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Biochemical Effect on Potato Tubers Irradiated by Gamma-Ray at Sprout-Inhibition Dose

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방사선 조사에 의한 감자 발아 억제시 생화학적 효과

전재홍 · 변시명 · 박영숙[1] · 정규회[2] · 조한옥[2]

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초 록

방사선 조사에 의한 발아 억제기작을 살펴보기 위하여 4℃에서 저온 처리한 감자에 0.12 kGy 감마선을 조사한 후 5주 동안 20±2℃, 상대습도 70~90%에서 저장하였다. 이 기간 중 발아에 관여된다고 생각되는 α-amylase, peroxidase, indole acetic acid (IAA) oxidase, IAA synthesizing enzyme의 활성 변화를 추적하였다.

시판 α-amylase와 peroxidase의 방사선 감수성은 0.94와 0.36 kGy의 감마선 조사하여 D_{37} 값을 얻었다. 감마선 조사를 행한 감자의 IAA oxidase 역가는 조사후 급격히 증가됐고 IAA 합성 효소의 역가는 조사직후 조금 증가하였으나 저장 기간에 따라 크게 감소하였다. 형태적인 변화에서 조사된 감자에서는 성장점의 고사와 변형된 눈이 관찰되었고 IAA 혹은 gibberellin 등의 처리에 의해 이들 자체로는 회복되지 않았고 그 주위로 새로운 눈의 성장이 관찰되었다.

결론으로 방사선 조사에 의해 야기된 감자의 호흡은 탄수화물이 α-amylase 등의 효소에 의한 분해로 이루어져 ATP를 공급하며 뒤이은 peroxidase 역가의 증가와 같은 효소의 합성은 방사선에 의한 손상을 치유하려는 과정에 속한다 할 수 있다. 또한 눈의 생장점에서의 고사 현상은 손상에 대한 방어 작용의 일종이라 해석되며 이로인한 발아 억제 현상을 페놀 대사와 관련지어 추측할 수 있었고 증가된 IAA oxidase와 IAA 합성계의 파괴에 의한 IAA의 고갈 역시 방사선 조사에 의한 발아 억제 현상의 원인이 됨을 알 수 있었다.

Introduction

One of the promising application of ionizing radiation is sprout inhibition of potato, because of its obvious technological feasibility at the level of low doses. Ionizing radiations bring about various biochemical and physiological changes.[1~4] During this transient period of metabolic activation the potatoes are capable of synthesizing new proteins and nucleic acids.[5] Earlier, it has been observed that the radiation-induced dormancy of potato tubers is reversed by the treatment with indole acetic acid (IAA) or gibberellin (GA) after irradiation. The mechanism for radiation-induced dormancy has been studied in relation to the metabolism of plant growth regulatory substances.[6~11]

In order to understand basic knowledges on the mechanism of sprout-inhibition by γ-ray irradiation, potato tubers pretreated at low temperature were irradiated with sprout-inhibition dose in this study. During the storage for 5 weeks after irradiation, changes in respiration rate, several enzyme activities such as α-amylase, peroxidase, IAA oxidase and IAA synthesizing enzyme, which are considered to have a relation with sprouting, were determined. In addition, the effects of plant hormones including IAA and GA on the irradiated pototo tubers were carried out.

Materials and Methods

Materials

One-month old Irish cobbler potatoes after harvest were purchased from a local market and treated at low temperature 4°C for 4 weeks before use. L-Tryptophan, polyvinylpyrolidone-40 (PVP-40), *o*-dianisidine, IAA, GA, (grade III), dichlorophenol, bovine serum albumin, pyridoxal-5-phosphate, α-amylase, peroxidase, were purchased from Sigma Chemical Co. (St. Louis, U.S.A.).

Irradiation and Storage

Potatoes pretreated were placed to room temperature before irradiation. The potatoes wrapped with a nylon bag were irradiated in air with a dose of 0.12 kGy in a 10,000 Ci ^{60}Co irradiator at a dose rate of 0.12 kGy/hr, by putting them aside 45cm from the source. The accuracy of the irradiation dose was within ±15%. The irradiated tubers were stored at 20±2°C, 70~90% humidity in a dark storage room during experiments. During the storage, some potato tubers were dipped in different concentrations of GA or IAA for 2hr and stored along with the control.

In Vitro Irradiation on Enzyme

The enzyme solutions with a concentration of 100 μg/m*l* in distilled water were irradiated with doses of 0.01, 0.12, 0.5, and 1 kGy at a dose rate of 16.7 kGy/min.

Determination of Respiration Rate

The respiration rate of the irradiated tubers during the storage after irradiation was determined by the measurement of CO_2 liberation according to the method of Okubo.[12]

Preparation of Peroxidase and IAA Oxidase[13]

Ten grams of peeled and frozen potato in liquid nitrogen, were homogenized in an electronic blender with 20m*l* of cold 0.02M sodium phosphate buffer, pH 7.0, containing 1% PVP-40, 0.01M ascorbic acid, and 1% Triton X-100. The pellet obtained after centrifugation at 15,000 rpm for 20min was reextracted for 30min with 10m*l* of the above-mentioned buffer and centrifuged. To the pooled supernatants, 1.6 volumes of chilled acetone (−25°C) was added. The precipitate was removed by centrifu-

gation and dissolved in a small volume of the phosphate buffer and reprecipitated by addition of 1.6 volumes of acetone. The pellet obtained after centrifugation was homogenized in a small volume of the buffer. The insoluble residue was removed by centrifugation at 9,000 rpm for 10 min. The clear supernatant was used as an enzyme preparation. The protein was estimated by the method of Lowry *et al.*[14] All experiments were done at 4°C.

Preparation of α-Amylase and IAA Synthesizing Enzyme

Twenty grams of potato peeling were homogenized in an electronic blender with 30 m*l* of cold sodium phosphate buffer, 0.1M, pH 7.0. The homogenate was filtered through a double layer of cotton cloth and centrifuged at 13,000 rpm for 20min. This supernatant was used as an α-amylase preparation. To the supernatant, ammonium sulfate was saturated to 75% with gentle stirring. This solution was kept for 10 min and the precipitated proteins were centrifuged out at 9,000 rpm for 10 min. The pellet obtained after centrifugation was homogenized in a small volume of buffer and the insoluble residue was removed by centrifugation at 15,000 rpm for 20 min. The supernatant was used as an IAA synthesizing enzyme preparation.

Enzyme Assay

dPeroxidase and α-amylase activities were etermined by the methods described in Worthington manual.[15] IAA synthesizing enzyme and IAA oxidase activities were measured by amodified method by Ussuf and Nair.[11]

Results and Discussion

Distribution of Enzyme Activities

The distribution of enzyme activities in the skin, cortex and pith of the nonirradiated potato tubers shown in Table 1. The activities of four enzymes examined were located mainly in

Table 1. Distribution of enzymatic activities in different tissues of the potato tubers.

Enzyme \ Tissues	Enzyme Activity	
	Skin+Cortex	Pith
α-Amylase(U/g fresh weight)	8.2	5.5
Peroxidase(mU/mg protein)	10,420	1,780
IAA oxidase(nmol IAA oxidized/mg protein)	36.8	16.6
IAA synthesizing enzyme (nmol IAA produced/mg protein)	2.7	0

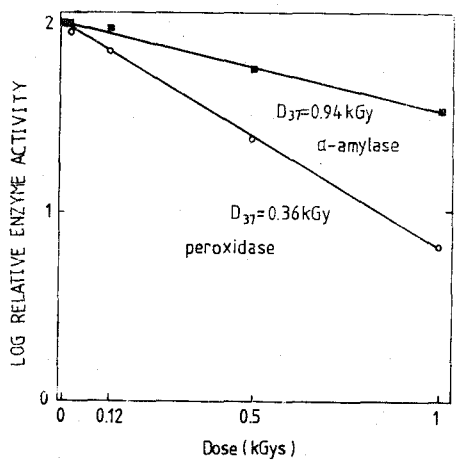

Fig. 1. Effects of γ-ray irradiation on the activities of standard α-amylase and peroxidase. Concentration of enzyme preparation, 100μg/m*l*; dose rate, 16.7 Gy/min. D_{37}=the dose necessary to cause 63% inactivation.

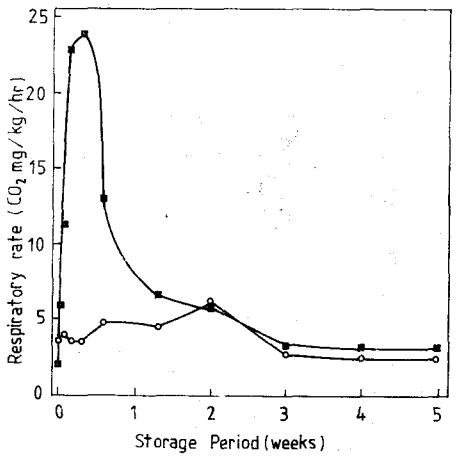

Fig. 2. Respiration rate of the irradiated potato tubers during storage at 20±2°C. ○, 0 kGy; ■, 0.12 kGy.

potato peeling. In the peeling, the activities of α-amylase, peroxidase and IAA oxidase were about 1.5, 6 and 2 times higher than in the pith. In addition, IAA synthesizing activity was not detected in pith at all. Therefore, peeling was used for the enzyme extraction at the further experiment.

Effect of *In Vitro* Irradiation and Enzyme Activities

γ-Ray irradiation inactivated the standard α-amylase and peroxidase as shown in Fig. 1. D_{37} value of α-amylase and peroxidase were 0.94 and 0.36 kGy, respectively. With 0.12 kGy the dose of sprout inhibition of potatoes, activities of α-amylase and peroxidase were inactivated 5% and 30%, respectively within 12hr after irradiation.

Respiration Rate of Potato Tubers Irradiated

When potato tubers exposed to radiation, effects of irradiation were immediate or somewhat delayed. Increase in respiration rate belongs to the class of immediate one. As shown Fig. 2, the respiration rate of irradiated potatoes decreased about 45% after irradiation and then suddenly increased thereafter. It increased to maximum 7-folds on 2-day storage after irradiation, compared with control and normalized thereafter. This remarkably increased respiration rate was the results of many biochemical events induced by radiation. It is likely that the metabolic pathways associated with respiration induced by radiation have two main roles in irradiated potatoes. One is to supply chemical energy in the form of ATP, the other is to provide the cells with precursors for the biosynthesis of primary and secondary plant products such as phenylpropanoids and phytoalexins. In fact, several enzymes associated with phenylpropanoid biosynthesis are synthesized in irradiated potatoes.[3,16~19]

Changes in α-Amylase Activity

In general, α-amylase was known as one of the hydrolase essential for providing energy for germination. The activity of α-amylase in non-irradiated potatoes was considered to increase along with sprouting, and did not change in irradiated potatoes. In this study, the activity of α-amylase in the non-irradiated potatoes increased about 2 times within one week after storage. Therefore, this indicated that the enzyme was related to the destruction of food reserves during germination. But, similar changes in the activity of the irradiated potatoes

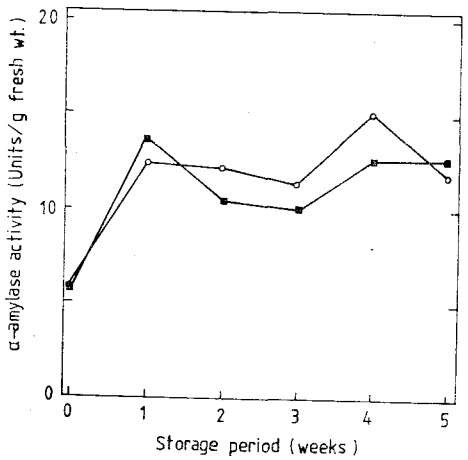

Fig. 3. Changes in α-amylase activity of the irradiated potato tubers during storage. ○, 0 kGy; ■, 0.12 kGy.

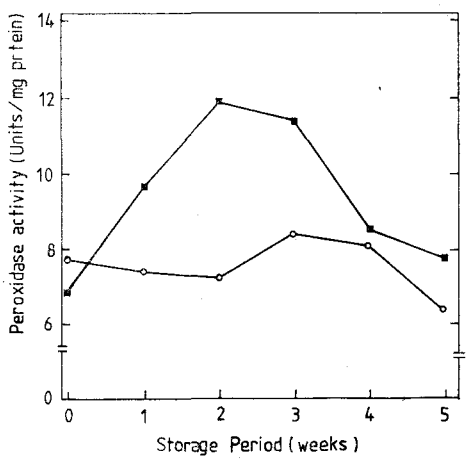

Fig. 4. Changes in peroxidase activity of the irradiated potato tubers during storage. ○, 0 kGy; ■, 0.12 kGy.

was determined as shown in Fig. 3. This result can be explained easily with relation to radiation induced respiration. The irradiated potato tubers decomposed carbohydrate actively to supply chemical energy in the form of ATP and stimulation of starch degradation in irradiated potatoes was ascribed to an increase in either α-amylase or phosphorylase activity.[2,3]

Changes in Peroxidase Activity

Plant peroxidase has multiple catalytic properties involved in growth and development. The interaction of the various factors which control growth development is paralled to their interaction in the control of this enzyme. Thus, its formation is dependent on hormonal level, external damage and other factors. It is interesting to consider its action in producing and inactivating auxin and interaction with gibberellin, cytokinin, and ethylene.[20,21] As shown in Fig. 4, the effect of irradiation on peroxidase are somewhat delayed. The peroxidase activity increased approximately two-fold in irradiated potatoes and its increase reached a maximum level during 2 to 3 weeks storage. It seemed that this increase is closely related to hormonal level such as auxin[21] and also wound-healing processes and defense reaction connected with phenolic metabolism[22].

Changes in IAA Oxidase and Synthesizing Activity

Free auxin in the tissue is very sensitive to ionizing radiation and it is in a dynamic pool maintained as a steady state system by uncomitant biosynthesis and degradation.[7,10] Regulationat both the synthetic and degradative level exists as per demand of this compound for growth purposes. The control of concentration of IAA in the various of the plant is complex, involving multiple processes. One means of regulation of IAA level is control of biosynthesis *in situ*. According to the result of our study, irradiation activated the IAA synthesizing system

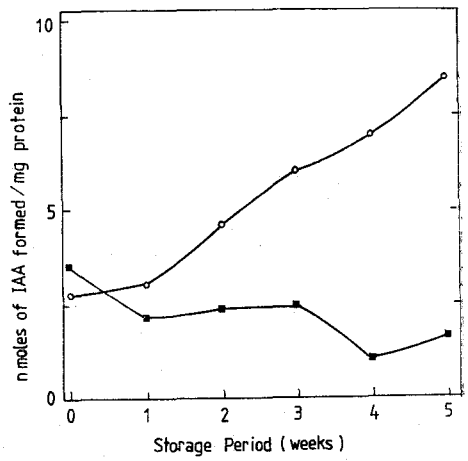

Fig. 5. Changes in indole acetic acid synthesizing enzyme activity of the irradiated potato tubers during storage.
○—○, 0 kGy; ■—■, 0.12 kGy.

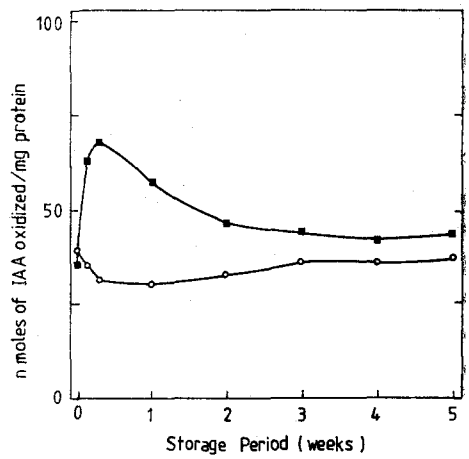

Fig. 6. Changes in indole acetic acid oxidase activity of the irradiated potato tubers during storage.
○—○, 0 kGy; ■—■, 0.12 kGy.

originally present in the tissue about 35% as shown in Fig. 5. It seemed that this is an immediate response to irradiation and then the activity decreased gradually 50~75% for 4 to 5 weeks after storage. However, IAA synthesizing activity increased normally along with sprouting in non-irradiated potatoes. This indicated that certain systems are interfered or repressed by γ-ray radiation, which related to the induction of IAA synthesizing enzyme, within

the period of metabolic activation induced by radiation. Another process by which auxin levels are regulated is degradation to inactive compounds. The most important oxidative process is enzymatic destruction catalyzed by an enzyme known as IAA oxidase or peroxidase. In Fig. 6, initial increase in IAA oxidase of irradiated potatoes was determined with a value of 2-fold. From the results, the depletion of IAA by the action of IAA oxidase induced by radiation and damage to the IAA synthesizing system seemed to possible cause to radiation-induced dormancy.

Reversal of Sprout Inhibition with GA or IAA Treatment

As shown in Table 2, the sprouting of tubers by γ-ray irradiated with a dose of 0.12 kGy was completely inhibited during storage for 7 weeks, while the control showed that all tubers are sprouted within 7 weeks after storage. When these were treated with 100 ppm of GA, it sprouted within 2 weeks. Radiation-induced dormancy in 0.12 kGy irradiated potatoes was reversed by treatment with GA somewhat incompletely (60%) during 7 weeks after storage, the optimum concentration required was 100 ppm. When these are treated with IAA with a concentration of 5, 10, 20, 50, 100, or 250 ppm, respectively, the reversal was less effective than with GA. The optimum concentration required for 40% reversal was 20 ppm of IAA. At high concentration of IAA and 250 ppm of GA, there was less effective reversal of sprout inhibition than low ones and the rotting rate increased. This results were not well agreed

Table 2. Reversal of sprout-inhibition of potato tubers irradiated by the treatment with gibberellin or indole acetic acid.

Sample	Sprouting Rate (%) Time of Storage (weeks)					
	1	2	3	4	5	7
Control (nonirradiated)	0	55	65	70	75	100
Control+GA 100 ppm	60	100				
0.12 kGy Irradiated	0	0	0	0	0	0
+GA 5 ppm	0	5	10	10	20	35
10 ppm	0	0	5	5	10	35
20 ppm	0	5	10	10	20	40
50 ppm	0	10	25	25	30	50
100 ppm	0	10	25	30	40	60
250 ppm	0	10	15	20	30	35
0.12 kGy Irradiated	0	0	0	0	0	0
+IAA 5 ppm	0	0	0	0	5	30
10 ppm	0	5	5	5	5	30
20 ppm	0	5	5	5	15	40
50 ppm	0	0	0	0	10	25
100 ppm	0	0	0	0	10	25
250 ppm	0	0	0	0	15	25

Twenty potatoes were selected in each lot. The irradiated tubers were dipped in different concentration of GA or IAA for 2hr and stored at 20±2°C and 70~90% relative humidity.

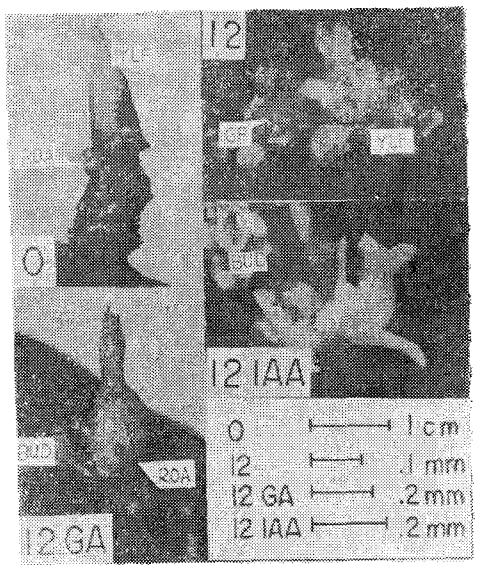

Fig. 7. Sprout buds of potato tubers for one month after storage. Abbereviation: 0, non-irradiated; 12, irradiated with a dose of 0.12 kGy; 12GA, GA was treated with a concentration of 100ppm to the irradiated tubers; 12IAA, IAA was treated with a concentration of 20 ppm to the irradiated potato tubers; BUD, deformed bud; GP, growing point, YLF, young leaf; ROA, adventitious root.

described by Ananthaswany.[10]

Morphological Changes in Irradiated Potato Tubers

Growing points in buds of freshly harvested potato tubers were in the dormant state and each of several tiny dormant buds in an eye had young leaves over its growing points. When tubers were exposed to radiation, remarkable morphological changes in dormant buds were induced to deformed buds and necrosis at growing points during storage.[23,24] As shown in Fig. 7, non-irradiated tubers developed normal bud with growing points covered by young scaly leaves, which were grown to the stem with an adventitous root. Irradiated tubers, however, developed the deformed buds with severe necrosis in the growing points; these surrounded by young swollen and abnormal leaves. These deformed buds were uncapable to grow further. This indicated that the radiation-induced dormancy was associated with inhibition of cell division and an aberrant enlargement at growing points, resulting in the formation of deformed buds. On the other hand, the reversal of radiation-induced dormancy by the post-irradiation treatment with 100 ppm GA or 20 ppm IAA was observed. After this treatment, new buds were found adjacent to deformed buds, and these new buds underwent the normal developmental stages of sprouting and formed the stem and root. But abnormal form of buds described previously were not able to form the stem and root and it remained as it was.

In conclusion, the roles of the metabolic pathway associated with radiation-induced respiration accompanied by increased induction of α-amylase activity seemed to supply chemical energy which is needed for biosynthesis of materials. The depletion of indole acetic acid by the combined actions of indole acetic acid oxidase induced and indole acetic acid synthesizing enzyme inactivated seemed be possible cause to radiation-induced dormancy of potato tubers.

Abstract

Potato tubers treated at 4°C for 4 weeks were irradiated with a dose of 0.12 kGy from ^{60}Co source and stored at 20°C, 70~90 humidity for 5 weeks. Changes of α-amylase, peroxidase, indole acetic acid oxidase, indole acetic acid synthesizing enzyme activities were determined. In addition, treatment of gibberellin or indole acetic acid to tubers irradiated were carried out to examine reversal of sprout-inhibition of tubers irradiated. Results are as follows;

1. Irradiation by γ-ray at 0.12 kGy dose inactivated easily the enzyme acivities *in vitro*. D_{37} values obtained were 0.94, 0.36 kGy for α-amylase and peroxidase, respectively.

2. Complete inhibition of the tuber sprouting was resulted by the irradiation of tubers with a dose of 0.12 kGy.

3. The indole acetic acid oxidase activity increased 2 times immediately after irradiation. Meanwhile, indole acetic acid synthesizing activity decreased about 50~75% for 5-week storage in irradiated potatoes, whereas the activity increased about 3.5 times along with sprouting in non-irradiated tubers.

4. In morphological aspects, deformed buds with necrosis in the meristmatic tissue were developed in irradiated tubers. Treatment of gibberellin or indole acetic acid at the concentration of 100 or 20 ppm to the irradiated tubers reversed the sprout-inhibition partially. Nevertheless, the deformed buds remained without change.

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