

Seasonal Changes in Jasmonic Acid Contents of Yam Leaves

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ABSTRACT

This study confirmed that the initiation time of tuberization was well consistent with the activation time of JA. The consistency was also confirmed in the tuberization of yam plants under the altered condition of natural day length. The final yield of JA from 500g fresh leaves was 89.3 μ g

Key words: jasmonic acid, yam, seasonal change, tuberization

INTRODUCTION

Yam has widely been cultivated and utilized as a staple food and vegetable in the wet tropical regions of the world and the temperate regions of East Asia. Tuberization in yam is predominantly under the control of photoperiod. Short days promote the tuberization, whereas long days inhibit the process. When we cultivate East Asia native yam plants in Japan in spring, tubers are formed at seedling stage of early growth. The growth of tubers is ceased for a long time and only leaf and stems grow vigorously. Tubers start to enlarge from early fall and such growth pattern is unique compared to other tuber crops. However, the chemical nature of the stimulus remains unknown. Koda and Okazawa(1988) suggested that cytokinin and ABA have some roles in the tuberization process in potato but they are not the tuberization stimulus which triggers the process. Koda and Kikuta(1991) designated jasmonic acid(JA) to be involved in the tuberization of yam plants. Koda et al(1994) suggested that tuberization of Jerusalem artichoke plants is controlled by JA and related compounds. Chang et al.(1997) demonstrated the possible involvement of JA in the tuberization of yam plants using a bioassay for tuber-inducing activity.

This report describes a seasonal changes in JA contents of yam leaves to clarify the possible involvement

of JA in the tuberization of yam plants.

MATERIALS AND METHODS

Seed tubers of yam(*Dioscorea alata* L.) were introduced from Indonesia and planted in an experimental field on April 25, 1996 and raised in the usual manner. The tubers germinated between May 20 and May 25. The new underground tubers began to grow early in June. The leaves were respectively harvested in the middle of July, August, September, and October. The leaves were subjected to extraction of JA-related compounds.

The leaves(usually 500g fresh weight) were homogenized immediately after harvest with sufficient methanol to give a final extract in 80% methanol. The homogenate was kept overnight at 4 $^{\circ}$ C and then filtered. The filtered extract was concentrated in vacuo at 35 $^{\circ}$ C and was added with distilled water and extracted three times with hexane. The filtered extract was concentrated and the resultant aqueous residue was acidified to pH3.0 with 1M HCl and extracted three times with ethyl acetate. The ethyl acetate fraction was concentrated in vacuo at 35 $^{\circ}$ C and passed through charcoal column and eluted with acetone. The fractions were dried over anhydrous sodium sulfate and evaporated to dryness. The acidic ethyl acetate fraction was dissolved in 100ml of 30% ethanol sonicated for a few minutes and filtered. The filtrate was passed through a

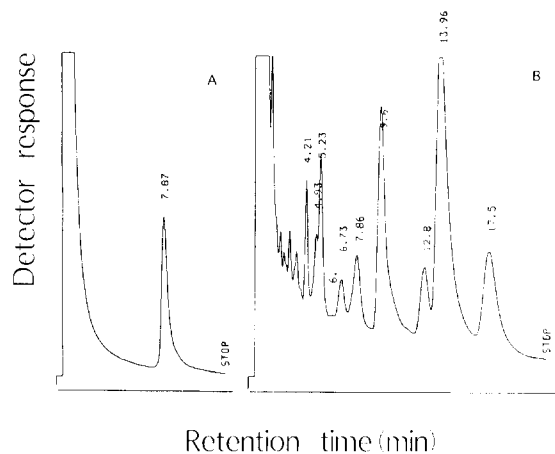


Fig. 1. Gas chromatograms of the methylated samples extracted from leaves under 10 hour day length(B) and of the methyl jasmonate 0.1 μ g(A). The samples was eluted with n-hexane from Si-gel column. GC conditions: glass column(3mm \times 2m) packed with 5% SE; N₂ flow rate, 50ml/min; column temp. 165°C

100ml column of silica gel and then eluted with n-hexane-EtoAc. Determination of the level of JA in acidic ethyl acetate fraction by GC was carried out as reported previously(Chang et al., 1997).

RESULTS AND DISCUSSION

Presence of JA in yam leaves

To examine the nature of JA-related compounds in yam leaves, the eluate from the charcoal column after chromatography of the acidic ethyl acetate fraction obtained from the third harvest was fractionated on a silica gel column and assayed for tuber-inducing activity. The elution profile showed the presence of a sharp peak that correspond to the retention time(7.87min.) of JA(Fig. 1). Thus, the occurrence of JA in yam leaves was confirmed. The final yield of JA from 500g fresh leaves was 89.3 μ g.

Changes in levels of JA-related compounds in yam leaves during the growth of the plant

The tuber growth of yam grown in the field was retarded by July, August, and early September but started to regrow on the middle of September(Fig. 2). The shortening of the day length was the main factor causing the growth

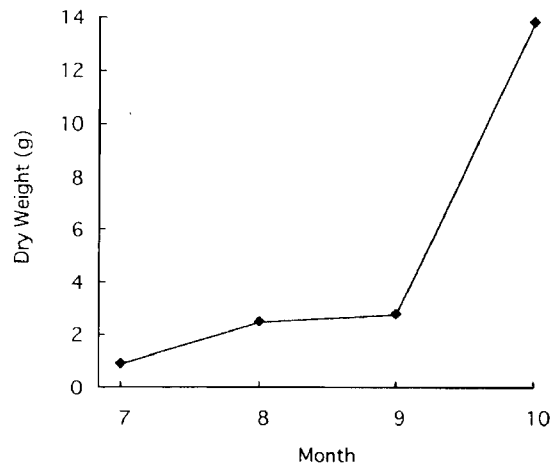


Fig. 2. Monthly changes in the growth and enlargement of tubers in yams(*D. alata* L. cv. Solo Yam).

and enlargement of tubers in yam. The time of commencement of the thickening growth was consistently in September regardless of the different time of planting(Hayashi & Ishihata, 1990; 1991). Changes in the levels of JA in the acidic ethyl acetate fraction were examined by gas chromatography. A close relationship was observed between the shift in growth of the yam tuber and the activity of substances which is active. The marked increase of JA content was found at the shift in growth of yam plants from growth retardedness to regrowth. The level of JA increased highly in the leaves harvested on the middle of September(Fig. 3). JA was not detected in the leaves harvested before July but detected in the leaves harvested on August. Thereafter, the level of JA decreased sharply as shown in Fig. 3. After Gregory's suggestion(1956) about the occurrence of a specific tuberization stimulus in potato leaves, many studies were done to identify the stimulus in the root crops(Melis and Van Staden, 1984; Ewing, 1985; Koda and Okazawa, 1988; Koda et al., 1988; Koda and Kikuta, 1991; Nakatani, 1997). They are most concerned on the effects of known plant hormones on the tuberization. Kumar and Warening(1988) demonstrated that tuberization begins just after cessation of stolon elongation and the tuberization stimulus, which moves acropetally, inhibits the shoot growth Koda and Kikuta(1991) isolated JA from yam leaves. In a previous paper(Chang and Hayashi, 1995),

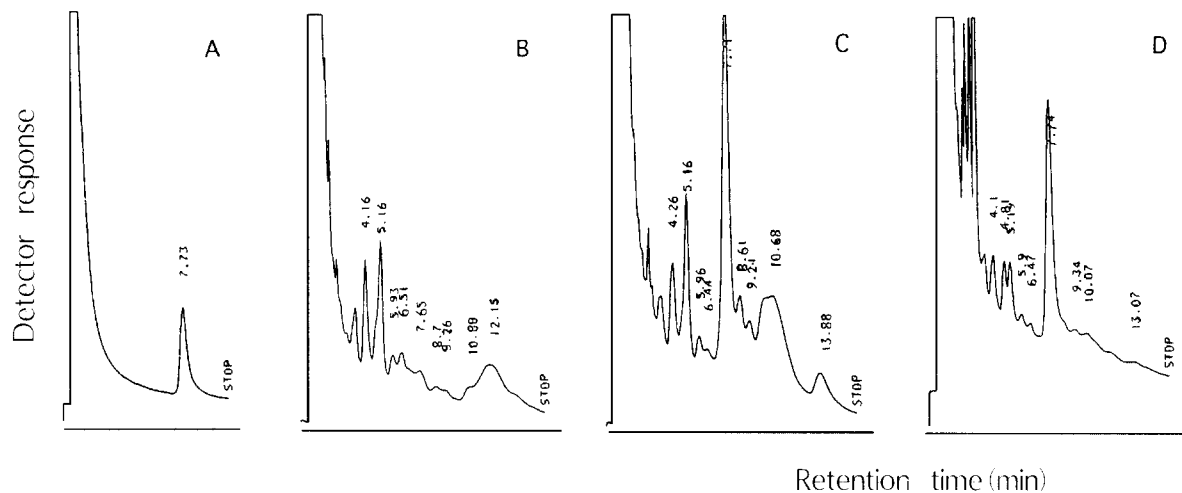


Fig. 3. Gas chromatograms of the methylated samples extracted from leaves August (B), September(C), October(D) and of the methyl jasmonate 0.1 μ g(A).

we developed a bioassay method for testing activity of plant hormones using microtubers of yams and made the analysis of physiological mechanism for tuberization possible.

This study confirmed that the initiation time of tuberization was well consistent with the activation time of JA. The consistency was also confirmed in the tuberization of yam plants under the altered condition of natural day length. Consequently, it seems that tuberization of yam occurs by the activation of JA, which is possible when yam plants respond to short day length. From this result, it is expected that chemical control using JA can be used for the year round cultivation and utilization of yam as a vegetable in tropical region.

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