Endogenous Gibberellins in Bulbils of Chinese Yam during Growth and Storage

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Abstract: Five gibberellins in the early-13-hydroxylation pathway (GA15, GA44, GA19, GA20 and GA1), and six gibberellins in the non-13-hydroxylation pathway (GA12, GA15, GA24, GA9, GA36 and GA4), were detected in the bulbils of Chinese yam. This indicated the presence of two gibberellin biosynthetic pathways in bulbils. The total endogenous gibberellins were dramatically increased in enlarged bulbils. The endogenous level of bioactive GA4 was always higher than that of GA1. A rapid increase in endogenous gibberellins including bioactive GA1 was observed during a 30-day storage period. These results show that gibberellins are closely related to bulbil enlargement and dormancy in Chinese yam plants. However, further research is needed for better understanding of the fluctuation of gibberellin levels in bulbils of Chinese yam during storage.

Keywords: Bulbils, Chinese yam, Dioscorea opposita, Gibberellins, Growth, Storage.

Gibberellins are required for the growth and development of Chinese yam (Dioscorea opposita Thunb.). In many plants, exogenous gibberellin induces dormancy breaking and enhances seed germination and tuber sprouting (Okagami and Nagao, 1973; Okagami and Tanno, 1977). By contrast, in some plants gibberellins also induces dormancy. One of the most characteristic physiological features of Chinese yam is that dormancy of tubers and bulbils is induced by application of gibberellin (Okagami and Nagao, 1971). The sprouting of aerial tubers (bulbils) of Begonia evansiana (Nagao and Mitsui, 1959), like that of resting buds of some woody plants (Brian et al., 1959; Weaver, 1959), is delayed by gibberellin treatment, while exogenous gibberellins (GAs) have a dormancy-breaking effect in most plants. Apart from the physiological role of gibberellin in relation to dormancy, endogenous gibberellins are closely related to tuber enlargement of Chinese yam and gibberellin treatment increases tuber yield (Kim et al., 2003a, 2003b). Endogenous gibberellins in tubers and leaves during the tuber enlargement stage in Chinese yam in which the concentration of GA1 is higher than that of GA15. This suggests that endogenous gibberellins are basically required for tuber growth and development.

However, little is known about the biosynthesis pathway of gibberellins in the vegetative and reproductive tissues of the Chinese yam because their concentrations are low. To date, eight endogenous GAs, i.e., GA15, GA20, GA24, GA9, GA36, GA4, GA4, GA53, GA19, GA20, GA12, GA24, GA9, GA36 and GA4, have been identified in the bulbils of the Chinese yam (Tanno et al., 1992), suggesting that two different gibberellin biosynthetic pathways are involved in the ripening of bulbils of Chinese yam.

To our knowledge, the endogenous gibberellins have not been quantitatively analyzed in relation to developmental stage and storage periods of bulbils in Chinese yam.

In this study, we determined the levels of endogenous gibberellins in the bulbils at various harvest dates and after various storage periods in the Chinese yam.

Material and Methods

1. Plant material and growth conditions

Two different experiments were conducted to quantitatively analyze the changes of endogenous gibberellins in bulbils during growth and storage periods. In the first experiment, tubers of Chinese yam (Dioscorea opposita cv. Tsukune) were cut into three or six pieces (50-60 g FW). Then the pieces were pre-sprouted on a mixture of vermiculite and sand (1:1, v/v) in plastic pots (50 × 30 × 15 cm), and kept in a chamber at 25°C under a continuous dark...
condition. On 15 to 20 June 2003, after 30 to 35 days of incubation, the sprouted tuber pieces were planted to a natural growth condition in a field by the standard method used at the Institute for Bioresources Research at Gyeongbuk Provincial Agricultural Technology Administration. Bulbils were sampled between 4:00 p.m. and 4:20 p.m. from the plants grown for 85 (August 7), 105 (August 27), 125 (September 15), 145 (October 5) and 165 (October 25) days after planting. In the second experiment, fully ripened bulbils collected on November 22 in a greenhouse were stored in a temperature-controlled room in the dark at 4 ± 0.5°C at an approximate relative humidity of 85 ± 1% until gibberellin analysis. Bulbils were sampled at 30, 60, 90, and 120 days after the start of storage.

2. Extraction and HPLC of endogenous gibberellins

Bulbils (0.5 g, dry weight) were extracted as described elsewhere (Lee et al., 1998). After methanolic extraction, the GAs in the extracts were chromatographed on a 3.9 X 300 mm µ-BondaPak C18 column (Waters Associates Co., USA) and eluted at flow rate of 1.5 mL min\(^{-1}\) with the following gradient: from 0 to 5 min, isocratic 28% MeOH in 1% aqueous acetic acid; from 5 to 35 min, linear gradient from 28 to 86% MeOH; from 35 to 36 min, 86 to 100% MeOH; and from 36 to 40 min, isocratic 100% MeOH. Fifty fractions of 1.5 ml each were collected. Small aliquots (15 µl) from each fraction were taken, and the radioactivity was measured by liquid scintillation methods (Beckman, LS 1801) to determine accurate retention times of each GA based upon the elution of \(^3\)H-GA standards and labeled (deuterated) GA standards (obtained from Prof. Lewis N. Mander, Australian National University, Research School of Chemistry, Canberra, Australia), previously determined.

3. GC-MS-selected ion monitoring (GC-MS-SIM)

Each concentrated GA fraction was redissolved in 100% methanol, transferred to a 1 mL vial and dried under N\(_2\) at 40°C. The sample was dissolved in 35 µl of methanol, and the GA methyl ester was prepared with ethereal diazomethane. The sample was dried under N\(_2\), redissolved in methanol and methylated once more. The sample was dissolved in 35µl pyridine, and silylated for 30 min at 65°C with the same amount of N, O-bis (trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% TMCS (Pierce Chemical Co.). The sample was then reduced to dryness in N\(_2\) and solubilized in anhydrous dichloromethane. One µl of each sample was injected into a column (30 m X 0.25 mm (i.d.), 0.25 µm film thickness DB-1 capillary column; J & W Co.). The GC (Hewlett Packard 6890 Series) oven temperature was programmed 60°C for 1 min, then to rise at 15°C min\(^{-1}\) to 200°C followed by 5°C min\(^{-1}\) to 285°C. Helium carrier gas was maintained at a head pressure of 30 kPa. The GC was directly interfaced to a mass selective detector with an interface and source temperature of 280°C, an ionizing voltage of 70 eV and a dwell time of 100 ms.

4. Quantification of endogenous GAs

Collection and analysis of the GC-MS data was accomplished with a GC-MS (Finnigan Mat GCQ and Hewlett Packard 6890 Series). Three major ions of the supplemented [\(^2\)H\(_2\)]GA internal standards (obtained from Prof. Lewis N. Mander, Australian National University, Canberra, Australia) and the endogenous GA were monitored simultaneously. Retention time was determined by using the hydrocarbon standards to calculate the KRI value (Kim et al., 2003a). Quantification of gibberellin was based on the peak area ratios of endogenous (non-deuterated, sample) to deuterated GAs, after correcting for any contribution from the deuterated standard to non-deuterated GAs. The endogenous contents of GA\(_{53}\), GA\(_{12}\), GA\(_{15}\), GA\(_{19}\), GA\(_9\), GA\(_{24}\), GA\(_{60}\), GA\(_{20}\), GA\(_{9}\) and GA\(_6\) were calculated from the peak area ratios of 450/448, 302/300, 241/239, 434/432, 316/314, 436/434, 300/298, 420/418, 286/284 and 508/506, respectively.
Results

1. Changes in endogenous GAs during bulbil growth

We analyzed the GAs extracted from the extract of bulbils at intervals of 5 days after bulbil formation (Aug 7) to bulbil ripening (Oct 25). Five of these gibberellins were members of the early-13-hydroxylation pathway (GA53, GA44, GA19, GA20, and GA1), and six were members of the non-13-hydroxylation pathway (GA12, GA15, GA24, GA9, GA36, and GA4). Fig. 1 shows the changes in the contents of biologically inactive gibberellins that are members of the early C-13 hydroxylation pathway (upper) and members of the non C-13 hydroxylation pathway (lower) during development of bulbils. Although all endogenous gibberellins showed different changes, there was a tendency to reach a maximum level on Sept 15, and decrease.

Fig. 2A shows the change in endogenous content of two groups of GAs in different biosynthetic pathways during bulbil growth. The content of GAs that are members of the early C-13 hydroxylation pathway (ECH) was about two times higher than that of the non C-13 hydroxylation pathway (ECH) from Aug 7 to Sept 15. Two bioactive gibberellins were also detected during bulbil growth (Fig. 2B). Endogenous GA4 content was always higher than GA1 content, and GA1 content was always kept low. A maximum endogenous GA4 content was found on Sept 15.

Fig. 3 shows the changes of total gibberellin content and fresh bulbil weight during bulbil growth. Bulbils began to set on Aug 2. Fresh bulbil weight slightly increased from Aug 7 to Aug 27. However, bulbil dramatically enlarged thereafter and reached a plateau on Sept 15. A sharp increase in total endogenous gibberellins was found at this time. A rapid decline in total gibberellin content was also observed during the storage period.
bulbil ripening from Oct 5 to Oct 25.

2. Changes in endogenous GAs during bulbil storage

Fig. 4 shows the changes in endogenous gibberellins included two gibberellin biosynthetic pathways (A) and endogenous gibberellins and two bioactive gibberellins (B) during bulbil storage. Fresh bulbils were harvested on November 22 and then stored in a temperature-controlled room in the dark at 4 ± 0.5°C at an approximate relative humidity of 85 ± 1%. Bulbils were sampled every 30 days after the beginning of storage. The total gibberellin content that was initially very low increased rapidly during the early days of storage and decreased slightly thereafter. A similar tendency was also observed in the two bioactive GAs (GA₄ and GA₃₉). During the storage periods, the GA₄ content was always much higher than the GA₃₉ content. GA₄, which was below 1 ng g⁻¹ DW.

Discussion

In the present study, we first quantified the endogenous gibberellins in bulbils of Chinese yam during growth and storage by GC-MS-SIM. Tanno et al. (1992) identified eight endogenous gibberellins including a bioactive GA₄ in bulbils of Chinese yam, and suggested that two different gibberellin pathways might operate in the bulbils. We also confirmed that two different gibberellin pathways operate in the bulbils of Chinese yam. The total gibberellins and bioactive GA₄ increased rapidly with the increase in bulbil size or weight.

We previously reported that the gibberellin content of tubers and leaves increased simultaneously with increasing tuber weight and that two gibberellin biosynthetic pathways operate in the tuber and leaves (Kim et al., 2003a). The rapid increase in endogenous gibberellins, especially GA₄, suggested that the GAs participate in the enlargement of bulbils and tuber. The present study shows that the major gibberellin biosynthetic pathway in the bulbils of Chinese yam is the non C-13 hydroxylation pathway, resulting in higher level of bioactive GA₄ during bulbil growth. This means that the endogenous bioactive GA₄ is essential for bulbil enlargement. These findings are consistent with our previous findings (Kim et al., 2003a). In practice, tuber yield was markedly increased by gibberellin treatment in Chinese yam in the growing season (Kim et al., 2003b).

In general, gibberellin breaks seed dormancy and promotes seed germination in higher plants. In contrast, the most characterized physiological features in Chinese yam are that dormancy of tubers and bulbils is induced by the application of gibberellin (Okagami and Nagao, 1971). It means that high endogenous gibberellin level is necessary for the dormancy of the bulbils of Chinese yam.

In the present study, the amount of total endogenous gibberellins and that of bioactive GA₄ increased rapidly during the early period of storage and decreased slightly. This sudden increase in total gibberellins and GA₄ during the early days of storage seems to play an important role in induction of dormancy of bulbils. Exposure of seeds to a low temperature (typically 4°C) is widely used to break seed dormancy and to improve the frequency of germination, and it is speculated that cold storage may increase the endogenous gibberellin content. However, the mechanism of the low temperature-induced acceleration of germination in relation to endogenous gibberellins is largely unknown. It is considered that larger amounts of endogenous gibberellins in tubers or bulbils of Chinese yam lead to a deeper dormancy. However, Dioscorea alata L., known as the greater yam, D. cayenensis Lam., the yellow yam, and D. rotundata Poir., the white yam, have different features in the response to dormancy (Craufurd et al., 2001). These tubers have a distinct dormancy period, which can be extended with curing and the application of gibberellic acid. Passam (1978) suggested that differences in the dormancy among yam species are the result of the difference in ecological environments in which they evolved, although dormancy is both a specific and varietal characteristic. It is supposed that a rapid increase in the amount of total endogenous gibberellins including bioactive GA₄, for a short period after harvest might be necessary to maintain stable dormancy induction for a long period. The above results suggest that the increase in endogenous gibberellins is closely associated with the bulbil growth and storage of Chinese yam, although we cannot fully elucidate how rapidly the content of endogenous gibberellins in the bulbils increased or fluctuated in.

Further research is needed for a better understanding of the mechanism of gibberellin biosynthesis in response to thermal alteration during short storage periods.

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