Involvement of Jasmonic Acid and Related Compounds in the Tuberization of Jerusalem Artichoke Plants (*Helianthus tuberosus* L.)

Yasunori KODA, Kiyoshi TAKAHASHI and Yoshio KIKUTA
(Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan)

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Abstract : Using a bioassay for tuber-inducing activity, which was carried out with cultures of single-node segments of potato stems *in vitro*, jasmonic acid (JA) was isolated from the leaves of Jerusalem artichoke (*Helianthus tuberosus* L.), and identified by both HPLC and mass spectrometry. The level of endogenous JA in the leaves of young plants was very high (4.0×10^{-6} mol $\cdot$ kg $^{-1}$) but decreased sharply with the growth of the plants. By contrast, the level of water-soluble derivatives of JA increased with the growth of plants and reached a maximum near the time at which tuberization was initiated. The effect of exogenous JA on the tuberization of Jerusalem artichoke plants was examined *in vitro*, and JA was found to have strong tuber-inducing activity. These results suggest that tuberization of Jerusalem artichoke plants is controlled by JA and related compounds.

Key words : *Helianthus tuberosus*, Jasmonic acid, Jerusalem artichoke, Tuberization.

キクイモ塊茎形成におけるジャスモン酸類の役割: 幸田泰則・高橋 淳・喜久田嘉郎 (北海道大学農学部)

要 旨 : 野生型と塊茎収量の高い都城在来型の2種のキクイモを用いて、キクイモの塊茎形成を制御する内生要因について検討した。葉に含まれる塊茎形成促進物質を、バレイショ塊茎形成活性検定法を用いて追求した結果、ジャスモン酸 (JA) が単離・同定された。生育初期の都城在来型の葉中の JA 含量は野生型のものより7倍程高く (4.0×10^{-6} mol $\cdot$ kg 生重 $^{-1}$)、植物体の生育に伴って急激な減少を示した。これに対し、水溶性分画に含まれる JA 誘導体と見られる活性は生育と共に増加し、地下部における塊茎形成開始時にやや遅れて最大となり以降は減少を示した。野生型の JA 類の葉中の含量はかなり低く推移したが、全体としての消長はほぼ同様であった。この2種のキクイモの茎断片に JA を与えて培養したところ、JA は強い塊茎形成活性を示した。これらの結果はキクイモの塊茎形成は JA 類によって制御されていることを示唆している。葉で合成された JA は水溶性の誘導体に変化した後、地下部へと送られ塊茎形成を誘起するものと考えられる。

キーワード : 塊茎形成, キクイモ, ジャスモン酸。

Various herbaceous plants have developed tubers for their vegetative propagation. Tuberization in potato plants is known to be controlled by both tuberonic acid (TA) and its glucoside^{8,9)}. The chemical structure of TA is closely related to that of jasmonic acid (JA)¹⁴⁾, and JA also has strong potato tuber-inducing activity^{5,6)}. Furthermore, JA also considered to be involved in the tuberization of yam plants⁴⁾. Henceforth, we designate TA, JA and related compounds as JA-related compounds.

Tuberization in Jerusalem artichoke plants (*Helianthus tuberosus* L.) is under the control of the photoperiod, as is potato tuberization^{2,3,15)}. Short days stimulate the process while long days inhibit it. Zimmerman and Hitchcock¹⁵⁾ suggested that some hormone-like substances produced in leaves under short-day conditions might be responsible for the tuberization. It seems, therefore, very possible that JA-related compounds are involved in the tuber-

ization of Jerusalem artichoke plants. Using bioassay for potato tuber-inducing activity, Matsuura et al¹⁰⁾ have isolated four compounds, including JA and methyl glucosyl TA, from leaves of wild type of Jerusalem artichoke plants.

If JA-related compounds are indeed involved in the tuberization of Jerusalem artichoke plants, the levels of these compounds in the leaves should show characteristic changes that are associated with tuberization. Furthermore, obviously, these compounds must be capable of inducing tuberization of Jerusalem artichoke plants. This report describes a study of the possible involvement of JA-related compounds in the tuberization of Jerusalem artichoke plants.

Materials and Methods

1. Plant material

Two types of Jerusalem artichoke plants (*Helianthus tuberosus* L.) were used. One was

the wild type, which is characterized by long stolons and a low yield of tubers. Seed tubers of this type were collected on the campus of Hokkaido University early in May 1992. The other type was the Miyakonojo variety, which is characterized by shorter stolons and a high yield of tubers. This variety was obtained from the National Agriculture Center (Tsukuba, Ibaraki, Japan). Tubers of both types were planted in an experimental field on May 13, 1992, and plants were raised in the usual manner. The new tubers began to grow early in August in both cases. The leaves were harvested four times during the growth of the plants. The leaves were subjected to extraction of JA and related compounds.

2. Culture of segments of stems *in vitro*

Seed tubers of both types were sterilized with a 1% solution of sodium hypochlorite for 15 min and rinsed thoroughly with running tap water. Then they were planted in a plastic pot that contained vermiculite and grown in the dark at 25°C. After 4 weeks, the etiolated shoots were harvested and cut into single-node segments of about 2 cm in length.

The single-node segments were sterilized with a 1% solution of sodium hypochlorite for 1 h and then washed with sterile water. Then a portion of 5 mm in length was removed from the end of each segment. Three segments were placed in a 100-ml Erlenmeyer flask that contained 20 ml of Murashige-Skoog medium¹²⁾, solidified with 0.6% Bacto-agar (Difco) and supplemented with JA. The concentration of sucrose in the medium was either 2% or 9% (w/v). Five replicates of each experimental culture were prepared. The cultures were maintained at 25°C in the dark. After 2 months in culture, the rate of tuberization was calculated as the number of segments with tuberized laterals divided by the number of segments with laterals that had emerged.

3. Extraction of JA-related compounds from the leaves

Leaves, usually 200 g fresh weight, were homogenized immediately after harvest with sufficient ethanol to give a final extract in 70% ethanol. The homogenate was allowed to stand overnight at 4°C and then filtered. The filtered extract was concentrated and the resultant aqueous residue was acidified to pH 3.0 with 1 M HCl and extracted three times with ethyl acetate. The ethyl acetate fraction

was separated into acidic and neutral/basic fractions by extraction with 1 M sodium bicarbonate in the usual way. The fractions were dried over anhydrous sodium sulfate and evaporated to dryness. The acidic ethyl acetate fraction was fractionated by chromatography on a column of charcoal, and JA was purified from the eluate of the column by chromatography on a cartridge of Sep-Pak C₁₈ (Waters) as reported previously⁴⁾.

The neutral/basic ethyl acetate fraction, in which we expected JA-Me to be present, was hydrolyzed to free acids with 1 M NaOH for 5 h at room temperature. After acidification to pH 3.0 with 1 M HCl, the acidic ethyl acetate fraction was recovered from the aqueous solution. JA was purified from this fraction as described above.

The aqueous fraction that remained after the first ethyl acetate extraction contained water-soluble derivatives of JA-related compounds, such as methyl glucosyl JA¹⁰⁾. The aqueous fraction was purified to some extent by chromatography on a charcoal column as reported previously⁴⁾. The level of JA-related compounds in this fraction was estimated by a bioassay for potato tuber-inducing activity.

4. Determination of the level of JA by HPLC

The level of JA was determined by HPLC as reported previously⁴⁾. The amount of JA in the sample was calculated from a standard curve constructed from measurements of peak areas generated by known amounts of JA. The recovery of JA by this purification procedure, which was calculated by the addition of a standard preparation of JA was $65 \pm 5\%$ ($\pm$ SD, $n=5$). No corrections were made of any values cited in the Results.

5. Bioassay for JA-related compounds

Since JA-related compounds have strong potato tuber-inducing activity⁶⁾, a bioassay for JA-related compounds was carried out using cultures of single-node segments of potato stems *in vitro*, as reported previously⁸⁾.

Results

1. Presence of JA in leaves of Jerusalem artichoke plants

To examine the nature of JA-related compounds in the leaves of var. Miyakonojo, the eluate from the charcoal column, after chromatography of the acidic ethyl acetate

Fig. 1. Potato tuber-inducing activities from an extract of Jerusalem artichoke leaves. The eluate from a charcoal column, after chromatography of the acidic ethyl acetate fraction obtained from the leaves, was fractionated on a silica gel ODS column in 60% methanol that contained 0.1% acetic acid. Fractions were assayed for tuber-inducing activity in cultures of single-node segments of potato stems *in vitro*. Bars indicate positions of elution of authentic TA and JA under the same conditions.

fraction obtained from the first harvest of the variety was fractionated on a silica gel ODS column (RQ-2, Fuji gel) as reported previously⁴⁾ and fractions were assayed for potato tuber-inducing activity. One large peak and three small peaks of activity were found, and the large peak was eluted with the same volume of elution as JA (Fig. 1). The presence of JA in this peak was confirmed by purification by HPLC and subsequent mass spectrometry. The fractions under this peak were combined and evaporated to dryness. The residue was dissolved in chloroform and insoluble substances were removed by filtration. The chloroform-soluble fraction was fractionated on a Novapak C₁₈ column (Waters) in 60% methanol that contained 0.1% acetic acid. Fractions of 0.5 ml each were collected and assayed for tuber-inducing activity. A single peak of activity was found in the fractions that corresponded to JA (elution volume, 8–9 ml). The active fractions were combined and rechromatographed on the same column in 26% acetonitrile that contained 0.1% acetic acid. The elution pro-

Fig. 2. EI-Mass spectra of authentic JA (A) and that of the compound isolated from the leaves of Jerusalem artichoke (B).

file, monitored in terms of absorbance at 210 nm, revealed the presence of a sharp peak at a position that corresponded to the retention time of JA (25.0 min), and the activity was found only in this fraction. The fractions under the peak were collected, purified again on the same column with the same solvent and subjected to electron impact (EI) mass spectrometry, which was carried out at the GC-MS & NMR Laboratory of the Faculty of Agriculture, Hokkaido University. The spectrum revealed that the purified substance had a molecular ion peak at m/z 210 (Fig. 2). The molecular weight and the fragmentation pattern were identical to those of authentic JA. Thus, the occurrence of JA in the leaves of var. Miyakonojo was confirmed. The final yield of JA from 200 g of fresh leaves was 108 μ g.

Free TA could not be detected among the compounds responsible for the second small peak of activity shown in Figure 1.

Fig. 3. Changes in the levels of JA (●), water-soluble derivatives of JA (○) and methyl jasmonate (■) in leaves of Jerusalem artichoke (var. Miyakonojo) during the growth of the plant. The levels of JA were determined by HPLC and those of methyl jasmonate were determined by HPLC after hydrolysis to JA. Levels of water-soluble derivatives were determined by the bioassay. The arrows indicate the time at which tuberization became apparent in the plants.

2. Changes in levels of JA-related compounds in Jerusalem artichoke leaves during the growth of the plant

Changes in the levels of JA in the acidic ethyl acetate fraction were examined by HPLC. The level in the leaves of young plants of var. Miyakonojo was very high (540 ng g fresh weight⁻¹) and the level decreased sharply with the growth of the plants (Fig. 3). The level of JA-related compounds in the water-soluble fraction, as determined by the bioassay, increased with the growth of the plants and reached a maximum about one month after the start of tuberization. The level of methyl jasmonate, as determined by HPLC after hydrolysis to JA, was low and showed the same pattern of changes as that of JA-related compounds in the water-soluble fraction.

The level of JA and that of JA-related compounds in the water-soluble fraction in leaves of the wild type showed similar changes

Fig. 4. Changes in the levels of JA (●) and water-soluble derivatives of JA (○) in leaves of Jerusalem artichoke (wild type) during the growth of the plant. The arrows indicate the time at which tuberization became apparent in the plants.

to those of var. Miyakonojo (Fig. 4). However, the levels of JA and related compounds were much lower in the wild type than in var. Miyakonojo.

3. Effect of JA on the tuberization of Jerusalem artichoke *in vitro*

The effect of exogenously applied JA on the tuberization of Jerusalem artichoke was examined in cultures of single-node segments of stems *in vitro*. JA induced the tuberization of both types of plant in a similar manner. Increases in the concentration of sucrose in the medium increased the ability of JA to induce tuberization. When 2% sucrose was added to the medium, JA exhibited tuber-inducing activity at concentrations above 3×10^{-5} M. In the presence of 9% sucrose, JA exhibited tuber-inducing activity at concentrations above 10^{-6} M. The rates of tuberization of the wild type induced by JA at concentrations of 10^{-7} , 10^{-6} and 10^{-5} M under these conditions were 0, 0.46 and 0.67, respectively. The typical appearance of tubers of the wild type that were induced by JA is shown in Figure 5. Although tubers were not formed at a concentration of 10^{-7} M, the lateral shoots did become shorter and thicker.

Discussion

The data presented herein suggest that JA

Fig. 5. Typical appearance of Jerusalem artichoke tubers induced by JA. Numbers indicate the molar concentration of JA in the test medium. Single-node segments of stems were cultured on medium that contained JA at 25°C in darkness for two months. Arrows indicate tubers.

and related compounds, in particular water-soluble derivatives of JA, play an important role in tuberization of Jerusalem artichoke plants. Since Matsuura et al.¹⁰⁾ have isolated methyl glucosyl TA which is soluble in water from leaves of wild type of Jerusalem artichoke, a major JA-related compounds in water-soluble fraction appears to be this compound. A sharp decrease in the level of JA and a concomitant increase in the level of water-soluble derivatives was observed prior to the start of tuberization (Figs. 3 and 4). The results suggest that JA synthesized in the leaves is metabolized to water-soluble derivatives, which are suitable for long-distance transport, and that these derivatives are transmitted to underground parts of the plant to induce tuberization.

The level of JA in the leaves of var. Miyakonojo at the first harvest was 4.0×10^{-6} mol $\cdot$ kg⁻¹ if the value is corrected by the recovery of JA (65%). The level was much higher than that in leaves of the wild type (Figs. 3 and 4). Although we did not carried out exact yield

surveys of these two varieties, a large difference was found in the final yield of tubers in this experiment. The yield per plant was ca. 1,700 g fresh weight in var. Miyakonojo and ca. 300 g fresh weight in the wild type, when average yields from three individual plants were calculated. The level of JA in the leaves seems to be related to the final yield of tubers.

The tuber-inducing activity of JA is lower in Jerusalem artichoke plants than in potato plants. As reported previously, JA was capable of inducing potato tuberization *in vitro* at concentrations above 10^{-7} M when the medium contained 2% sucrose^{5,6)}. However, under analogous conditions, tuberization of Jerusalem artichoke plants *in vitro* was induced by JA at concentrations above 3×10^{-5} M. The tuberization of many plants appears to be controlled by a balance between the level of gibberellins, which inhibit the process¹³⁾, and that of JA-related compounds, which stimulate it. The considerable height (more than 2 m) and the long inter-node length of Jerusalem artichoke plants suggest the presence

of high levels of gibberellins in the plants. Murakami¹¹⁾ reported the presence of considerable amounts of gibberellins in tubers of this plants. The difference in tuber-inducing activities of JA between potato and Jerusalem artichoke may be attributable to a difference in the levels of endogenous gibberellins.

The involvement of JA-related compounds in the tuberization in potato plants^{8,9,14)}, yam plants⁴⁾ and Jerusalem artichoke plants suggests that tuberization in many other tuber-forming plants may also be controlled by JA-related compounds. It appears that tuberization is initiated by the radial expansion of cells with subsequent cell division^{1,7)}. JA-related compounds seem to trigger tuberization by inducing the radial expansion of cells.

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* In Japanese.