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Effect of GA_3 and ABA on Peroxidase and Catalase Activities and Isoperoxidase Patterns in Mung Bean Seedling

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녹두의 발아과정 중 GA_3 및 ABA의 처리가 Peroxidase, Catalase 활성변화와 Isoperoxidase Pattern에 미치는 영향

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초 록

식물 생장조절제에 의한 녹두의 부위별 peroxidase, catalase 활성과 전기영동상 peroxidase 동위효소의 변화를 조사하였다. 녹두의 생육이 진행됨에 따라 하배축을 제외하고는 peroxidase 활성도는 증가하였으나 catalase 활성도는 감소하여 서로 상반된 결과를 나타내었다. 암발아시킨 녹두에 GA_3와 ABA를 각각 처리하면 peroxidase 활성도의 증가에 대하여 자엽에서만 GA_3가 ABA 보다 더 큰 영향을 끼쳤으나 catalase 활성도 변화는 ABA가 GA_3 보다 더 큰 영향을 끼쳐 모든 부위에서 활성도를 증가시켰다. GA_3와 ABA를 혼합하여 처리한 효소활성도의 변화 경향은 GA_3 보다 ABA에 더 큰 영향을 받았다. Peroxidase 동위효소의 수는 발아, 생육이 진행됨에 따라 모든 부위에서 계속 증가하여 활성도와는 무관하게 발아 후 6일째에 가장 많이 나타났으며, 호르몬의 처리에 따른 동위효소의 변화는 동위효소의 수에 대해서 보다는 intensity에 대하여 더 큰 영향을 받은 것으로 나타났다.

Introduction

Peroxidases have been widely distributed throughout the biological world. They have been found in many plants, in some animal tissues, and in microorganisms[1] and they have been known to carry out a variety of biosynthetic and degradative functions for the use of peroxides, specially H_2O_2, as an oxidant.

Peroxidase might play an important role in the regulation of plant growth, development,[2,3,4] and more specifically in metabolic activities concerned with germination[5,6] although its precise function was as yet not understood.

Stimulation of germination in dark-imbibed light-sensitive seeds by gibberellic acid(GA_3) has been widely reported. More recently the observation has been made that this promotion can be reversed by abscisic acid(ABA).[7] However, it is not well understood how the hormone regulates the activities of enzymes involved in the growth and differentiation.

In order to understand the functions of peroxidase and catalase in plant, it is needed to monitor the enzyme activities and isozyme forms during the development of the plant. This study was undertaken to examine the effect of various

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combination of hormone(GA_3 and ABA) on the changes in peroxidase and catalase activity, and isozyme patterns in different parts of mung bean seedling.

Materials and Methods

Chemicals

O-dianisidine dihydrochloride, tris(hydroxy-methyl) aminomethane, bovine serum albumin, gibberellic acid and abscisic acid were purchased from Sigma chemical Co. Electrophoresis reagents were from Bio-Rad. All other chemicals were used of analytical grade.

Germination and enzyme extraction

Mung bean(*Phaseolus aureus Roxb.*) seeds were germinated for a different period in a growth chamber in the dark at 27°C with the application of 10^{-7}M or 10^{-5}M hormones(GA_3 and ABA) one time a day, and that of tap-water 4 times a day after imbibition for 12 hours. Depending upon germination time, mung bean sprout was divided into 3 sections described by Lee et al.[8]

Crude enzymic extracts were prepared by grinding with 2.5 volumes(w/v) of phosphate buffer(pH 5.5) and then centrifuged at $10,000 \times g$ for 10 min. The supernatant was used as the enzyme.

Determination of enzyme activity

The activity of peroxidase was determined by the procedure described in the Worthington enzyme manual.[9] The assay mixture consisted of 0.1 ml of crude extract and 2.9 ml of 46.8 mM phosphate buffer(pH 6.0) containing 0.05 ml of 0.26 mM H_2O_2 and 0.05 ml of 0.34 mM o-dianisidine. Perkin-Elmer spectrophotometer was used to measure the rate of decomposing the peroxide at 460nm. One unit of the enzyme activity was defined as the amount of enzyme decomposing 1 μmole of peroxide per minute at 30°C, and the specific activity expressed as unit of enzyme activity per mg protein.

The activity of catalase was determined by the procedure of Omran.[10] The reaction was carried out at pH 7.0 using 4.1 ml of 10 mM phosphate buffer, 0.8ml of 240 mM H_2O_2 and 0.1 ml of crude extract at 30°C and the reaction stopped by the addition of 2N H_2SO_4. The assay mixture was titrated with 10 mM potassium permanganate. One unit of the enzyme activity was defined as the amount of enzyme decomposing 1 μmole of hydrogen peroxide per minute. The amount of protein was determined by the method of Lowry et al.,[11] with bovine serum albumin as standard. Disc electrophoresis in polyacrylamide gel was performed as described by Davis[12] using 6% acrylamide gel.

Results and Discussion

Changes in peroxidase activity

The application of GA_3 increased the enzyme activity, while that of 10^{-5} M ABA decreased the enzyme activity as compared with control at the 2nd day after germination(Fig. 1). The enzyme activity in root was particularly higher than those in any other parts. In all parts except in hypocotyl at the 2nd day after germination, GA_3 and ABA did not influence the enzyme activity was higher than that of control and continued to increase until the 6th day.

An increase in enzyme activity following hormonal treatment may be caused by de novo synthesis or activation of existing enzyme.

The combinated treatment with GA_3 and ABA resulted in the changes of the enzyme activity, which was much more affected by ABA than by GA_3(Fig. 2). Irrespective of the kind and concentration of these hormones, peroxidase activity in root increased at an early stage of germination.

Changes in catalase activity

As Shown in Fig. 3, the catalase activity in cotyledon and hypocotyl was decreased by GA_3 and increased by ABA. The catalase activity in

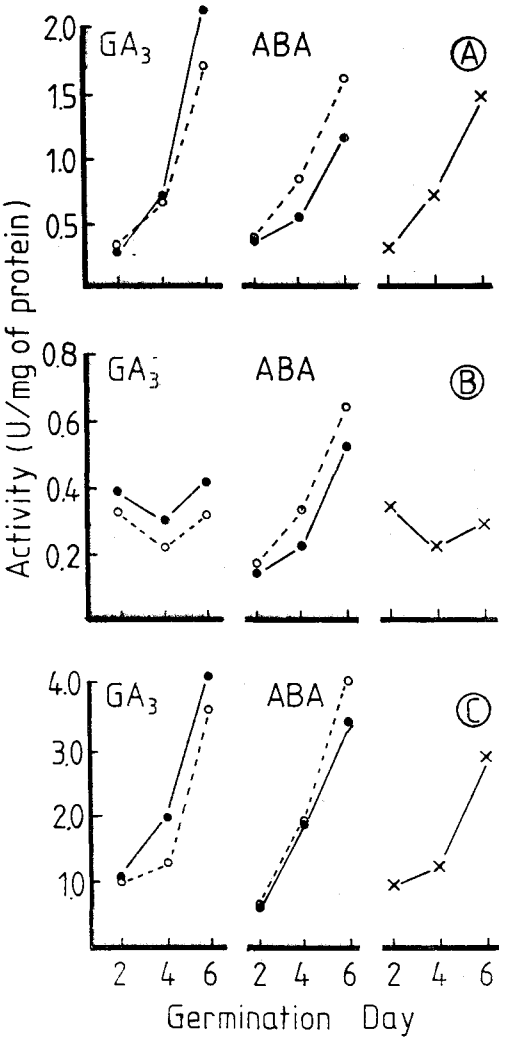

Fig. 1. Time course of peroxidase activity in cotyledon (A), hypocotyl (B) and root (C) with and without GA_3 and ABA
(●—●), 10^{-5}M; (○···○), 10^{-7}M
(x—x), non-treatment

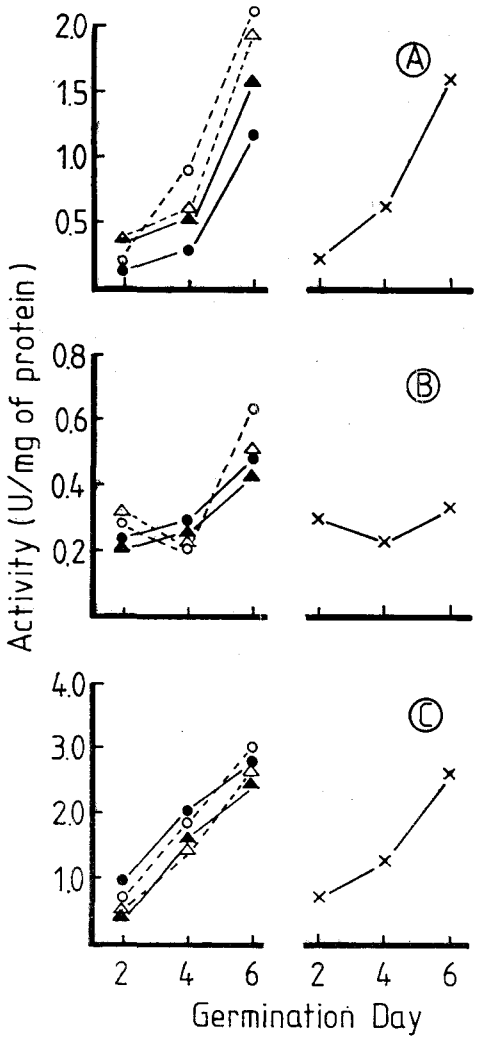

Fig. 2. Time course of peroxidase activity in cotyledon (A), hypocotyl (B) and root (C) with and without GA_3/ABA
(●—●), $GA_3 10^{-5}$M/ABA 10^{-5}M
(○—○), $GA_3 10^{-5}$M/ABA 10^{-7}M
(▲—▲), $GA_3 10^{-7}$M/ABA 10^{-5}M
(△···△), $GA_3 10^{-7}$M/ABA 10^{-7}M

cotyledon showed a tendency to decrease continuously during germination in all treatments. The enzyme activity in hypocotyl was decreased to the 3rd day after germination and increased thereafter in control and GA_3, but the reverse was the case in ABA.

Although it was reported that the changes of peroxidase and catalase activity generally conflicted with each other, different results were observed when GA_3 was applied at hypocotyl in this study.

Catalase activity in combined treatment with GA_3 and ABA was higher than that in control (*Fig.* 4A). As shown in *Fig.* 4B, the changes of activity in hypocotyl were more affected by ABA than by GA_3, while the activity in root affected GA_3 and ABA(Fig. 4C).

Isoperoxidase patterns in different parts of mung bean

Fig. 5 illustrated the electrophoretic patterns

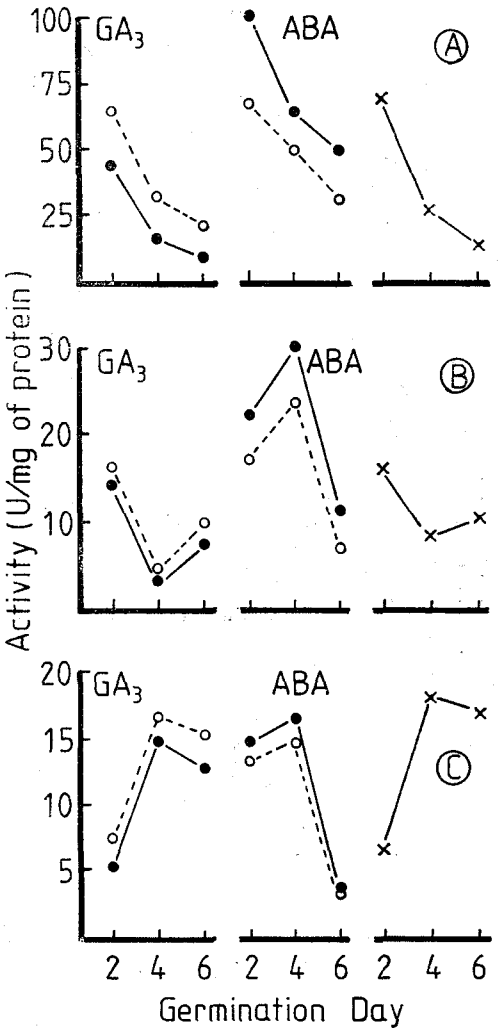

Fig. 3. Time course of catalase activity in cotyledon (A), hypocotyl (B) and root (C) with and without GA_3 and ABA
(●—●), 10^{-5}M; (○···○), 10^{-7}M
(x—x), non-treatment

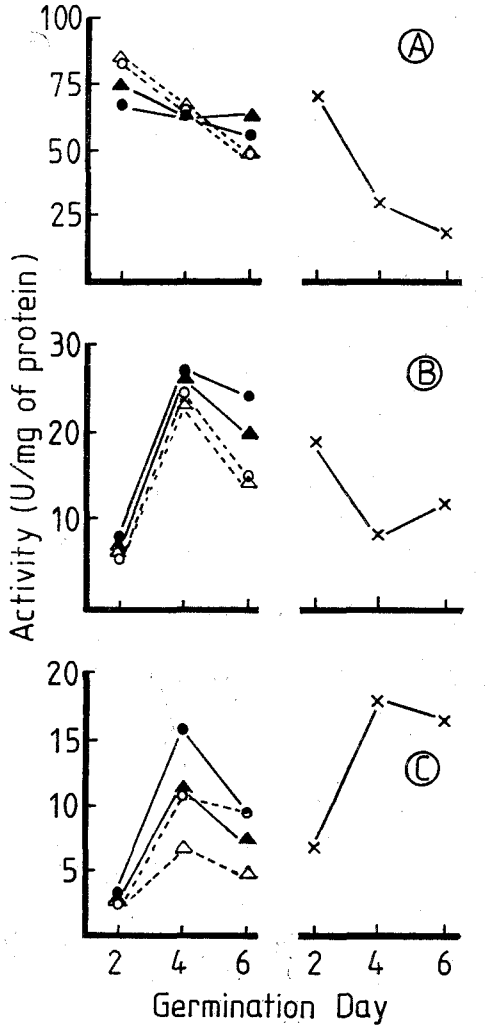

Fig. 4. Time course of catalase activity in cotyledon (A), hypocotyl (B) and root (C) with and without GA_3/ABA
(●—●), $GA_3 10^{-5}$M/ABA10^{-5}M
(○···○), $GA_4 10^{-5}$M/ABA10^{-7}M
(▲—▲), $GA_3 10^{-7}$M/ABA10^{-5}M
(△···△), $GA_3 10^{-7}$M/ABA10^{-7}M

of crude extract from cotyledon of mung bean seedling, which was treated with GA_3 or ABA. The application of GA_3 resulted in the appearence of two isozymes(Rm 0.43 and 0.51) at the 2nd day after germination but that of ABA did not have an effect.

In combination of two hormones, two isozymes(Rm 0.43 and 0.51) did not appear at the 2nd day and the intensity of other isozyme decreased except by the treatment with 10^{-7}M of GA_3 and ABA(Fig. 5B). By the treatment with ABA, the activity of the peroxidase was affected but the isozyme pattern was not.

Fig. 6 illustrated the isozyme patterns in hypocotyl. The number of isozymes was gradually increased afterwards as germination went on, and no change could be observed in the isozyme pattern by GA_3 and ABA. However, the isozyme having Rm value of 0.59 was occurred at the 6th day after germination in combination of two hormones(Fig. 6B).

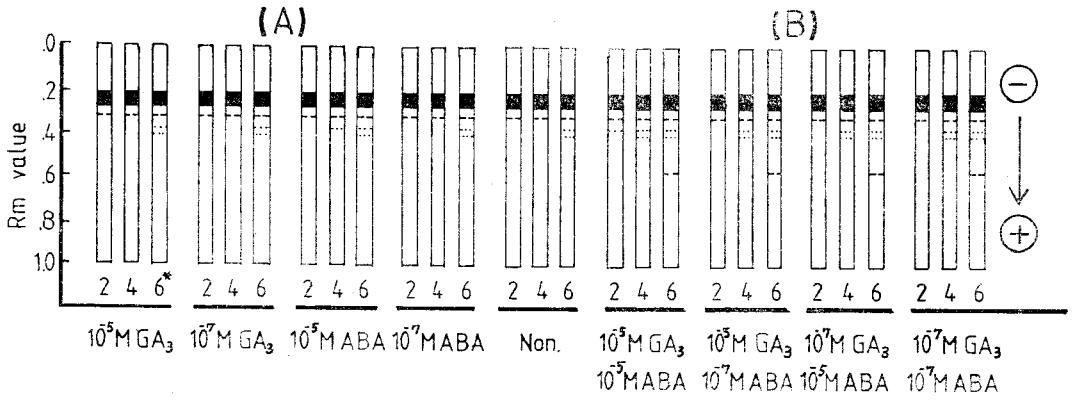

Fig. 5. The isozyme patterns of peroxidase in cotyledon treated with GA_3 and ABA, alone (A) and in combination (B)

* 2, 4, 6; Germination day

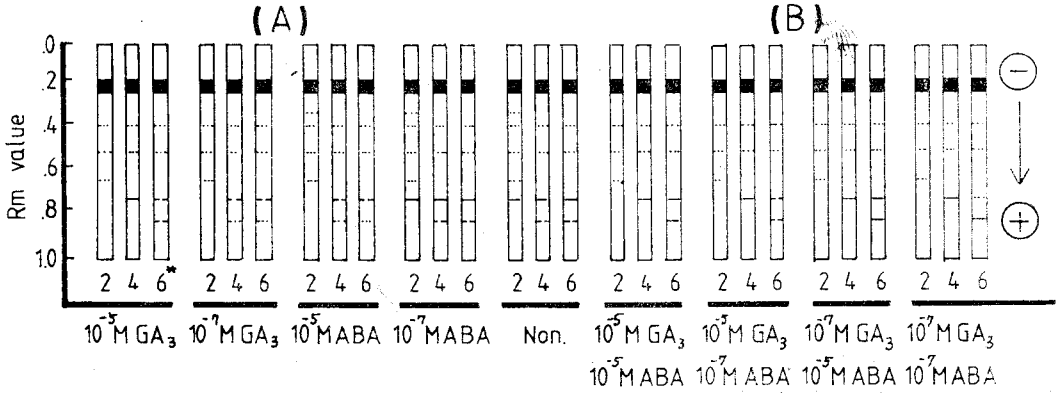

Fig. 6. The isozyme patterns of peroxidase in hypocotyl treated with GA_3 and ABA, alone (A) and in combination (B)

* 2, 4, 6; Germination day

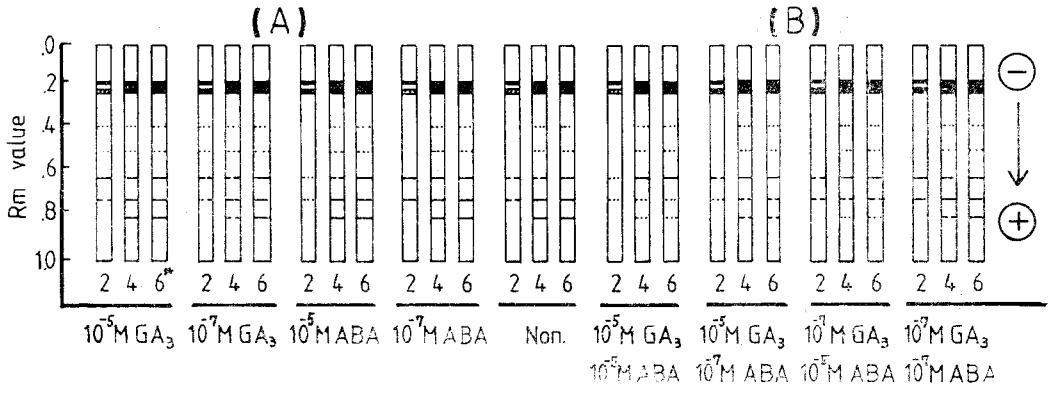

Fig. 7. The isozyme patterns of peroxidase in root treated with GA_3 and ABA, alone (A) and in combination (B)

* 2, 4, 6; Germination day

The effects of GA_3 and ABA, alone and in combination, on the patterns of isozyme in root were shown in Fig. 7A and 7B.

In this case there was also a marked change

in the number of isozymes during germination. The maximal number could be seen in an early stage of germination. The isozyme having Rm value of 0.22 occurred throughout development and remained constant throughout 6 days; band that has seen at this site was also present in the other two organs. These zymograms shown that it might be used isozyme patterns as well as the changes of peroxidase activity as predictable indicators of growth and cellular differentiation.

Hormones, such as GA_3, kinetin and ABA, have been shown to induce or repress individual isoperoxidases.[4,13,14] In the present study the effects of GA_3 and ABA on isozymes were also observed in mung bean seedling.

As the time elapsed after germination, the maximal number of isozyme occurred, being 6, 5 and 7 in cotyledon, hypocotyl and root, respectively. Regardless of increase and decrease in enzyme activity, the number of isozyme increased as the seedling developed. These large number of isozymes found in plant may suggest that different metabolic functions are associated with the different tissues. Such changes have been shown to in maize,[15] in developing pea cotyledons,[2] and in the germinating seeds of wheat[16] and barley.[17]

Abstract

The changes in peroxidase and catalase activities, and isoperoxidase patterns in different parts of mung bean seedling caused by the treatment with plant growth substances, GA_3 and ABA, were examined. As germination proceeded, the activity of peroxidase in all part except hypocotyl was increased, while that of catalase decreased. The separate application of GA_3 and ABA increased the activity of peroxidase which was more influenced by GA_3 than by ABA only in cotyledon, while that of catalase was more affected by ABA than by GA_3. Electrophoretic study revealed that the number of isoperoxidase was increased continuously in all parts during development. A greater influence was exerted on the intensity of isozyme than the number of isozyme by the hormonal treatment.

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