Effect of Storage Time of Potatoes after Harvest on Increase in the Ascorbic Acid Content by Wounding

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Although the ascorbic acid (AsA) content in most vegetables is decreased by wounding, that in potato tubers tends to be increased by wounding. The change of AsA content in wounded potatoes was investigated in connection with the storage time after harvest to clarify the cause of the AsA increase. Whereas the AsA content in wounded potato tubers did not increase within 4 weeks after harvest, the content slightly increased during 5–6 weeks and remarkably increased after 7 weeks. The AsA content in potato tubers stored more than 7 weeks after harvest decreased to approximately half the value on the beginning of storage, but it returned to the original value by wounding. These results suggested that potato tubers wounded after a constant storage time increase the AsA content to overcome the damage generated by wounding stress.

Introduction

Wounding of vegetables, which is an environmental stress, on post–harvest handling is an undesirable phenomenon and lowers the nutritional value of vegetables. Although the ascorbic acid (AsA) content in most vegetables tends to be decreased by wounding, the AsA content in potato tubers was not decreased but increased by wounding1. However, the cause of the AsA increase in wounded potato tubers has not been resolved. In our previous paper4), we reported the changes in AsA content and enzyme activities concerning AsA synthesis in potato tubers during storage and assumed that the remarkable changes in AsA content and related enzyme activities occur in order to maintain the AsA level during dormancy. In this paper, the effect of the storage time of potato tubers after harvest on the changes in AsA content and L-galactono-\(\gamma\)-lactone dehydrogenase (EC 1.3.2.3) activity in wounded tuber tissues is described to clarify the cause of AsA increase in the wounded tissues.

Materials and Methods

1. Experimental materials and preparation of wounded tissue

Potato tubers (Solanum tuberosum, var. May queen) cultivated in Obihiro, Hokkaido, were stored at 20°C until use. Tubers were sterilized with 1% NaClO, and then the parenchymatous tissues were cut into small disks (12 mm diameter, 5 mm thickness). The disks were put on the net in moist containers and incubated at 20°C for 48 h to prepare wounded tissues.

2. Determination of total AsA content

Both types of AsA, AsA and dehydro AsA were extracted from 10 g of tuber disks with 100 ml of 5% metaphosphate at 4°C. Both AsAs in the extract were changed to dinitrophenylhydrazone of AsA by 2,4-dinitrophenyl-hydrazine method, and the derivative content was determined by silica gel TLC (solvent system : toluene-acetone-5% acetic acid, 2:1:1) according to Fujita et al.9). The AsA derivative amount on TLC plate was measured at 530 nm using flying spot scanner CS–9000 (Shimazu). The AsA content described in the result and discussion indicated the total amount of both AsAs.

3. Extraction and assay of L-Galactono-\(\gamma\)-lactone dehydrogenase

Ten grams of tuber disks were homogenized with 20 ml of 0.1 M potassium phosphate buffer (pH 7.5) containing 2.5 mM dithiothreitol at 4°C. The homogenate was centrifuged at 500 × g for 10 min. The supernatant was applied on
Sephadex G-25 column and the protein fraction of the eluate was collected as enzyme solution.

L-Galactono-γ-lactone dehydrogenase activity in the enzyme solution was determined by the modification of method of Mapson et al. In a final volume of 2 ml of 0.1 M Tris-HCl buffer (pH 7.5) were placed the enzyme, 2.5 mM L-galactono-γ-lactone and 20 mg of cytochrome c. This mixture was incubated at 25°C for 10 min. The reaction was stopped by addition of 0.2 ml of 50% TCA. After centrifugation, 0.2 ml of 85% orthophosphate, 2.5 ml of 0.5% α,α'-dipyridyl and 0.5 ml of 1% ferric chloride were added to 0.5 ml of the supernatant of reaction mixture. The reaction product was measured at 525 nm. L-Gulono-γ-lactone oxidase activity was determined by the method of Nishikimi et al. L-Galactono-γ-lactone and L-gulono-γ-lactone were purchased from Extrasynthese (Genay, France).

Results and Discussion

1. Change in AsA content by wounding

Fig. 1 and Fig. 2 showed the change in the AsA content of wounded potato tubers, whose storage period after harvest affected the AsA content. The increase in AsA content was not observed in the disks of tubers stored within 4 weeks after harvest (Fig. 1). The AsA content in disks of tubers stored during 5-6 weeks was slightly increased by wounding (Fig. 2). A remarkable increase in AsA content was observed in disks of tubers stored more than 7 weeks (Fig. 2). On the other hand, the AsA content in tubers without wounding continued a gradual decrease during 6-10 weeks after harvest, and the profile of the change was same as the result of our previous paper. Therefore, these results implied that the active synthesis of AsA in tuber tissues is induced by wounding in spite of the AsA decrease in tubers stored more than 7 weeks after harvest.

Fig. 1 Change in ascorbic acid content in wounded tissues of tubers stored within 4 weeks after harvest
The time after harvest when tubers were used was as follows.
- ○ - 1 week ; - - - 2 weeks ; - - - 3 weeks ; - - - 4 weeks

Fig. 2 Change in ascorbic acid content in wounded tissues of tubers stored during 5-8 weeks after harvest
The time after harvest when tubers were used was as follows.
- ○ - 5 weeks ; - - - 6 weeks ; - - - 7 weeks ; - - - 8 weeks
The AsA content in tubers stored more than 7 weeks after harvest decreased to approximately one third of the value at the beginning of the storage, but the content in the disks incubated for 48 h increased closely to the value immediately after harvest (approximately 25 mg/100 g fr. wt.).

2. Change in L-galactono-γ-lactone dehydrogenase activity by wounding and the property of the enzyme

Fig. 3 and Fig. 4 show the change in L-galactono-γ-lactone dehydrogenase activity of wounded tissues. Whereas the activity in tubers stored less than 4 weeks was not increased by wounding (Fig. 3), the enzyme activity in those stored more than 7 weeks obviously increased for 48 h incubation (Fig. 4). The wounding of tissues which were prepared from tubers stored during 5 - 6 weeks showed a slight effect on the increase of the enzyme activity. Whereas the enzyme activity in wounded tubers scarcely increased after 24 h incubation in the report by Oba et al.7), the enzyme activity of tubers stored more than 7 weeks obviously increased during 48 h incubation in the present experiment. The difference may depend on the storage time of used potato tubers.

The pattern of the change in L-galactono-γ-lactone dehydrogenase activity coincided in the storage time of potato tubers with that of the change in AsA content. Although changes in other enzyme activities concerning AsA production were not investigated, the increase of L-galactono-γ-lactone dehydrogenase activity may considerably contribute the increase in AsA content of wounded tubers.

While L-galactono-γ-lactone dehydrogenase in cauliflower10) occurred in mitochondria, the enzyme activity in fresh potato tubers was

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Fig. 3 Change in L-galactono-γ-lactone dehydrogenase activity in wounded tissues of tubers stored within 4 weeks after harvest

The time after harvest when tubers were used was as follows.

- ○-, 1 week; -●-, 2 weeks; -△-, 3 weeks; -▲-, 4 weeks

Fig. 4 Change in L-galactono-γ-lactone dehydrogenase activity in wounded tissues of tubers stored during 5-8 weeks after harvest

The time after harvest when tubers were used was as follows.

- ○-, 5 weeks; -●-, 6 weeks; -△-, 7 weeks; -▲-, 8 weeks
widely distributed in cytosol and microsomal fractions as well as mitochondrial fraction. Moreover, although L-galactono-γ-lactone dehydrogenase in cauliflower\(^{10}\) did not exhibit L-gulono-γ-lactone dehydrogenase activity, the enzyme solution purified partially from potato tubers having a strong L-galactono-γ-lactone dehydrogenase showed a weak L-gulono-γ-lactone dehydrogenase activity. These results were the same as the property of the potato L-galactono-γ-lactone dehydrogenase in the report of Oba et al.\(^7\) and was similar to that of enzyme in Euglena gracilis\(^{12}\) in the point of subcellular distribution and substrate specificity.

3. The effect of the storage time on the AsA increase in wounded potatoes

Both of the AsA content and L-galactono-γ-lactone dehydrogenase activity in potato tubers stored more than 7 weeks were obviously increased by wounding. However, those increases were not observed in tubers stored within 4 weeks. Since the resting period of 'May Queen' is about 2 months\(^8\), 7 weeks after harvest is close to the end of the dormancy. Therefore, the active physiological response to environmental stress may be induced at that time, and then the increase in L-galactono-γ-lactone dehydrogenase activity which is a main enzyme producing AsA may be occurred by wounding.

The AsA content which had considerably decreased in potato tubers stored more than 7 weeks after harvest was returned to the significant value, the AsA content immediately after harvest, by wounding. The constant amount of AsA may be required for the protection of tissues against wounding stress which probably induce peroxidation reaction in wounded tissues. Therefore, it is suggested that the synthesis of AsA was stimulated to overcome the damage by wounding at the end of the resting period.

References


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