Stimulation of Root Thickening and Inhibition of Bolting by Jasmonic Acid in Beet Plants

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Abstract: Beet is a biennial plant. In the first season, it develops leaf rosette on a dwarf stem and forms a thick storage root. Based on the idea that some growth-inhibiting compound may participate in these developmental events, the effects of jasmonic acid (JA) on root thickening and stem elongation (bolting) were examined using beet plants cultured in vitro. JA failed to induce thickening of the tap root, but greatly promoted thickening of lateral roots. Furthermore, JA strongly inhibited bolting induced by chilling or exogenous gibberellic acid. The presence of JA in the tops (leaves and dwarf stems) of field-grown plants was confirmed by mass spectrometry. Although the content of JA seems to be insufficient to account for the induction of root-thickening and for the inhibition of bolting, the results suggest that JA-related compounds are involved in the thickening growth of storage root and in the formation of leaf rosette.

Key words: Beet, Bolting, Jasmonic acid, Leaf rosette, Storage root formation.

Several root crops such as carrot, radish, turnip and beet are biennial cold-requiring long-day plants. In the first season, the plant develops leaf rosette on a dwarf stem and accumulates assimilation products in a thick storage root under short days in the autumn. After the cold winter season, rapid growth of the dwarf stem called "bolting" occurs under long days in the spring and flowers are formed on the elongated stem. Unseasonable bolting that sometimes occurs in the first season causes severe problems in these crops. For example, it markedly lowers the sucrose content in storage roots of beet plants.

In many rosette long-day plants, an application of gibberellins (GA) under short-day conditions causes both bolting and flowering (Pharis and King, 1985). Furthermore, the rate of GA biosynthesis in the plants is higher under long-day conditions, when bolting and flowering take place, than during vegetative growth in the rosette stage under short-day conditions (Zeevaart and Gage, 1993). These results have led to the idea that rosette plants under short days are deficient in GA while the plants under long days produce enough GA which in turn would stimulate bolting and flowering. Although there is no doubt that GA play crucial roles in controlling bolting of the plants (Suge and Rappaport, 1968; Graebe, 1987), its participation in the control of flowering is a controversial matter (Pharis and King, 1985; Bernier, 1988).

Garner and Allard (1923) observed that the thickening growth of storage root in beet was stimulated by short-days. They also found that the overwintered beet plants soon ceased bolting and produced leaf rosette when exposed to short days. The final result of the short-day treatment was the formation of "aerial beet" (thickened stem) at the base of the leaf rosette. This observation suggests a close correlation between formation of leaf rosette and thickening growth of root under short days. The formation of aerial beet may not be explained by mere deficiency of GA. One possible explanation for this phenomenon is that some growth inhibitor that is formed under short-day conditions counteracts the effect of GA, resulting in the inhibition of bolting, formation of leaf rosette, and subsequent thickening of the root.

Tuberization in potato plants is induced by short days and is initiated by thickening growth at the sub-apical region of stolon. We previously reported the involvement of tuberonic acid (TA) and jasmonic acid (JA) in tuberization in potato (Koda and Okazawa, 1988; Koda et al., 1988; Yoshihara et al., 1989), am (Koda and Kikuta, 1991) and Jerusalem artichoke (Koda et al., 1994) plants. JA is thought to induce tuberization by inducing expansion of cells (Takahashi et al., 1994). Since JA has various inhibitory effects on plant growth (see Koda, 1992) and appears to be involved in the termination of stem growth of indeterminate types of soybean plants under short days (Koda et al., 1991), JA can be a candidate for the inhibitor that is responsible for the formation of leaf rosette and for the thickening growth of storage roots in beet plants.

In the present study, we examined the effects of JA both on the thickening growth of roots and on the bolting of beet plants cultured in vitro. We also examined the change in the content of endogenous JA.
during the growth of the field-grown plants, to elucidate roles of JA in these developmental events.

Materials and Methods

1. Culture of seedlings in vitro

Effects of exogenous JA on root thickening and bolting of beet plants (*Beta vulgaris* L. var.saccharifera Alef. cv. Monohikari) were examined using seedlings cultured in vitro. For simultaneous germination, the seeds were soaked in concentrated sulfuric acid for 30 min and rinsed with running tap water overnight. The seeds were then sterilized with a 1% solution of sodium hypochlorite for 1 h. After thorough washing with sterile water, the seeds were germinated in a 300-mL culture bottle containing 50mL of Murashige-Skoog medium (Murashige and Skoog, 1962) with a half-strength of inorganic salts (1/2 MS medium). The medium was solidified with 0.6% Bacto-agar. The cultures were maintained under a photoperiod of 16 h light and 8 h darkness (long-day conditions) at 25°C for one week. Illumination (1.9 mW cm⁻²) was provided from white fluorescent lamps.

2. Effect of JA on root thickening

The seedlings were cultured on the medium containing JA for 2 weeks under the same conditions as above. For light microscopy, the roots of the plants were fixed in a mixture of formalin, acetic acid, ethanol and water (2:1:10:7 v/v/v) and processed in the usual way for sectioning on a microtome and sectioned at 10 μm thickness (Jensen, 1962). The preparations were stained with a safranin-fast green combination.

3. Effects of JA and GA₃ on bolting

In vitro bolting can be induced either by chilling the 1-week-old seedlings for one month at 4°C, or by removal of both roots and cotyledons from the seedlings. The chilled seedlings were used to examine sole effect of JA on bolting. After the chilling, roots were removed from the seedlings to facilitate uptake of JA and cultured on medium containing JA at various concentrations for one month. To examine effects of JA and gibberellic acid (GA₃) on bolting, we removed the roots and cotyledons from the seedlings and transferred the remaining shoots to medium that contained GA₃ and JA at various concentrations. The shoots were cultured for one month. The criterion for bolting was visible elongation of the epicotyl above the cotyledonary node. The rate of bolting was calculated as the number of bolted shoots divided by the number of total shoots.

All in vitro experiments were repeated twice, each with at least 15 seedlings and the results showed high reproducibility.

4. Determination of the content of JA in the tops of beet plants

The seeds were sown in paper pots and grown in a green-house for five weeks. The seedlings were transplanted in an experimental field early in May, and grown in the usual manner. Tops (leaves and dwarf stems) were harvested from the plants three times during the growth and subjected to extraction of JA and ABA. In a preliminary experiment, we compared content of JA-like compounds in the tops of field-grown plants with those in the roots by bioassay for tuber-inducing activity as reported previously (Koda and Okazawa, 1988). The amount of JA-like compounds in the tops was always larger than that in the roots during the growth of the plants. Therefore, only the tops were subjected to the determination of the JA content. Tops (usually 300 g in fresh weight) were homogenized immediately after harvest with a sufficient amount of ethanol to give a final extract in 70% ethanol. JA was separated from the ethanolic extract, and the amount of JA was determined by HPLC as reported previously (Koda and Kikutu, 1991). The recovery of JA which was calculated by the addition of standard preparation of JA was 50%±8% (± SD, n=5). No corrections were made for any values presented in the Figure. In addition to the contents of JA, the content of abscisic acid (ABA) in the same samples was also determined. ABA fractions obtained from silica gel ODS column chromatography were purified with two successive procedures of HPLC on a cartridge of Novapak C₁₈ (Waters) using the following solvents that contained 0.1% acetic acid; 60% methanol (retention time of authentic cis-ABA was 6.5 min.), 30% acetonitrile (10.5 min.). The authenticity of the isolated JA and ABA was confirmed by electron impact (EI) mass spectrometry, at the GC-MS & NMR Laboratory of the Faculty of Agriculture, Hokkaido University.

Results

1. Effect of JA on root thickening

JA at concentrations below 10⁻⁶ M did not induce any appreciable morphological changes in the seedlings except for a slight inhibition of growth. At a concentration of 10⁻⁵ M, JA induced thickening of lateral roots (Fig. 1) and changed the color of the roots from white to brown. When the concentration was raised to 10⁻⁴ M, JA induced both a slight thickening of tap root and nodulation of lateral roots (data not presented). Microscopic observations of the transections of lateral roots showed that the thickening of the root in response to 10⁻⁵ M JA was due to both expansion of cells in the cortical tissue and an increase in the number of cell in the cortical tissue and the central cylinder (Table 1).

2. Effects of JA and GA₃ on bolting

JA inhibited the chilling-induced bolting of the seedlings; the rate of bolting at concentrations of 0, 10⁻⁶, 10⁻⁵ and 10⁻⁴ M being 1.0, 0.9, 0.8 and 0, respectively (data not presented).

Fig. 2A shows the effects of JA and GA₃ on the bolting of seedlings from which roots and cotyledons had been
removed. \( \text{GA}_3 \) stimulated bolting, while \( \text{JA} \) strongly inhibited it dose-dependently. In addition, \( \text{GA}_3 \) promoted the growth of epicotyl whereas \( \text{JA} \) inhibited it (Fig. 2B). The typical appearance of shoots after the culture on the medium containing \( 10^{-6} \) M \( \text{GA}_3 \) and \( \text{JA} \) at various concentrations for one month is shown in Fig. 3.

3. Presence of JA and ABA in tops

The mass spectrum of the isolated JA-like compound revealed that the compound had a molecular ion peak at \( \text{m/z} \) 210 and major peaks at \( \text{m/z} \) 192, 181, 163, 151, 142, 133, 121, 109, 95, 83, 67, 60, 55 and 41. The molecular weight and the fragmentation pattern were identical to those of authentic JA. Thus, the presence of JA in beet tops was confirmed.

Since ABA has various inhibitory effects on plant growth, ABA can be another candidate for the factor that induces the formation of leaf rosette. The presence of ABA in beet tops was also confirmed by mass spectrometry; the isolated ABA had a molecular ion peak at \( \text{m/z} \) 264 and major peaks at \( \text{m/z} \) 246, 231, 204, 190, 162, 149, 136, 121, 108 and 93, which were identical to those of authentic ABA.

4. Changes in the contents of JA and ABA during the plant growth

If JA is indeed involved in the thickening of roots, the JA content in the tops should be associated with the

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**Table 1. Effects of jasmonic acid on thickening of lateral roots of beet plants. One-week-old seedlings of beet were cultured on the medium with or without \( 10^{-5} \) M JA for 2 weeks.**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Addition</th>
<th>Width of the tissue along radius (μm)</th>
<th>Number of cells along radius</th>
<th>Diameter of cells (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>None</td>
<td>23.5±4.9 (100)</td>
<td>2.3±0.1 (100)</td>
<td>10.3±1.9 (100)</td>
</tr>
<tr>
<td></td>
<td>( 10^{-5} ) M</td>
<td>74.0±2.9 (314)</td>
<td>3.8±0.5 (165)</td>
<td>19.2±4.4 (186)</td>
</tr>
<tr>
<td>Central cylinder</td>
<td>None</td>
<td>12.0±2.6 (100)</td>
<td>3.0±0.3 (100)</td>
<td>4.1±0.6 (100)</td>
</tr>
<tr>
<td></td>
<td>( 10^{-5} ) M</td>
<td>27.8±3.1 (231)</td>
<td>5.2±0.8 (173)</td>
<td>5.7±0.5 (139)</td>
</tr>
</tbody>
</table>

(±SD, \( n = 5 \))

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**Fig. 1. Typical appearance of beet root thickened in response to \( 10^{-5} \) M JA (A, bottom), and sections of lateral roots after a 2-weeks culture on the medium without JA (B) or with \( 10^{-4} \) M JA (C). TR, tap root; LR, lateral root. Bars indicate 100 μm.**

**Fig. 2. Effects of JA and \( \text{GA}_3 \) on bolting (A) and growth of the epicotyl of beet (B). Cotyledons and roots were removed from 1-week-old seedlings and the remaining shoots were cultured for 1 month on the medium supplemented with JA and \( \text{GA}_3 \) at various concentrations. The error bars represent ±SD (\( n = 15 \)).**
thickening of roots. In the field-grown plants, thickening of roots became prominent in August (3 months after transplanting) (Fig. 4A). The content of JA per fresh weight was more or less constant from July to August and then decreased (Fig. 4B). The maximum level of JA in the tops that was corrected by the recovery rate was $5.3 \times 10^{-7}$ mol (kg fresh weight)$^{-1}$. The amount of JA per plant reached a maximum in August. The content of ABA was much lower than that of JA at any stage of growth.

**Discussion**

The rosette plants have shoot apices and leaves close to the soil surface in the first season. The rosette habit appears to be acquired by plants during their evolutionary processes in order to protect themselves from detrimental environmental conditions such as a strong wind and large daily temperature fluctuation. The plants must produce growth inhibitor(s) to keep the rosette style. Wareing and El-Antably (1970) reported that the growth-inhibiting activity, that was determined by wheat coleoptile elongation test, in the extract from spinach plants grown under short days was stronger than that from the plants exposed to long days. Zeewaart (1971) found that the inhibitor is not ABA, since ABA content increased rather than decreased when the plants were transferred from short days to long days. The low ABA content in the tops of beet plants (in the order of $10^{-8}$ - $10^{-9}$ M, Fig. 4B) suggested that ABA does not participate in the control of rosette style in beet plants.

Exogenous JA strongly inhibited chilling- or GA$_3$-induced bolting in beet plants (Figs. 2 and 3.), and JA was found in the tops of the plants. The results imply the participation of JA in the inhibition of bolting, in other words, in the maintenance of rosette style. Although the content of endogenous JA (in the order of $10^{-7}$ M, Fig. 4B) in the tops of field-grown plants may be insufficient to inhibit bolting (Fig. 2), it is possible that enough JA is localized in the dwarf stem.

Exogenous application of JA to beet seedlings failed to induce thickening growth of tap roots, but induced marked thickening of lateral roots (Fig. 1C). An increase in the number of cells in the JA-treated roots (Table 1) suggests that JA stimulates cambial activity and participates in the formation of storage root in beet. It is well known that thickening of storage roots is attributable to the activity of vascular cambium, and the cambial activity of excised roots can be induced by both auxin and cytokinin (Torrey and Roomis, 1967; Peterson, 1972; Webster and Radin, 1972). Loomis and Torrey (1964) succeeded in activating cambium in isolated radish roots by introducing hormone mixture from the basal cut end. On the other hand, Nakatani (1994) demonstrated the tuberous root formation of sweet potato in vitro by introducing JA from the basal cut end and cytokinin from the root tip. Their results suggest that establishment of an elaborate culture technique is necessary to demonstrate the stimulative effect of JA on storage root formation of beet in vitro.

The findings that exogenous JA caused thickening of roots and inhibited bolting are not enough to prove the
participation of endogenous JA in these events. However, the results presented herein raise a possibility that JA-related compounds play important roles both in the maintenance of rosette style and in the thickening of storage roots in beet plants.

References


