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# Effects of Triacontanol on Growth and Peroxisomal Enzyme Activities in Radish (*Raphanus sativus* L.) Seedlings

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## 무우 유식물의 생장과 Peroxisome 효소 활성에 미치는 트리아콘타놀의 효과

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## ABSTRACT

The present study investigated the effects of triacontanol (TRIA) on plant growth and peroxisomal enzyme activities in radish seedlings.

The optimum concentration of TRIA with respect to radish seedling bioassay was decided to 1.0 mg $l^{-1}$. In comparison to untreated controls (including Tween 20 treatment), 1.0 mg $l^{-1}$ TRIA treatment caused an increase in seed germination rate and root growth, but no stimulation in hypocotyl growth. Chlorophyll accumulation in cotyledon during early development stage increased rapidly, and degradation of chlorophyll in later stage due to the cotyledon senescence was noticeably retarded. Increase of soluble protein content in cotyledon at early period of development was observed. Isocitrate lyase and catalase activity was not significantly different in both the treated and the untreated plants. But, glycolate oxidase activity was inhibited by TRIA down to 20% against controls. Also, the increase of the activity of peroxidase, a leaf-senescence marker enzyme, was continuously retarded over control for 8 days of development. Based on above results, TRIA was found to be active in both the growth and the peroxisomal enzyme activities of radish seedlings.

## INTRODUCTION

Triacontanol (TRIA), a 30-carbon primary alcohol, is present in small quantities in the soil and animal waxes, as well as in many plant species (Chibnall *et al.*, 1933). Since the plant growth regulating activity of TRIA was first observed in some crop plants in 1977 (Ries *et al.*, 1977; Ries and Wert, 1977), there have been many attempts to elucidate the action mechanism of TRIA in regulating growth and enzymatic changes

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within growing plants. However, the history of TRIA has had several ups and downs (Maugh II, 1981).

The first growth responses reported for TRIA was for increase in dry weight, leaf area, and water uptake of rice (Ries *et al.*, 1977). Early researches which tried to demonstrate the effect of TRIA on seed germination of several species did not provide any positive consistent results (Ries *et al.*, 1978; Hoagland., 1980; Steffens and Worley, 1980; Lewak and Skowronska, 1982). Also, Henry and Gordon (1980) pointed out that the effect of TRIA on hypocotyl growth appeared to be species-specific and Singletary and Foy (1980) found that TRIA at 1.0 mg $l^{-1}$ significantly stimulated the root growth.

In addition, TRIA was known to be effective both in causing the retention of chlorophyll in oat leaf senescence assay (Steffens and Worley, 1980; Bhalla, 1981) and in promoting chlorophyll increase of corn and rice (Ries *et al.*, 1981). However, the mechanism of the effects of TRIA on chlorophyll retention is not known.

Although there is in a controversy for the photosynthesis promoting effect of TRIA (Ries and Wert, 1977), Devata and Murty (1981) found that TRIA was efficient in photosynthesis and mobilization of photosynthates. Especially, Eriksen *et al.* (1981) showed the TRIA-induced photorespiration inhibition in tomato plants and Haugstad *et al.* (1983) reported the same result in *Chlamydomonas*.

The above results implicate photorespiration inhibition as an important factor in the growth response to TRIA.

In the previous screening for TRIA responses on enzyme systems, the total activity of several enzymes was studied after TRIA treatment.

Lesniak and Ries (1982) found the increase of isocitrate dehydrogenase and 6-phosphogluconate dehydrogenase activity in TRIA treated corn seedlings and Ries *et al.* (1981) obtained the result that starch phosphorylase and PEP carboxylase activity was also increased by TRIA up to 40% over untreated controls *in vitro* cell free studies. Also, Henry and Gordon (1980) reported that the effect of TRIA on peroxidase activity appeared to be cultivar specific with respect to varieties of peas. However, there is still no research on the peroxisomal enzymes with TRIA application.

At the present time, TRIA would be considered to be a plant growth regulator (Noggle and Fritz, 1983; Ries *et al.*, 1983). Up to the present, however, there were insufficient studies of physiological responses to TRIA in plants. Moreover, much of previous researches conducted with TRIA have been concerned with yield and with dry weight accumulation of established seedlings. Therefore, the objectives of this study were to discover the physiological effects and the distinctive biochemical marker for the TRIA response in radish seedlings.

## MATERIALS AND METHODS

**Plant materials and growth conditions.** Radish seed (*Raphanus sativus* L. cv. Jinju-

daepycong) was surface-sterilized with 1% solution of sodium hypochlorite. The seeds were sown and allowed to germinate for 8 days in glass-covered plastic containers which contained 3-layers of filter paper (Toyo No. 2) moistened with distilled water or TRIA solution receiving Tween 20. The radish seedlings in growth chamber were grown at 25± 1°C and continuously irradiated with daylight tube (General Electric, U.S.A.) giving an approximate intensity of 7,000 lux.

**Preparation of treatment solutions.** Treatment solutions consisted of aqueous 0.1% (v/v) Tween 20 containing TRIA (Sigma Chemical Co.) prepared from stock dissolved in acetone. The amount of stock added to distilled water was adjusted to achieve a final concentration of 0.1% Tween 20 and 0.01 to 10 mg $l^{-1}$ TRIA.

**Measurement of plant growth.** Growth characteristics of light-grown radish seedlings were investigated for 8 days after germination. Cotyledons were harvested at daily intervals from day 0 to day 8. Fresh weight was determined on samples of 20 cotyledons and dry weight was measured after drying the same samples for 24 hr at 90°C. Hypocotyls and roots were harvested everyday and the length-growth was measured. All determinations were made with 3 replicate samples and the mean value is shown.

**Determination of chlorophyll and protein.** Chlorophyll content was determined by the modified method of Arnon (1949) and soluble protein content in cotyledons was determined by the method of Lowry *et al.* (1951).

**Extraction and assay of enzymes.** Fifteen pairs of cotyledons were ground on ice with 0.5 g quartz sand in 4 ml of 70 mM K-phosphate buffer (pH 8.0). After the homogenate was centrifuged at 18,000 *g* for 30 min, the clear supernatant was used for enzyme assays. Isocitrate lyase (EC 4.1.3.1) was assayed according to Bajracharya and Schopfer (1979). The 1.0 ml volume of reaction mixture contained 55 μmol phosphate buffer (pH 7.6), 3.3 μmol phenylhydrazine HCl, 8.75 μmol cysteine, 22 μmol $MgCl_2$, 17.5 μmol DL-Na-isocitrate, and 0.15 ml of enzyme extract. The reaction was started with isocitrate after 5 min preincubation. Enzyme activity was calculated from the linear increase of absorbance at 334 nm using an extinction coefficient of $13.43\times10^3$ $M^{-1}cm^{-1}$. Glycolate oxidase (EC 1.1.3.1) was assayed with a Clark-type oxygen electrode by a modification of the method of Tolbert *et al.* (1949). The reaction mixture contained 2.5 ml of 0.1M K-phosphate buffer (pH 8.0), 0.5 ml of 0.04M glycolate (titrated with KOH to pH 8.0) and 0.1 ml of enzyme extract. The reaction was started with glycolate after 5 min preincubation at 25°C. Enzyme activity was calculated from the decrease of dissolved oxygen. Catalase (EC 1.11.1.6) was assayed spectrophotometrically by a modification of the method of Chance and Maehly (1955). In total volume of 3.0 ml, the reaction mixture contained 0.05M K-phosphate buffer (pH 7.0), 10 mM $H_2O_2$ and 0.05 ml of enzyme extract. The reaction was started with $H_2O_2$. Enzyme activity was estimated from the decrease of absorbance at 240 nm using an extinction coefficient of $0.44\times10^2$ $M^{-1}$ $cm^{-1}$ (Chance and Maehly, 1955). Peroxidase (EC 1.11.1.7) was assayed by a modification of the method of Chance

and Maehly (1955). In a total volume of 3.0 ml, the reaction mixture contained 0.07M K-phosphate buffer (pH 6.0), 5 mM guaiacol, 10 mM $H_2O_2$, and 0.1 ml of enzyme extract. The reaction was started with $H_2O_2$ and estimated from the increase of absorbance at 430 nm.

## RESULTS AND DISCUSSION

**Effect of TRIA on chlorophyll and soluble protein.** In general, a variation in chlorophyll and soluble protein content can be considered to be a marker of noticeable physiological response in plant tissue to exogenous stimulus.

Figure 1 shows the effect of TRIA treatment with various concentrations on chlorophyll accumulation in the cotyledons during the development of radish seedlings. The appearance of the TRIA effect, specially at 1.0 mg $l^{-1}$ concentration, was divided into two phases according to the time course of radish seedlings. As compared with controls (water treatment and Tween 20 treatment), chlorophyll accumulation during the early development stage increased rapidly, and degradation of chlorophyll in the later stage by the cotyledon senescence was noticeably retarded. The result of this study implicates the correlations with both the report of TRIA-induced chlorophyll retention in oat and rice leaf senescence (Bhalla, 1981; Debata and Murty, 1981) and the observation of TRIA-promo-

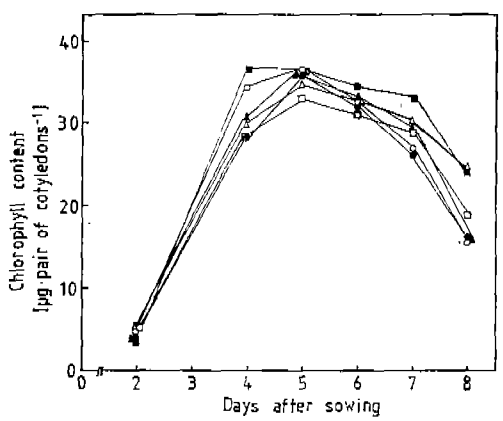


Fig. 1. The effects of triacontanol on chlorophyll content in the cotyledons during the development of radish seedlings: -○-, D.W. only; -●-, 0.1% tween 20 only; -▲-, 0.01 mg $l^{-1}$; -△-, 0.1 mg $l^{-1}$; -■-, 1.0 mg $l^{-1}$; -□-, 10.0 mg $l^{-1}$ triacontanol added.

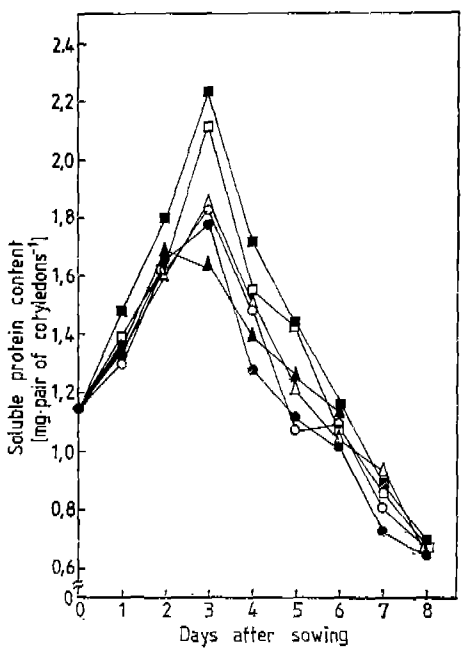


Fig. 2. The effects of various triacontanol concentrations on soluble protein content in the cotyledons during the development of radish seedlings: -○-, D.W. only; -●-, 0.1% tween 20 only; -▲- 0.01 mg $l^{-1}$; -△-, 0.1 mg $l^{-1}$; -■-, 1.0 mg $l^{-1}$; -□-, 10.0 mg $l^{-1}$ triacontanol added.

ting chlorophyll increase in corn and rice (Ries *et al.*, 1981). Such an effect of TRIA on chlorophyll retention in senescing leaf may be explained with respect to the inhibition of peroxidase activity following the application of TRIA (Fig. 11).

The effect of TRIA at various concentrations on the level of soluble protein in the cotyledons during the development of radish seedlings was shown in Figure 2.

The overall fluctuation patterns in soluble protein were similiar to each other in both the controls and TRIA treatments. However, TRIA most rapidly increased the soluble protein content after 3 days of incubation at concentration of 1.0 mg $l^{-1}$ than in any other treatments. In previous studies, TRIA has been shown to increase the protein content of rice seedling within 3 to 6 hrs in the light and dark (Ries and Wert, 1977; Ries *et al.*, 1978). Especially, Ries *et al.* (1981) observed soluble protein increase within 4 minutes after foliar application of TRIA in corn and rice (Ries *et al.*, 1981). Based on the previous research results and our present data, it can be suggested that TRIA participates in early metabolic events of germination. Also, this concentration of 1.0 mg $l^{-1}$ TRIA was chosen for the following tests described in this paper because it was found to be optimum for TRIA response in plants.

**Effects of TRIA on growth responses.** The previous researches conducted with TRIA showed lack of consistent results on seed germination. Hoagland (1980) and Steffens and Worley (1980) observed no significance of TRIA on seed germination and Lewak and Skowronska (1982) found the inhibition effect in lettuce seeds on the contrary. However, Chowdhury *et al.* (1980) and Ries *et al.* (1978) reported TRIA induced germination increase. Nevertheless, the promotion of seed

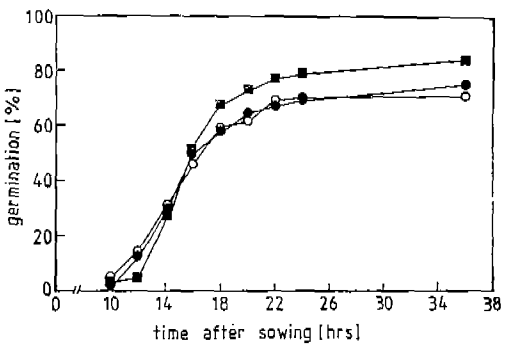


Fig. 3. Germination of radish seeds in response to triacontanol: -o-, D.W.; -•-, 0.1% tween 20; -▪-, 1.0 mg $l^{-1}$ triacontanol + 0.1% tween 20.

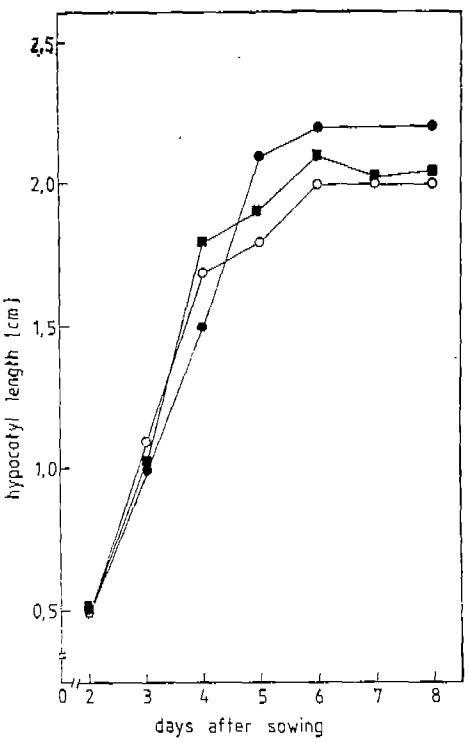


Fig. 4. Hypocotyl growth of radish seedlings in response to triacontanol: -o-, D.W.; -•-, 0.1% tween 20; -▪-, 1.0 mg $l^{-1}$ triacontanol + 0.1% tween 20.

germination can be considered to be an important factor for the primary growth response in plants. From this point of view, our research was carried out to reveal the effect of TRIA on germination of radish seeds. As a result of our experiment, the data presented in Figure 3 showed that TRIA caused a 10% germination rate increase compared to untreated controls.

In the cucumber hypocotyl bioassays, TRIA was found to be active (Bhalla, 1981). However, our result presented in Figure 4 showed that hypocotyl length was not significantly affected in germinating radish seedlings. In an early study (Henry and Gordon, 1980), it has been shown that the effect of TRIA on hypocotyl growth appeared to be species-specific.

Singletary and Foy (1980) observed that TRIA singnificantly stimulated root growth and the total accumulation of $K^+$ ion in root of corn receiving the nutrient solution which contained N, P and K. From this result, he suggested the correlation between TRIA and availibility of inorganic nutrient salts in root of corn. However, Henry and Gordon (1980) reported that the effect of TRIA on root growth appeared to be cultivar-specific.

Response obtained in our study (Fig. 5) in radish seedlings showed the root growth promoting effect of TRIA for 8 days of incubation. From these data, TRIA appears to be a biologically active material in root growth.

Hangarter and Ries (1978) obtained that TRIA caused to increase the fresh weight of cell

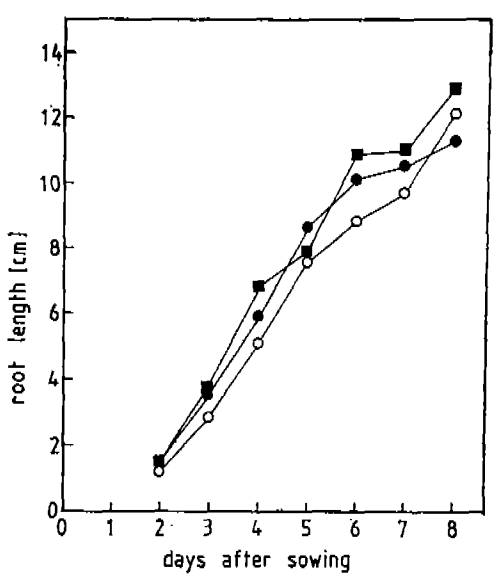


Fig. 5. Root growth of radish seedlings in response to triacontanol: -o-, D.W.; -•-, 0.1% tween 20; -▪- 1.0 mg $l^{-1}$ triacontanol + 0.1% tween 20.

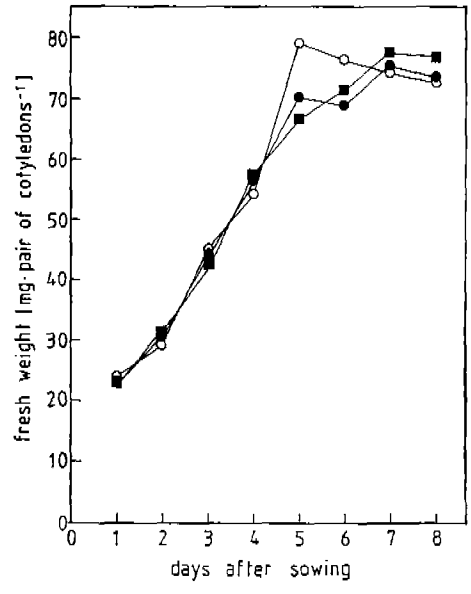


Fig. 6. Developmental changes of fresh weight of cotyledons from radish seedlings in response to triacontanol; -o-, D. W.; -•-, 0.1% tween 20; -▪- 1.0 mg $l^{-1}$ triacontanol + 0.1% tween 20.

cultures of tomato, bean, potato and barley. Henry and Gordon (1980) showed that water uptake in pea tissue treated at 1.0 mg $l^{-1}$ TRIA was higher than that of control. This water uptake promoting effect of TRIA appears to be one of important factors to plant growth response. In our data presented in Figure 6, the increase tendency of fresh weight in the TRIA-treated cotyledons was not observed. In our growth experiment, the germinating radish seedlings were only watered with distilled water or treatment solution receiving no nutrients.

The developmental changes in dry weight of cotyledons for 8 days of incubation were shown in Figure 7. The changes of dry weight in controls were constantly decreased for 8 days of incubation, while the decrease of dry weight in TRIA-treated group was significantly retarded.

For the reason of this phenomenon, water incorporated via hydrolysis, hydration and oxidation reactions will perhaps contribute to dry weight retardation.

**Effects of TRIA on enzyme activity.** In order to explain the growth responses and photorespiration to TRIA, the total activities of several enzymes in radish seedlings were examined after TRIA treatment.

**Isocitrate lyase.** Isocitrate lyase (ICL) is one of typical glyoxysomal enzymes which participates in the glyoxylate cycle during the early metabolic events after germination. The development of ICL follows the well known pattern that has been described in a large number of fat-storing seeds (Becker *et al.*, 1978). In order to discover a distinctive bio-

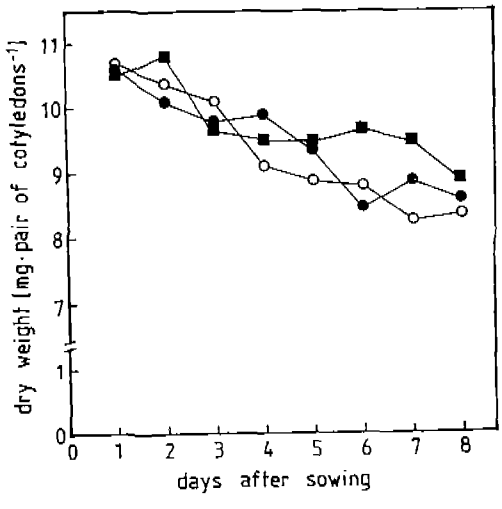


Fig. 7. Developmental changes of dry weight of cotyledons from radish seedlings in response to triacontanol: -○-, D.W.; -●-, 0.1% tween 20; -■-, 1.0 mg $l^{-1}$ triacontanol + 0.1% tween 20.

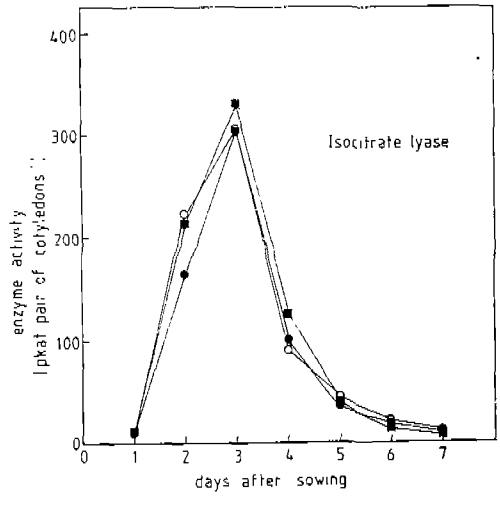


Fig. 8. The effects of triacontanol on isocitrate lyase activity in the cotyledons during the development of radish seedlings: -○-, D.W.; -●-, 0.1% tween 20; -■-, 1.0 mg $l^{-1}$ triacontanol + 0.1% tween 20.

chemical maker for the TRIA response, therefore, the authors investigated the development of ICL according to the time course during 7 days of incubation. However, ICL development pattern in TRIA-treated groups was not significantly different from that of untreated controls as shown in Figure 8. Although TRIA seemed to have a small tendency to enhance the ICL activity, the effect of TRIA on ICL was not noticeably significant.

**Glycolate oxidase.** The peroxisomal enzyme, glycolate oxidase (GO), is a distinctive marker enzyme of photorespiration which participates in glycolate oxidation metablism. The previous studies on TRIA effects on photorespiration were as follows; Eriksen *et al.* (1981) observed the TRIA reduction in the oxygen inhibition of photosynthesis (photorespiration) in tomato plants, and Haugstad *et al.* (1983) found the same result in *Chlamydomonas.* From these studies, they suggested that an enhanced growth was the result of an increase in net production which could be caused by an inhibition in the rate of photorespiration.

On the other hand, our experiment investigated the development of GO activity during 7 days of incubation in both the TRIA treatment and the untreated controls. As presented in Figure 9, TRIA did not affect the level of GO activity in the early interval from day 1 to day 3, but GO activity in later stage was inhibited by TRIA down to 20% against controls. From the data obtained in our present research and in previous studies (Eriksen *et al.*, 1981; Haugstad *et al.*, 1983), we can assume that TRIA in photorespiration regulation affects processes related to growth response. Therefore, we suggest that GO may be a useful biochemical marker of TRIA response for physiological studies.

**Catalase.** Catalase is an enzyme common both to the glyoxysomes during early stages and to the peroxisomes at later stages (Becker *et al.*, 1978). The decomposition of toxic $H_2O_2$ derived by $\beta$-oxidation of storage fats and oxidation of glycolate requires this enzyme. In order to discover the TRIA effect on catalase activity in radish seedling, its activity

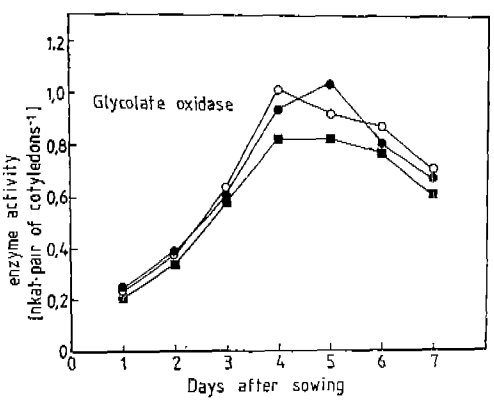


Fig. 9. The effects of triacontanol on glycolate oxidase activity in the cotyledons during the development of radish seedlings: -o-, D.W.; -•-, 0.1% tween 20; -▪-, 1.0 mg $l^{-1}$ triacontanol + 0.1% tween 20.

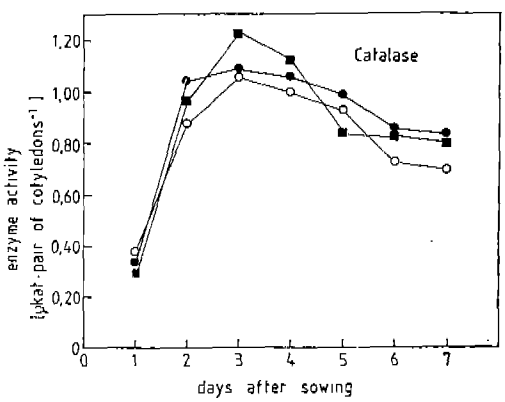


Fig. 10. The effect of triacontanol on catalase activity in the cotyledons during the development of radish seedlings: -o-, D.W.; -•-, 0.1% tween 20; -▪-, 1.0 mg $l^{-1}$ triacontanol + 0.1% tween 20.

profile was investigated for 7 days of incubation.

As shown in Figure 10, catalase activity was increased a little by TRIA over controls in the interval from day 3 to day 4. As compared with the ICL activity profile, it is of interest that the time of catalase activity increase over controls was in accordance with that of ICL. To explain growth promotion response to TRIA, however, the effect of TRIA on catalase is still obscure.

**Peroxidase.** Peroxidase is relatively rare in animal tissues, but all higher plants, in contrast, is rich in peroxidase activity (Dunleavy and Urs, 1978). Especially, peroxidase is well known to be a marker enzyme in leaf senescence. Lesniak and Ries (1982) observed that peroxidase activity after application of TRIA in corn and rice seedlings was not significantly affected. However, Henry and Gordon (1980) found that pea tissue treated with 0.1 mg $l^{-1}$ TRIA plus 10 $\mu$M $GA_3$ showed a decrease in peroxidase activity. In our experiment using the radish seedlings treated with 1.0 mg $l^{-1}$ TRIA, the results of TRIA treatment on peroxidase activity was presented in Figure 11. The increase of peroxidase activity due to the development of cotyledons was conspicuously inhibited by TRIA at later development stage. In relation to inhibition of cotyledon senescence via chlorophyll retention after application of TRIA (Fig. 1), the above result suggests that the peroxidase activity to TRIA may become a biochemical marker for radish seedling growth.

Up to present time, the precise mechanism of action of TRIA in promoting or inhibiting growth and enzymic changes within growing seedlings remains to be ascertained. However, the present study indicates that a specific level of TRIA has specific promotive effects on radish tissue with respect to germination, senescence retardation, and photorespiration inhibition (expressed by peroxisomal enzymes activities).

From the results obtained in our research, we suggest specially that glycolate oxidase may be a useful biochemical marker of the TRIA response for physiological studies.

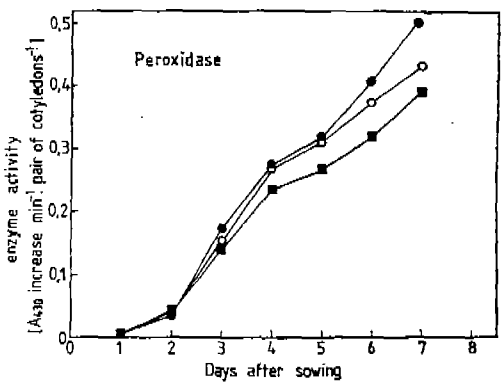


Fig. 11. The effects of triacontanol on peroxidase activity in the cotyledons during the development of radish seedlings: -o-, D.W.; -•-, 0.1% tween 20; -■-, 1.0 mg $l^{-1}$ triacontanol + 0.1% tween 20.

## 摘 要

무우 유식물의 생장과 peroxisome 효소의 활성에 미치는 트리아콘타놀의 효과를 조사하였으며, 그 결과 1.0 mg $l^{-1}$의 농도에서 최적 활성이 나타났다. 1.0 mg $l^{-1}$ 트리아콘타놀 처리시, 대조구에 비해서 발아율과 뿌리의 생장이 증가되었으나 하배축 생장은 유의한 차이가 없었다. 또한 자엽내의 엽록소 함

량은 발아초기의 빠른 축적 현상과 후기에서의 감소 억제 효과를 보였으며 수용성 단백질 함량의 증가를 일으켰다. 또한 isocitrate lyase 활성과 catalase 활성은 유의한 차이를 보이지 않았으나 광호흡의 marker 효소인 glycolate oxidase 활성은 20% 정도 억제되었다. 잎의 노화 현상 marker 효소인 peroxidase의 활성 증가율은 8일간의 배양기간 동안 계속 대조구에 비해 억제되었다. 따라서 트리아콘타놀은 발아을 촉진, 발아초기의 자엽의 엽록소 함량 증가, 발아후기의 노화 현상 지연, 광호흡 활성 억제 등 무우 유식물의 생장에 관여된다고 생각된다.

## REFERENCES


Arnon, D. J. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* **24** : 1~15.

Bajracharya, D. and P. Schopfer. 1979. Effect of light on the development of glyoxysomal functions in the cotyledons of mustard (*Sinapis alba* L.) seedlings. *Planta* **145** : 181~186.

Becker, W. M., C. J. Leaver, E. M. Weir and H. Riezman. 1978. Regulation of glyoxysomal enzymes during germination of cucumber. *Plant Physiol.* **62** : 542~549.

Bhalla, P. R. 1981. Triacontanol as a plant biostimulant. Proc. 8th Ann. Plant Growth Regulator Soc. Florida. pp. 184~195.

Chance, B. and A. C. Maehly. 1955. Assay of catalase and peroxidase. *Methods Enzymol.* **2** : 764~775.

Chibnall, A. C., E. F. Williams, A. L. Latner and S. H. Piper. 1933. The isolation of n-triacontanol from lucerne wax. *Biochem. J.* **27** : 1885~1888.

Chowdhury, I. R., K. B. Paul and D. Sasseville. 1980. The emergence and yields of beans, okra and squash as influenced by cytex, triacontanol and threl. Proc. 7th Ann. Meeting of PGR Working Group. Texas. pp. 76~82.

Devata, A. and K. S. Murty. 1981. Effect of growth regulators on photosynthetic efficiency, translocation and senescence in rice. *Indian J. Exp. Bio.* **19** : 986~987.

Dunleavy, J. M. and N. V. R. Urs. 1978. Peroxidase activity in roots and leaves of soybeans. *Crop Sci.* **18** : 104~108.

Eriksen, A. B., G. Sellden, D. Skogen and S. Nilsen. 1981. Comparative analyses of the effect of triacontanol on photosynthesis, photorespiration and growth of tomato ($C_3$-plant) and maize ($C_4$-plant). *Planta* **152** : 44~49.

Hangarter, R. and S. K. Ries. 1978. Effect of triacontanol on plant cell culture *in vitro*. *Plant Physiol.* **61** : 855~857.

Haugstad, M., L.K. Ulsaker, A. Ruppel and S. Nilsen. 1983. The effect of triacontanol on growth, photosynthesis and photorespiration in *Chlamydomonas reinhardtii* and *Anacystis nidulans*. *Physiol. Plant.* **58** : 451~456.

Henry, E. W. and C. J. Gordon. 1980. The effect of triacontanol on peroxidase, IAA and plant growth in *Pisum sativum* var. 'Alaska' and 'Little Marvel' *J. Exp. Bot.* **31** : 1297~1303.

Hoagland, R. E. 1980. Effects of triacontanol on seed germination and early growth. *Bot. Gaz.* **141** : 53~55.

Lesniak, A. P. and S. K. Ries. 1982. The effect of triacontanol application on enzyme activity in corn and rice seedlings. Proc. 9th Ann. Plant Growth Regulator Soc. California. p. 79.

Lewak, S. and E. Skowronska. 1982. Interaction of triacontanol with gibberellin in control of lettuce seed germination. *Physiol. Plant.* **55** : 183~186.

Lowry, H., N. J. Rosenbrough, A. J. Farr and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193** : 265~275.

Maugh Ⅱ, T. H. 1981. New chemicals promise larger crops. *Science* **212** : 33~34.

Noggle, G. R. and G. J. Fritz. 1983. Introductory Plant Physiology. Prentice-Hall, New Jersey. pp. 445~479.

Ries, S. K., R. Houtz and V. Wert. 1983. The current status of triacontanol as a plant growth regulator. (Unpublished).

Ries, S. K., T. L. Richman and V. F. Wert. 1978. Growth and yield of crops treated with triacontanol. *J. Amer. Soc. Hort. Sci.* **103**(3) : 361~364.

Ries, S.K. and V. Wert. 1977. Growth responses of rice seedlings to triacontanol in light and dark. *Planta* **135** : 77~82.

Ries, S. K., V. Wert, C. C. Sweely and R. A. Leavitt. 1977. Triacontanol : a new naturally occurring plant growth regulator. *Science* **195** : 1339~1341.

Ries, S. K., V. Wert and R. Houtz. 1981. Rapid *in vivo* and *in vitro* effects of triacontanol. Proc. 8th Ann. Meeting of PGR Working Group. Florida. p. 137.

Singletary, G. W. and C. L. Foy. 1980. Effect of triacontanol on growth and mineral nutrition of corn and soybean seedlings. Proc. 7th Ann. Meeting of PGR Working Group. Texas. pp. 29~30.

Steffens, G. L. and J. F. Worley. 1980. Triacontanol : Evaluation in several plant assays. Proc. 7th Ann. Meeting of PGR Working Group. Texas. p. 137.

Tolbert, N. E., C. O. Clagett and R. H. Burris. 1949. Products of oxidation of glycolic acid and l-lactic acid by enzyme from tobacco leaves. *J. Biol. Chem.* **181** : 905~914.

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