stem, the activity in leaves of water-treated and gibberellin-treated plants decreased after 20 days, and only treatment with GA$_{13}$ under 24 h or with GA$_{3}$ under 8 h photoperiods partially checked the fall in activity.

**Pattern of peroxidase isoenzymes**

Nine isoenzymes could be observed in stem tissue initially. These bands persisted until the termination of the experiment. Three new bands ($R_f$ 0.50, 0.71 and 0.75) of high activity, appeared in GA$_{3}$ as well as GA$_{13}$-treated plants under 24 h photoperiods. Two of the three isoenzymes ($R_f$ 0.50 and 0.75) could be detected with gibberellin treatment under 8 h photoperiods as well, but not the third. As in the stem, nine isoenzymes appeared in leaves also and these persisted until the end. It may be noted that a new band ($R_f$ 0.05) appeared in gibberellin-treated plants under 8 h but not under 24 h photoperiods. A comparison of the initial band patterns in stem and leaves interestingly reveals a few similarities and many differences in position, intensity of staining and size of bands (Fig. 1).

**DISCUSSION**

Impatiens balsamina is a qualitative short-day plant

The results presented in this paper confirm the earlier findings that both GA$_{3}$ and GA$_{13}$ cause floral bud initiation under non-inductive conditions and that while GA$_{3}$ enhances extension growth, GA$_{13}$ does not affect it (Nanda et al., 1969).

Total activities of amylase, catalase and peroxidase showed no marked regular trend associated with floral morphogenesis induced either by the 8 h inductive photoperiods or by gibberellin application under 24 h non-inductive photoperiods.

However, study of the patterns of isoenzymes of amylase, catalase and peroxidase showed that there was some correlation with floral induction. The inductive photoperiods caused the synthesis of a new isoamylase ($R_f$ 0.05) in both stem and leaves. The appearance of this isoenzyme in water-treated plants under inductive photoperiods indicates its possible association with flowering. This view is supported by the fact that both the gibberellins which can induce flowering under non-inductive photoperiods also induced the synthesis of this isoenzyme. However, the appearance of this enzyme under inductive photoperiods in the stem, which is non-photoperceptive, can be questioned. It is noteworthy that a new protein band, although with higher electrophoretic mobility, was observed in the stem of induced balsam plants (Sawhney, Sawhney and Nanda, 1976). The significance of such cases is not clear. The appearance of one additional isoenzyme in leaves of all plants, irrespective of gibberellin or photoperiodic treatment, indicates its non-involvement in floral induction. Induction of this isoenzyme in the stem only by GA$_{3}$ suggests that it may be responsible for the mobilization of reserve food material which thus becomes available for enhanced growth.

Although catalase could not be detected in stem tissue, its activity was high in leaves. The selective distribution of this enzyme is in accord with the earlier report
The lack of difference in the isoenzyme profiles of catalase in leaves of induced and vegetative plants suggests that this enzyme was not involved qualitatively in floral morphogenesis and that the changes in total catalase activity in response to gibberellin and photoperiod were due to alterations in turnover of isoenzymes rather than to the synthesis of new ones.

Peroxidase activities also appeared to be qualitatively indifferent to photoperiodic since their electrophoretic pattern was not altered in numbers, mobility or intensity of the individual constituent isoenzymes. However, gibberellins were effective in differentially inducing the synthesis of new peroxidase isoenzymes in stem and leaves and with some connexion with inductive and non-inductive photoperiods. Thus, treatment with gibberellins induced the synthesis of new isoenzymes of peroxidase in the non-photoperceptive stem, irrespective of the photoperiod, but in photoperceptive leaves only under inductive photoperiods. The appearance of a new isoenzyme (RF 0-50) in the leaves of gibberellin-treated plants, but not in water-treated ones, rules out the involvement of this isoenzyme in floral morphogenesis. Such alterations of the isoenzyme profiles may be induced as a consequence of interaction of the gibberellins with photoperiod. It is interesting, however, that the isoenzyme profiles of the stem and leaves of untreated plants differed even under identical conditions of photoperiod, pointing again to the tissue specific distribution of different molecular forms of the same enzyme.

References


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