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Effects of IAA on the Elongation and Cell Wall Glycosidase Activities in Excised Rape (*Brassica napus* L. cv. Yongdang) Hypocotyl Segments

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油菜 下胚軸 分節의 伸張과 細胞壁 分解酵素의 活性에 미치는 IAA의 效果

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ABSTRACT

Effects of IAA on the elongation and cell wall glycosidase activities were investigated in excised rape (*Brassica napus* L. cv. Yongdang) hypocotyl segments. IAA promoted the elongation of rape hypocotyl segments. In rape hypocotyls, the first 10-mm segments from the hook exhibited maximal elongation and the capacity of elongation was gradually decreased with increasing distance of each 10-mm from the hook. A good correlation has been obtained between the magnitude of endogenous growth and the activities of α, β-glucosidase and α, β-galactosidase. However, exogenous application of IAA did not seem to enhance the activities of these four glycosidases in rape hypocotyl segments. Treatment of the tissue with IAA resulted in acidification of the incubation medium. From these data, we can conclude that IAA seems to enhance elongation of the tissue segments, at least in part, by releasing hydrogen ion into the cell wall, some of which may participate in the cell wall extension process, but does not seem to trigger the activation of α, β-glucosidase and α, β-galactosidase.

INTRODUCTION

Hypocotyls of angiosperms respond to a number of naturally-occurring growth substances. Auxins promote the elongation of hypocotyls from mung beans, soybeans, and cucumbers among others (Kazama and Katsumi, 1976; Vanderhoef *et al.*, 1977; Prat, 1978).

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According to the acid secretion theory of auxin action (Cleland, 1983), auxins stimulate growth by causing cells to acidify their cell walls. The resulting pH drop causes cell wall loosening, allowing turgor-driven cell expansion to occur. This theory is supported by the observation that stem, coleoptile, and hypocotyl segments will extend rapidly in response to low pH (Rayle and Cleland, 1970; Yamamoto *et al.*, 1974; Vanderhoef *et al.*, 1977; Brummel and Hall, 1981). However, the mechanism by which protons stimulate cell elongation is yet unknown. One possibility is that protons may enhance the activities of cell wall-bound enzymes that hydrolyze wall polysaccharides (Johnson *et al.*, 1974; Murray and Bandurski, 1975; Goldberg, 1980), a condition which may lead to cell wall loosening. In contrast to this, several cell wall glycosidases did not correlate with elongation rate in bean hypocotyls (Pierrot *et al.*, 1982).

In this work, we tried to elucidate the possible relationships between growth reaction and activities of four major cell wall bound glycosidases in IAA-promoted growth using rape hypocotyl segments.

MATERIALS AND METHODS

Plant materials. Rape (*Brassica napus* L. cv. Yongdang) seeds were obtained from the Office of Rural Development, Mokpo branch. They were selected to have similar size, shape and color, and then sterilized in 1% sodium hypochlorite solution for 20 minutes and rinsed with running and distilled water. Rape seedlings were grown in the dark at 25±1°C for 5 days and cut into 10-mm segments from the hook in succession.

Growth measurements. Selected segments were starved for 1 hour in 1 mM potassium phosphate buffer at pH 6.2 by shaking (85 rev./min). Each 20 segments were placed in a petri dish containing 10 ml of incubation medium. Incubation medium was made of 1 mM potassium phosphate buffer with or without IAA at various concentrations. Buffer used in acid growth measurements was 1 mM citrate phosphate buffer at pH 6.2 or pH 5. Ten mM citrate phosphate buffer was used as acid solution of pH 4 or pH 3.2. Each petri dish containing 20 starved segments was placed on a shaker (30 rev./min) in the dark at 25±1°C for 24 hours. The length of segments was measured with micrometer under a dissecting microscope and the change in pH of the medium was determined by a pH meter.

Preparation of cell wall enzyme extract. Each incubated 20 segments were ground with 3 ml of potassium phosphate buffer (pH 6.2, 10 mM) in a mortar. The homogenate was centrifuged at 500 *g* for 10 minutes, and the resulting pellet was resuspended with 3 ml of the same buffer solution. This resupended solution and the supernatant of 500 *g* were used as crude enzyme extracts. The supernatant was centrifuged again at 15,000 *g* for 15 min and the resulting supernatant was used as another enzyme extracts.

Enzyme assay. Enzyme assay was accomplished by modifying the method of Johnson

et al.(1974). Each 1 ml of substrate and enzyme extracts were mixed and incubated at 30° C for 30 min. Each substrate was 2.5 mM α, β-p-nitrophenylglucopyranoside or α, β-p-nitrophenylgalactopyranoside dissolved in 10 mM potassium phosphate buffer (pH 6.2). These reactions were terminated by adding 2 ml of 200 mM Na_2CO_3 solution and absorbance was read at 400 nm with a Beckmann Model 24 Spectrophotometer.

RESULTS AND DISCUSSION

Growth response to IAA solution. In general, it is known that auxins promote the growth of excised elongating parts in dicotyledons (Hager *et al.*, 1971; Prat, 1978; Carpita and Tarmann, 1982). The same results were shown in rape hypocotyls. The optimum concentration of IAA was between 10^{-5} M and 10^{-4} M. In addition, buffer was more effective than distilled water as growth medium (Fig. 1).

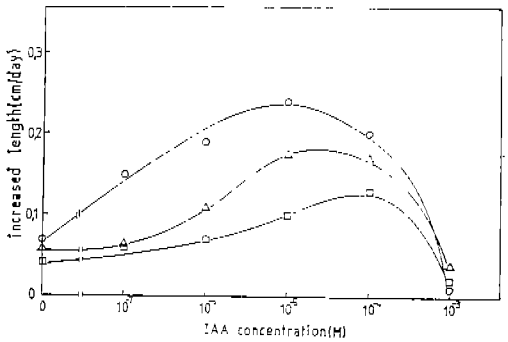

Fig. 1. Elongation of rape hypocotyl segments in solution of various IAA concentrations. ○—○; 1 mM potassium phosphate buffer with 50 mM sucrose, △—△; 1 mM potassium phosphate buffer(pH 6.2), □—□; distilled water.

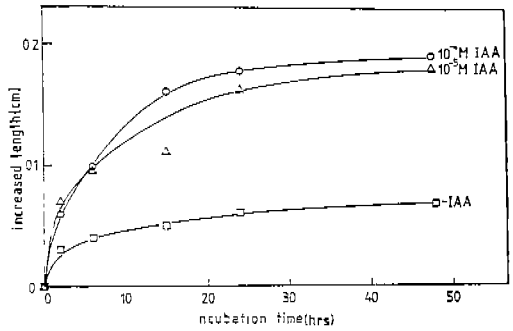

Fig. 2. Time course of elongation of rape hypocotyl segments in buffer solution with 10^{-4} M or 10^{-5} M, or without IAA.

At the optimum concentration hypocotyl segments elongated mostly within 6 hours, after which elongation rate was on the gradual decrease. As the segments were still elongating considerably until 24 hours of incubation, we used a 24 hour period as incubation time (Fig. 2).

When sucrose was added to the medium, the elongation was increased about 5% more than those without sucrose. This is because sucrose can be used as respiration materials and supply energies required for elongation. Moreover, it can prevent the decrease of turgor pressure by regulating the osmotic pressure of cells (Bonner, 1961). In our experiments, 50 mM sucrose was used as optimum since the effect of sucrose was similar where its concentration was between 20 mM and 80 mM (Fig. 3).

Ten-mm segments were excised from 4 different regions along the hypocotyl in

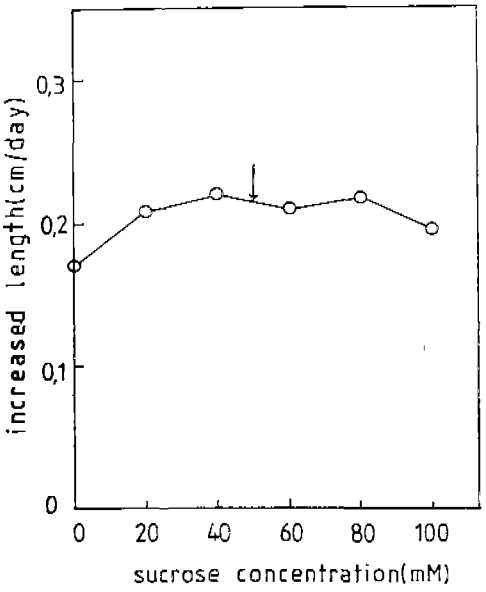

Fig. 3. Effect of sucrose on the IAA-induced elongation of rape hypocotyl segments.

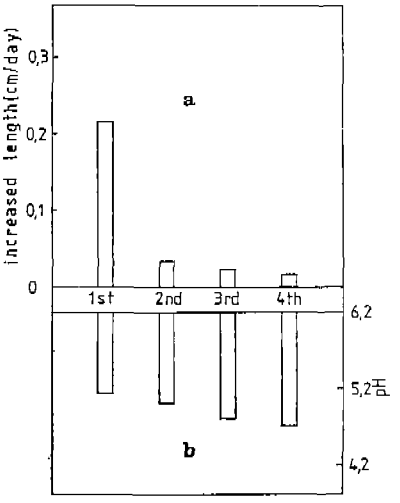

Fig. 4. Growth responses in different parts of rape hypocotyls. (a) Elongation in 10^{-5} M IAA solution. (b) Change in pH of the medium.

downward sequence starting from the base of the hook, and they were treated with IAA solution to investigate the effect of tissue age on the elongation. The elongation was much greater in the first segments than in other segments. On the contrary, pH of the medium dropped more in segments farther from the hook (Fig. 4). This results showed similar tendency to Goldberg's experiment (1980) with mung beans though there were some differences in the method of sectioning.

Growth response to acid solution. Recently many researches were made for the rapid cell elongation by acid solution, and this provided some good informations for studying the effects of auxin on the cell wall extension. Based on these facts, the responses of rape hypocotyl segments to various buffer solutions were investigated. In the buffer solution at pH 5, hypocotyl segments elongated most greatly, which exhibited similar trends from the view-point that pH of incubation medium with IAA was dropped near to 5 from 6.2 with the elongation of segments. But the amount of elongation was only 75% compared with that in the medium with IAA, suggesting the involvement of other factors besides acidification. In the buffer solution of pH 4 or pH 3.2, hypocotyl segments were less elongated than control, 1 mM potassium phosphate buffer of pH 6.2. Since they, with excessive protons, had tissues damaged by the irreversible breaking of wall linkage, there was less elongation than control. These results substanciate that cell wall loosening occurs by the reversible cleavage of cell wall materials which are resistant to alkali, but liable to acid. The optimum pH 5, in rape

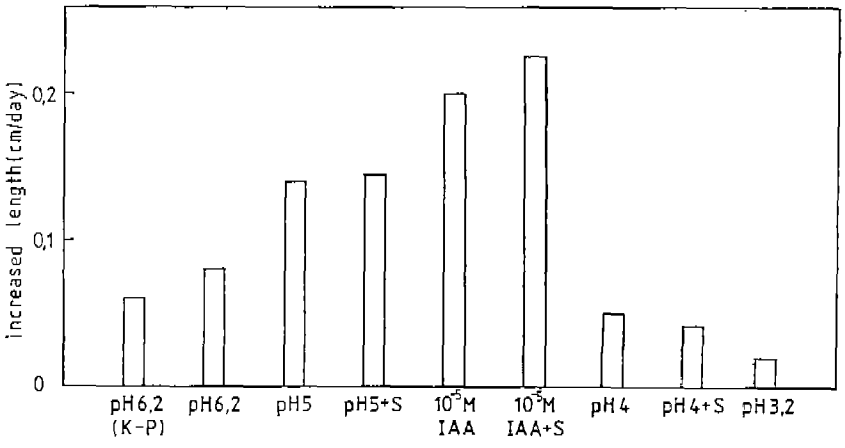

Fig. 5. Growth responses of the first 10-mm rape hypocotyl segments in various buffer solutions (S=50 mM sucrose, K–P=1mM potassium phosphate buffer). Other buffers are citrate phosphate buffers.

hypocotyls was higher than that of soybean hypocotyls and *Avena* coleoptiles (Rayle and Cleland, 1970; Vanderhoef *et al.*, 1977). While there were considerable effects of sucrose in IAA solution, the addition of sucrose to buffer solution exhibited no positive effects on elongation (Fig. 5).

At pH 6.2, citrate phosphate buffer was slightly more effective than potassium phosphate buffer (Fig. 5). By these experimental results, the acidification of medium, due to the IAA-induced proton excretion, seemed a major factor of wall loosening, but other factors might also be involved in the elongation of rape hypocotyl segments.

The activities of cell wall glycosidases with regard to the tissue age and the effects of IAA on them. Cell walls of higher plants contain several wall degrading enzymes including glycosidases (Klis *et al.*, 1974; Pierrot and Wieling, 1977). In pea seedlings, glycosidase activities were interrelated with cell wall extension (Murray and Bandurski, 1975), and the elongation of Avena coleoptiles and etiolated pea stems was well correlated with β-galactosidase activity (Johnson *et al.*, 1974; Tanimoto and Igari, 1976). Our results showed somewhat different aspects. When we used the supernatants, after centrifugations at 500 g or 15,000 g, as enzyme extracts, both β-glucosidase and β-galactosidase exhibited similar activities irrespective of excised parts. Moreover, the activities of both enzymes were not increased by IAA-treatment (Table 1 and 2).

In the case of α-glucosidase and α-galactosidase, the activities of both enzymes in the first segments were higher by 50% than those of other segments, but still no activation of these enzymes by IAA-treatment was shown (Table 3 and 4).

When we used resuspended solution of the pellets as enzyme extracts, the results obtained were different. The activities of all above-mentioned enzymes in the first segments were much higher than those in other segments. But IAA did not enhance

Table 1. Relative distribution of β-glucosidase activities in rape hypocotyl segments

Treatment			Relative enzyme activity(△OD/h)	
			Supernatant	Pellet
Without	IAA	1 st segment	0. 214	0. 648
		2 nd segment	0. 218	0. 328
		3 rd segment	0. 210	0. 312
10^{-5} M	IAA	1 st segment	0. 192	0. 732
		2 nd segment	0. 222	0. 364
		3 rd segment	0. 184	0. 288

Table 2. Relative distribution of β-galactosidase activities in rape hypocotyl segments

Treatment			Relative enzyme activity(△OD/h)	
			Supernatant	Pellet
Without	IAA	1 st segment	0. 294	1. 488
		2 nd segment	0. 288	0. 752
		3 rd segment	0. 294	0. 746
10^{-5} M	IAA	1 st segment	0. 222	1. 504
		2 nd segment	0. 224	0. 748
		3 rd segment	0. 256	0. 706

Table 3. Relative distribution of α-glucosidase activities in rape hypocotyl segments

Treatment			Relative enzyme activity(△OD/h)	
			Supernatant	Pellet
Without	IAA	1 st segment	0. 146	0. 057
		2 nd segment	0. 104	0. 030
		3 rd segment	0. 106	0. 032
10^{-5} M	IAA	1 st segment	0. 142	0. 053
		2 nd segment	0. 104	0. 033
		3 rd segment	0. 114	0. 033

the activation of α-glucosidase and β-galactosidase at all, while the activities of α-galactosidase and β-glucosidase were increased 10% by IAA-treatment (Table 1, 2, 3, and 4). Consequently, while our results suggest that the wall-bound glycosidases are involved in the extension growth (Nevins, 1970; Murray and Bandurski, 1975; Goldberg. 1980), they contradict previous results of Johnson and others that the wall glycosidase activities are much increased by auxin-treatment (Johnson *et al.*, 1974; Tanimoto and

Table 4. Relative distribution of α-galactosidase activities in rape hypocotyl segments

Treatment			Relative enzyme activity(△OD/h)	
			Supernatant	Pellet
Without	IAA	1st segment	0.317	0.446
		2nd segment	0.219	0.301
		3rd segment	0.206	0.309
10^{-5} M	IAA	1st segment	0.314	0.509
		2nd segment	0.213	0.307
		3rd segment	0.230	0.318

Igari. 1976). Our results, however, coincide with previous evidence of Evans against the involvement of galactosidase or glucosidase in auxin- or acid- stimulated growth (Evans, 1974). Thus we come to a conclusion from our results that the elongation of rape hypocotyl segments occurs primarily by the acidification of cell wall and activation of these four enzymes, at least, is not involved in IAA-induced elongation. To support our conclusion, more efforts including the use of inhibitors of these glycosidases shall be made. But the involvement of wall enzymes other than these four enzymes remains to be elucidated.

摘 要

油菜 下胚軸의 分節을 재료로 하여 IAA가 이들의 伸張과 細胞壁 分解酵素의 活性에 미치는 影響을 조사하였다. IAA는 下胚軸 分節의 伸張을 促進하였으며 절단부위에서의 처음 10-mm 分節이 最大의 伸張을 나타냈으며 절단부위로부터 멀어질수록 分節의 伸張은 적게 일어났다. 調査된 네가지 酵素, α, β-glucosidase와 α, β-galactosidase의 活性은 分節의 伸張과 잘 一致하여 첫번째 分節에서의 活性이 두세번째 分節에서의 活性보다 현저히 높았다. 그러나 IAA의 처리에 의해 이 酵素들의 活性이 增加되지는 않았다. 따라서 이 酵素들은 下胚軸의 伸張에는 重要한 作用을 하지만 IAA에 의한 分節의 伸張에는 關與치 않는 것으로 보인다.

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