バレイショ塊茎形成初期における内生生長物質の変動

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著者	幸田, 泰則 岡沢, 養三
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Characteristic Changes in the Levels of Endogenous Plant Hormones in Relation to the Onset of Potato Tuberization*

Yasunori KODA and Yozo OKAZAWA
(Faculty of Agriculture, Hokkaido University, Sapporo 060)
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The first sign of potato tuberization is a swelling at the sub-apical region of the stolon. At this early stage, the swelling mainly brought about by radial cell expansion in the region of the pith, and the hook of the stolon remains[1,2]. Subsequently, vigorous thickening growth due to cell division occurs and the hook is straightened out. The tuberization is completed when the straightened hook has incorporated into tuberous portion and form eye.

After an interesting finding that a specific tuber forming stimulus is responsible for the tuberization[3], a considerable attention has been directed to the role of endogenous plant hormones in the onset of the tuberization. Many investigations have been made as to the changes in the levels of the hormones, in order to elucidate the roles[1,7,9,12,13,14,15]. However, much more conclusive evidence about the changes in the levels of the hormones in relation to the changes in morphological and anatomical aspects is necessary to prove the involvement of individual hormone in the process of the tuberization.

In the present investigation, progressive changes in the levels of endogenous plant hormones; gibberellin (GA)-like substance, auxin, cytokinin and abscisic acid (ABA)-like substance were examined in relation to the morphological and anatomical changes occurred during the course of potato tuberization.

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Materials and Methods

Plant materials

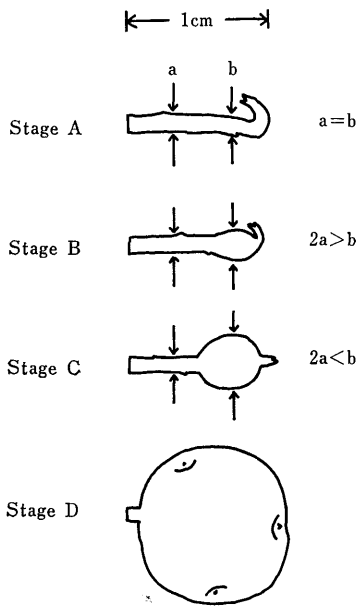
Seed tubers of potato (*Solanum tuberosum* L. cv. Irish Cobbler) were planted in an experimental field on 10 May, 1981 and raised in the usual manner. Early in June, the stolons began to outgrow, and about 2 weeks later they started to swell followed by forming tubers. The stolons and developing tubers were sampled 3 times on 8, 15 and 21 June. These harvested materials were divided into 4 groups in order of their developmental stages as shown in Fig. 1 : A, elongating stolon tips; B, swelling stolon tips; C, fully swelled stolon tips; D, young tubers (1–2 cm in diameter). The materials were washed thoroughly with running tap water, homogenized with a sufficient amount of ice-cold (−20°C) ethanol to give a final 70% ethanolic extract, kept overnight at 4°C and then filtered. The filtrate had been stored at −20°C until required for analysis.

Materials for cytological observation were fixed in formarin: acetic acid: ethanol: water (2:1:10:7 v/v), proceeded for microtomy in the usual way and sectioned at 10 μm. The preparations were stained with safranine-fast green combination[4].

Extraction and bioassay for cytokinin

Our previous finding that a decrease in the level of water-soluble cytokinin and an increase in that of butanol-soluble one occurred with the swelling of the stolon tips led us to the assumption that only butanol-soluble cytokinin is involved in the potato tuberization[7]. Therefore, the levels of this type of cytokinin were determined here.

The ethanol extract was adjusted to pH



Fi. 1. Diagram showing classificaion of developing stolons.

2.5 with 1 N HCl and passed through a Dowex 50W-X4 (50–100 mesh) cation exchange column. The column was washed with 70% ethanol, followed by distilled water and the adsorped substances were eluted with 3 N NH_4OH. The eluate was evaporated to eliminate ammonia and then extracted with 3 equal volumes of n-butanol. After the aqueous fraction was discarded, the combined butanol extracts were evaporated to dryness, and the resulting residue was chromatographed on papers with isopropanol: ammonia: water (10:1:1 v/v). Dried chromatograms were divided into 10 equal Rf strips which were assayed for cytokinin activity using soybean callus bioassay[5,10]. The amount of cytokinin was calculated from the growth curve for authentic zeatin.

Extractions and bioassays for auxin and ABA-like substance

The ethanol extract was evaporated to remove ethanol, adjusted to pH 2.5 with 1 N HCl and then partitioned 3 times against equal volumes of ethyl acetate. After removal of the aqueous phase, the ethyl acetate phase was partitioned 3 times against a half volumes of 0.5 M phosphate buffer (pH 8.0). The aqueous phase was adjusted to pH 2.5 with HCl and extracted with 3 equal volumes of ethyl acetate. The ethyl acetate was dried over sodium sulfate anhidride and then evaporated to dryness. The resulting residue was subjected to paper chromoatography developed with the same solvent as described above. Auxin activities on the paper chromatograms were detected by means of *Avena* coleoptile straight growth test. ABA-like activities were measured by inhibition of the coleoptile straight growth induced by 10^{-7} M indole-3-acetic acid (IAA). The amount of auxin was calculated from the dose-response curve for authentic IAA and that of ABA-like substance was calculated from the dose-response curve for authentic ABA.

Extraction and bioassay for GA-like substance

The procedure for extraction of GA-like substance was the same as that for auxin and ABA-like substance other than using 1 M phosphate buffer (pH 6.2) to prevent contamination of ABA-like substance. GA-like activities were determined by measuring the amount of reducing sugar liberated from *Avena* embryoless-endosperms[6]. The amount of this substance was calculated from the dose-response curve for authentic gibberellic acid (GA_3).

In preliminary experiments, it was found that partition coefficient of ABA to 1 M phosphate buffer (pH 6.2) was only 0.07, and moving rate of ABA to the buffer solution was only 5% even when subjected to 3 successive partitions. The value seems to be too low to mask GA activity in this bioassay system[6]. Therefore, GA activity detected here seems to represent the level of GA-like substance.

Results

Cytological observations of developing stolons

In order to assess whether classication of the developing stolons shown in Fig. 1 was suitable for investigating the correlation between the changes in the levels of endogenous plant hormones and cytological change occurred in the stolons, the median sections of the stolons at each developmental stage were prepared. The representative features

of the sections of stolons at stage A, B and C are shown in Fig. 2. No sign of swelling was found in stolons at stage A. A slight swelling was found at sub-apical region of stolons at stage B, and the hook of the stolon had not straightened out at this stage. At subsequent stage C, the swelling further proceeded and the hook was almost straightened out. In order to see whether the swelling occurred at stages B and C was brought about by cell expansion or cell division, comparisons were made between the number of cells across the diameter of swelled (position **b** in Fig. 1) and non-swelled part (position **a** in Fig. 1) of stolons (Table 1). In the median sections of stolons at the stage B, there found no significant difference of the number of cells between swelled and non-swelled part, however the swelling part exceeded in the width about 60% of that of non-swelled part. On the other hand, at subsequent stage C, the increase in the cell number kept pace with that in the width. These results indicate that the radial cell expansion growth was prominent in the swelled region at the stage B, while the cell division became to be evident with approaching to the stage C.

Changes in the level of GA-like substance

Time course changes in the level of GA-like substance in developing tubers are shown in Fig. 3. The level in elongating stolon tips (stage A) was relatively high. Subsequently, a drastic reduction of the level occurred with the swelling of the tips (stage B), and thereafter the level remained very low until the completion of the tuberization. In terms of GA_3 equivalent per kg fresh weight, the level at stage A was 2.2 μg. The levels in the following stages were below a critical threshold so that no reliable figures were given.

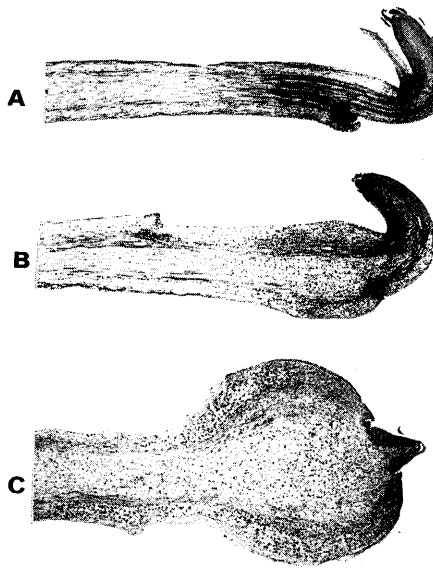


Fig. 2. Cytological comparison of median sections of stolon tips of potato at stage A, B and C.

Changes in the level of auxin

The level of auxin showed a little change during the course of the tuberization (Fig. 4). The level reached a maximum in the swelling stolons (stage B) and then somewhat decreased. The level at each stage was 5.9, 8.6, 6.5 and 3.7 μg in terms of IAA equivalent per kg fresh weight.

Changes in the level of cytokinin

Changes in the level of cytokinin are shown in Fig. 5. The level which was considerably low in the elongating stolon tips (stage A) began to increase with the swelling of the tips (stage B) and reached a maximum in the fully swelled stolon tips (stage C). Once the tuberization had been completed (stage D), the level decreased

Table. 1. Cytological comparison of stolons between its swelled and non-swelled parts at stage B and C.

Growing stage*	Number of cells across diameter			Width of stolon (mm)		
	Non-swelled part (a)	Swelled part (b)	Ratio b/a	Non-swelled part (a)	Swelled part (b)	Ratio b/a
B	52.0±4.3**	61.7±8.0	1.18	1.02±0.07	1.60±0.13	1.57
C	51.2±5.0	121.2±7.7	2.36	1.21±0.03	3.58±0.38	2.95

* Growing stages are refered to Fig. 1. ** Average of 5 replicates±SD.

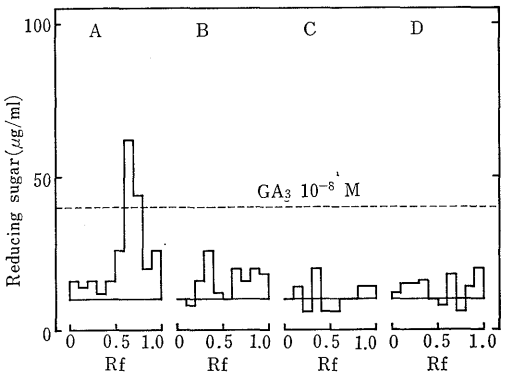


Fig. 3. Changes in the level of GA-like substance during the course of potato tuberization. Each extract, equivalent to 10 g fresh weight, was chromatographed on paper and assayed by *Avena* endosperm test. Broken line represents the amount reducing sugar liberated from the endosperms by 10^{-8} M GA_3.

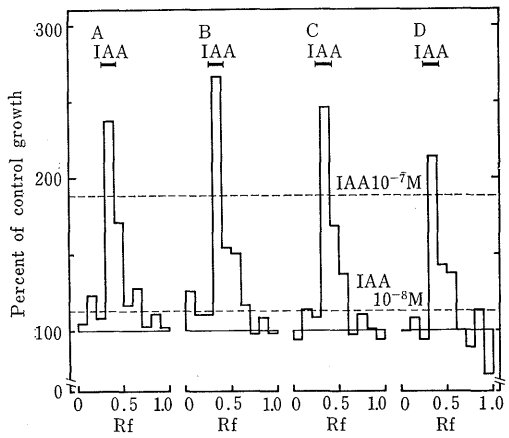


Fig. 4. Changes in the level of auxin during the course of potato tuberization. Each extract, equivalent to 10 g fresh weight, was chromatographed and assayed by *Avena* coleoptile straight growth test. Broken lines represent the percent of control growth by 10^{-8} and 10^{-7} M IAA. Markers in the upper part of figure indicate the position of authentic IAA.

slightly. In terms of zeatin equivalent per kg fresh weight, the level at each stage was 0.16, 0.25, 1.10 and 0.85 μg.

Changes in the level of ABA-like substance

The level in the elongating stolon tips (stage A) was extremely low (Fig. 6). However, the level began to increase with swelling of the tips (stage B). Thereafter, the level continued to increase vigorously until

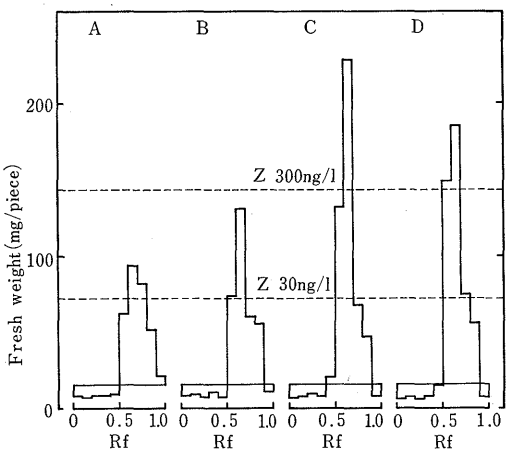


Fig. 5. Changes in the level of butanol-soluble cytokinin during the course of potato tuberization. Each extract, equivalent to 10 g fresh weight, was chromatographed and assayed using soybean callus. Broken lines represent the callus yields with 30 and 300 ng/l zeatin.

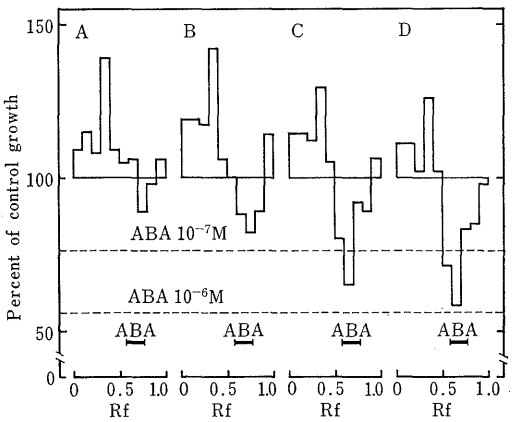


Fig. 6. Changes in the level of ABA-like substance during the course of potato tuberization. Each extract, equivalent to 10 g fresh weight, was chromatographed and assayed. The activities were measured by inhibition of *Avena* coleoptile straight growth induced by 10^{-7} M IAA. Broken lines represent the percent of control growth obtained by 10^{-7} and 10^{-6} M ABA. Markers in the lower part of figure indicate the position of ABA.

the completion of the tuberization. In terms of ABA equivalent per kg fresh weight, the level at each stage was 0.7, 2.1, 10.9 and 25.5 μg.

Discussion

As shown in Table 1 and Fig. 2, comparable median sections of stolons adduce a convincing evidence in support of the view proposed by BOOTH[1] and CUTTER[2] that an early tuber development was primarily caused by radial cell expansion followed by cell division. The changes in the levels of endogenous plant hormones seem to be closely correlated to such cytological changes occurred in the stolon tips. In view of a previous evidence that GA markedly enhance the elongation of potato stolons[11], a reduction of GA-like substance should be necessary for the onset of the tuberization. In fact, in the present experiment, the level of GA-like substance which was high in the elongating stolon tips (stage A) decreased markedly with the onset of the tuberization and kept very low during the course of the tuberization (Fig. 3). A maximum level of auxin was found at stage B, when the swelling was mainly caused by cell expansion (Fig. 4). Subsequently, an initiation of active cell division was observed at stage C, at which time the level of cytokinin reached a maximum (Fig. 5). On the other hand, the level of ABA-like substance showed a tendency to increase with advance of the tuberization process (Fig. 6). The increase in this substance seems to play important roles in cessation of stolon elongation at stage B and induction of dormant buds at stage D.

The experimental results presented herein appear to suggest that a shift of endogenous hormonal balances induce the potato tuberization. However, we have previously offered evidences suggesting that neither cytokinin nor ABA is the direct cause of the tuberization[8]. A maximum level of cytokinin was found at stage C when cell division became to be prominent (Fig. 5). This result seems to support our previous view that cytokinin is incapable of inducing the tuberization, but stimulate thickening growth of stolons to form tubers.

Summary

Changes in the levels of endogenous plant hormones; GA-like substance, auxin, cytokinin and ABA-like substance, in developing stolons and tubers were examined in relation to changes in their morphological and cytological aspects. With the advance of the tuberization process, transient accumulations of these substances were observed in order of GA-like substance, auxin, cytokinin and ABA-like substance, a maximum level being found in elongating stolon tips (stage A), swelling stolon tips (stage B), fully swelled stolon tips (stage C) and young tubers (stage D), respectively. Cytological observation revealed the cell elongation was predominant at the stage A and the radial cell expansion occurred at stage B. At subsequent stage C, the cell division became to be evident. These results seem to suggest as follows. (1), Firstly, GA behaves to enhance the stolon elongation as well as to inhibit the swelling. (2), Subsequently, auxin supports stolon swelling due to cell expansion. (3), Cytokinin stimulates cell division with aid of auxin. (4), Finally, a large accumulation of ABA impose the formed tubers into dormant state.

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〔和 文 摘 要〕

バレイショ塊茎形成初期における内生生長物質の変動

幸 田 泰 則・岡 沢 養 三
（北海道大学農学部）

バレイショ塊茎形成の初期におけるふく枝中の内生生長物質の変動と，ふく枝内で生ずる細胞学的変動との関連性について検討を加えた．

1．伸長中のふく枝先端部のジベレリン様物質含量は比較的高かったが，ふく枝の肥大に伴いその量は急減し，以降は塊茎形成完了期まで非常に低い値を示した．

2．オーキシン含量は，全体として大きな変動を示さなかったが，細胞肥大によって生ずるふく枝肥大の初期に最大のレベルに達した．

3．ブタノール可溶性サイトカイニンの含量は，伸長中のふく枝先端部では極めて低い値を示したが，ふく枝の肥大に伴いわずかに増加し，その後細胞分裂が活発化するふく枝肥大後期に最大となり，塊茎形成完了に伴ってわずかな減少をみせた．

4．アブシジン酸様物質の含量は，伸長中のふく枝先端部では極めて低い値を示したが，ふく枝の肥大に伴い増加しはじめ，以降急激な増加を続けた．

以上の結果は，ジベレリンはふく枝の伸長を促進することにより肥大を阻害し，オーキシンは細胞肥大によって生ずる初期のふく枝肥大に寄与していることを示すものと思われる．またサイトカイニンは細胞分裂を伴う後期のふく枝肥大を促進し，アブシジン酸はふく枝の伸長を阻止すると共に，後期においては，休眠芽の誘導に役立っているものと考えられる．