# EFFECT OF (2-CHLOROETHYL) PHOSPHONIC ACID [CEPA] ON CYTOKININ LEVEL OF POTATO TUBERS

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## Introduction

Control of potato sprouting through the exogenous application of chemicals has been receiving considerable attention. Cytokinin is a potent stimulator for releasing potato dormancy[3]. Accordingly, modification of endogenous cytokinin level of the tubers seemed to be closely associated with the sprouting behavior, and some data available lead us to assume that previous accumulation of cytokinins in quiescent potato tubers before resuming their sprouting would provide a favorable ambience for intensifing their sprouting potential[2]. In fact, a remarkable increase in cytokinin activity of the stored tubers was ascertained after releasing their dormancy[2]. In the previous paper[8], it appeared that no inhibition of sprout emergence resulted in the CEPA-treated tubers when those were subjected by kinetin application previously. Therefore, an inevitable question arises on possible effect of ethylene on cytokinin metabolism of their sprouts. In the present investigation, effect of CEPA on modification of cytokinin level in either intact tuber or their excised tissue was evaluated. In this context, CEPA-caused growth inhibition was further assessed on comparing with failure of potato sprouting during cold storage period.

## Materials and Methods

*Plant materials*: Potato tubers (*Solanum tuberosum* L. cv. Irish Cobbler) being harvested at the Experimental Farm of Hokkaido University on September 10, 1977 were used as materials as previously described[8]. Frequency of sampling for cytokinin assay of intact tubers varied with the phase of tuber age, and three times of the sampling were at; the harvesting stage

(September 10), the end of the rest period (November 10) and the seniled stage (July 10, next year).

*Preparation of excised tuber tissue for the CEPA treatment*: At approximately four months after storing in a cellar, tissue plugs (10 mm diameter and 10 mm thick) including apical buds were excised from the tubers, washed and blotted dry. Fifty $\mu$l of 50 mM citrate phosphate buffer (pH 4.0) with or without 1 mg of CEPA were applied on the defined area around the apical buds and subsequently those were incubated in the dark at 20°C for 24 hr with continuous supply of fresh air at flow rate of 30 ml/min.

*Extraction of cytokinin*: Disc-shaped tuber tissues (10 mm diameter and 2 mm thick) were excised from intact tubers and the plugs. These tissues were obtained from apical region of tuber, especially, the latter tissues were collected from uppermost region of the plugs.

Twenty five grams of the fresh tissues from each group were homogenized with 80% of ethanol and extracted for 48 hr at 3°C. After filtration the extracts were reduced to be aqueous and adjusted to pH 2.5 with HCl. The acidified extracts were partitioned three times against equal volumes of petroleum ether to remove impurities. After discarding the ether fraction, the aqueous fraction was passed through Dowex 50 W-X 8 cation exchange column (2.2 × 15 cm, $H^+$ form, 50-100 mesh). The column was washed with 50 ml of 70% ethanol, followed with 750 ml of distilled water and finally the absorbed substances were eluted with 250 ml of 3 N $NH_4OH$. After removal of ammonia, the aqueous phase was adjusted to pH 8.0 prior to partitioning with five equal volumes of water-saturated butanol. Both the butanol and aqueous fractions were evaporated to dryness *in vacuo* individually, and each of the residues was taken up in small volume of 80% ethanol, streaked onto Toyo No. 50 chromatography paper and then separated with iso-propanol : $NH_4OH$ : water (10 : 1 : 1, v/v). Dried chromatograms divided into 10 Rf strips and each of them was directly placed into cytokinin assay medium described below.

*Bioassay of cytokinin activity*: The chromatographic sections were placed in 50-ml Erlenmeyer flasks and bioassayed for cytokinin using soybean callus tissues cultured on 20 ml of MILLER's nutrient medium containing 3 mg of IAA per liter[7]. The assay cultures were maintained at 28°C for 6 weeks in light. All experiments were performed at least twice with replicate cultures each, and representative data were shown in the tables.

## Experimental Results

*Changes in cytokinin level of the stored tubers*: It is a well established

fact that dormancy of potato tubers is one of the conspicuous characteristics and those will not sprout even if placing under the favorable condition. According to BURTON's opinion[1], an essential feature of the dormancy is that the tubers will be able to acquire potential for future growth once certain changes have taken place. These changes in internal physical and/or chemical conditions could occur even in a constant environment at low temperature. In fact, changes in cytokinin levels of the tubers seem to be important for resuming the sprouting, citing from the fact described previously[8]. Attempts to further understand the dormancy at hormonal level, therefore, are focused on elucidating the nature of cytokinin metabolism in the potato tubers during the storage period.

As illustrated in Table 1, cytokinin activities of two chromatographic regions were recorded in terms of kinetin equivalents, and those were distributed to the Rf values at 0.2–0.4 and 0.6–0.8 both butanol and aqueous fractions. Henceforth the former is referred to as slow moving cytokinin and the latter as fast moving one. However, as a whole, each activity in the aqueous fractions was less than one tenth of that from the butanol ones. Considerable increase in their activities was observed at the time when the tubers were released from their dormancy on November 10. Afterward, drastic reduction of all their activities occurred except a marked increase

TABLE 1. Changes in cytokinin activities of apical tissues excised from potato tubers stored at 3°C in darkness for different period of time. The cytokinin of the tissue extract were divided into two fractions based on their solubility: butanol and water, each fraction was further divided based on their mobility on paper chromatograms; a slow-moving region 0.2–0.4, Region A and a fast-moving region Rf 0.6–0.8, Region B. Each cytokinin activity is expressed as $\mu g$ kinetin equivalents $kg^{-1}$, fresh material. Total activity means sum of them distributed over the entire chromatograms.

| Stage | Butanol fraction | | | Aqueous fraction | | |
|---|---|---|---|---|---|---|
| | Total activity | Region | | Total activity | Region | |
| | | A | B | | A | B |
| The harvesting stage | 20.06 | 2.51 | 17.10 | 0.59 | 0.28 | trace |
| The end of the rest period | 59.70 | 0.05 | 59.22 | 3.50 | 1.21 | 1.86 |
| The senile stage | 103.13 | 0.59 | 101.16 | 1.56 | 0.14 | trace |

TABLE 2. Effect of CEPA treatment on cytokinin level in the apical plugs excised from potato tubers. The treatment was incubated at 20°C for 24 hr in darkness. See legends of Table 1.

| Fractions | | Total activity | Region | |
|---|---|---|---|---|
| | | | A | B |
| Butanol fraction | Control | 70.4 | trace | 60.8 |
| | Treated | 62.8 | 14.4 | 39.6 |
| Aqueous fraction | Control | 7.3 | trace | trace |
| | Treated | 20.4 | 12.0 | 8.4 |

in the fast moving cytokinin of the butanol fraction, if the storage period was prolonged untill July next year. It is worth notice that the fast moving cytokinin activity of butanol fraction showed a gradual but consistent increase upto more than five times of the initial value, whereas a temporary increase in the activity of aqueous fractions occurred at the end of dormancy, thereafter the activity decreased again. Generally, no significant qualitative alteration of cytokinin form was appeared throughout the experiments.

*Effect of CEPA treatment on cytokinin levels*: The tissue plugs including apical buds were excised from the bud end of the stored tubers in the middle of January when the tubers were previously released from the endogenous dormancy, and immediately those were treated with CEPA. After the incubation at 20°C for 24 hr, the cytokinins were extracted, fractionationed and assayed. At the end of the incubation, no different appearance was observed between the treated and control plugs with no visible sign of sprout growth. As listed in Table 2, it appears that a perceptible increase in the aqueous cytokinin activities due to the treatment, which increase seems to be depend greatly on the activity of the slow moving cytokinin. Concerning the butanol fraction, the level of the fast moving ones in the treated plugs reduced to near half of the control level, whereas slight but significant increase in the slow moving ones resulted in the treated tissues. Consequently, no significant difference of the total cytokinin levels was observed between the both groups of plugs.

## Discussion

In the present investigation, it appears that the activities of the butanol soluble cytokinins in the apical plugs of potato tubers displayed a tendency to increase with time over the entire period of storage, while those of the

water soluble ones reached its maximum at the end of the dormant period and then declined as the tubers became to be senile. The data at present available indicate that potato tubers on their growing stage contained at least six different forms of cytokinins[6], and there were eight cytokinins present in storing potato tubers[14]. ENGELBRECHT and BIELINSKA-CZARNECKA [2] reported that cytokinin levels in the apical region of potato tubers increased considerably during the storage at 8°C and an additional increase occurred when moved to moist and warm condition. These facts may alluded to feasible that potato tubers would be able to readily interconvert between various forms of cytokinins owing to alteration of circumstance. As showing in Table 1, a pronounced increase in the fast moving cytokinin on the chromatogram of the butanol soluble fraction was ascertained at the end of dormancy, and these cytokinins seem to be zeatin and its riboside, quoting from the evidences by VAN STADEN and DIMALLA[14], and ENGELBRECHT and BIELINSKA-CZARNECKA[2]. Though we did not evaluated the process critically, previous workers have interpreted it as a consequence of conversion of the bound form, particularly cytokinin glucoside, to free forms[12]. During the prolonged storage period, additional increase in butanol soluble cytokinins as zeatin and/or its riboside may strengthen the previous findings[4,10]. However, the fact that an accumulation of these active cytokinins around the apical buds was concomitant with the disappearance of water soluble cytokinin is not yet enough to conclude whether it is a consequence of *de novo* synthesis of free cytokinin or interconversion from its precousor, even citing from the assumption proposed by KANNANGARA and BOOTH[5] and VAN STADEN and BROWN[12].

It has already been shown that exposure to ethylene resulted in a decrease of the endogenous cytokinin level in the treated potato stolons, and suggested that low concentration of ethylene could bring about cytokinin glucosylation[13]. Concerning the additional evidence that the glucosylated cytokinin may be inactive for stimulation of meristematic activity[9,11], the inhibitory effect of CEPA treatment on the development of potato sprouting is assumed to be as a result of cytokinin inactivation due to ethylene released from CEPA. As showing earlier[8], potato tubers decreased in endogenous auxin level of their apical tissues when treated with the low concentration of ethylene. The above consideration necessitates that lowering in attracting capacity of the tissue around the apical eye due to the lack of auxin should be taken into account. In addition, the ethylene-caused cytokinin lowering may conceivably lead to likewise decline the capacity, citing the assumption proposed by ENGELBRECHT and BIELINSKA-CZARNECKA[2]. On the other hand,

this provided a unfavorable circumstance for meristimatic activity of potato sprout.

In conclusion, unlike gibberellin, cytokinin did not directly influence on releasing potato dormancy but is prerequiste for acquiring sprouting potential at the end of dormancy.

## Summary

The present investigation was concerning changes in cytokinin levels in the potato tissues around apical buds of their tubers during cold storage period and in their tissue plugs treated with CEPA. The ethanolic extracts of the tissues were separated in to butanol- and water-soluble fractions. Each fraction contained both slow- and fast-moving cytokinins on paper chromatograms. During the cold storage period, total cytokinin level of the stored tubers increased with time, of which increase was mainly due to the activity of fast-moving cytokinin of butanol fraction. At the end of the dormant period, the water soluble cytokinin level reached to maximum and then declined. This decline was concomitant with an additional increase in the butanol soluble one. These facts may allude that the tubers would have a capacity to interconvert from inactive cytokinin to active one under unfavorable circumstance for sprouting when acquired their sprouting activity after releasing their dormancy.

In response to the CEPA treatment, the tissue plugs of potato tuber increased in the water soluble cytokinin depended mainly on the activity of the slow-moving cytokinin. Although the increase in the slow-moving cytokinin in response to CEPA treatment hardly conclude as consequence of interconversion between the two forms of butanol soluble cytokinins, it can be conceivably deduced that ethylene-caused accumulation of water soluble cytokinin may participate in lowering sprouting activity as well as the lack of endogenous auxin.

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