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Sensitivity Changes of Auxin Transport System in Maize Coleoptile Segments

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옥수수 (*Zea mays* L.) 자엽초 조직 절편에서 옥신 이동계의 감수성 증가

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ABSTRACT

In maize coleoptile segments where auxin transport capacity decreases with time following excision, susceptability of the tissue to transport inhibitors such as N-1-naphthylphthalamic acid (NPA), 3,4,5-triiodobenzoic acid (TIBA) or high concentrations of IAA was found to be rather increased. A time-dependent increase in the sensitivity to NPA can be postulated since the dose-response curve for NPA was shifted in the 'aged' tissue to the left (i.e. lower concentration). Preincubation of the tissue at a low temperature abolished the time-dependent sensitivity change, suggesting that cellular metabolism could be involved. The NPA-sensitive state was also brought about by calcium depletion of the tissue, which can be partially reversed by addition of calcium. Presence of exogenous IAA in the preincubation medium kept the auxin transport system from decay, implicating auxin as an endogenous controlling factor. Results of our experiments indicate a reversible, time-dependent changes of auxin transport system in which transport capacity and sensitivity to NPA are tightly coupled. Changes in the sensitivity to NPA were also seen in auxin action as well.

INTRODUCTION

Auxin transport system has been well charaterized by both *in vivo* and *in vitro* studies (Goldsmith, 1977; Hertel, 1983). The saturability, specificity and polarity found in *in vivo* auxin transport (Goldsmith, 1977) can be explained by operation of two carrier systems in the plasma membrane at a cellular level (Hertel, 1986). However mechanism for regulation of auxin transport remain to be elucidated although many factors, both endogenous and environmental, affecting auxin transport are known (Goldsmith, 1977). Modulation of specific carrier should be involved in the transport regulation. The putative auxin efflux carrier which constitue a rate-limitting element in the transport system is thought to be a site of regulation. A very complex model of efflux carrier with at least three different binding domains envisions a possibility of multiple regulation (Rubery, 1987). Evidences that the NPA receptor, i.e. the auxin efflux carrier is a functional entity for physiologcal activity of polar auxin transport are accumulating (Kang, 1986; Suttle, 1988, 1991; Morris and Johnson, 1990). Both positive and negative regulation could be involved in those cases. Physiological relevance of the NPA receptor could be further supported be possible occurence of endogenous ligands (Rubery and Jacobs, 1991).

Polar auxin transport is an integral part of a tissue responsive to IAA (Goldsmith, 1977). Phytotropins, polar

transport inhibitors which specifically bind to the auxin efflux carrier (Katekar and Giessler, 1980), interfere with auxin-regulated responses (Lee *et al.*, 1984; Vesper and Kuss, 1990; Kang *et al.*, 1992). In corn coleoptile segments, homeostatic control of auxin level by a mechanism involving autoregulation of auxin transport system is suggested (Yoon and Kang, 1992). Evidence presented here indicated a reversible change in the sensitivity of auxin transport system to NPA. Its implications on the regulation of auxin transport and action will be discussed.

MATERIALS AND METHOD

Plant material. Presoaked corn (*Zea mays* L. var. Merit) seeds were planted on wet paper towels in plastic trays and placed vertically in dark at 28℃ with 100% relative humidity. Three mm subapical segments were excised from 4-day-old seedlings with a double blade cutter with primary leaves removed and used for transport experiments unless indicated otherwise. For experiments measuring ethylene production and cell elongation, 10 mm subapical segments were used instead.

Radiochemicals. (5-^{3}H)-IAA (28 Ci/mmole) was purchased from CEA (Gif-sur-Vvette, France).

Auxin transport test. Agar blocks, 3 mm×3 mm×1 mm, (1.5% agar buffered with 50 mM sodium phosphate at pH 6.8) containing 38 nM ^{3}H-IAA were used as donor. Receiver blocks contained plain buffered agar and test chemicals where indicated. Individual segments were placed vertically, the basal end down, between donor and receiver blocks. At the end of the transport period, radioactivity in the receiver blocks was counted with a liquid scintiallation spectrometer.

Ethylene production. Ethylene production was measured according to Kang *et al.* (1971). Ten subapical coleoptile segments were incubated with 3 mL of medium (1% sucrose buffered with 10 mM sodium phosphate at pH 6.8) in a 25 mL Erlenmyer flask sealed with a silicon rubber cap. After an 18 h incubation in the dark with gentle shaking, 1 mL of air samples were withdrawn from the flask with a hypodermic syringe and ethylene content was measured with a gas chromatograph (Simadzu, GC-3BF, flame inonization detector, alumina column).

Elongation test. Ten subapical coleoptile segments were incubated in a medium (1% sucrose buffered with 10 mM sodium phosphate at pH 6.8) with test chemicals where indicated. After an 18 h incubation in the dark with gentle shaking, the length of each semgents was measured under the dissecting microscope (×10).

Thin-layer radiochromatography. Ten subapical coleoptile segments were incubated for 6 h with 2 mL of medium (buffered with 10 mM sodium phosphate at pH 6.8) containing 10 nM ^{3}H-IAA. The segments were washed twice with 10 mL of cold buffer, blotted to filter paper and radioactivity was cluted with 95% ethanol for 24 h. After concentrating the ethanol fraction with a vacuum evaporator, 0.1 mL of the solution was spotted on the TLC plate (Merck, Silicagel 60) and developed for 2 h with developing solution as follows: chloroform/ethylacetate/formic acid (5/4/1). Free IAA was identified under UV light. The spot was removed by scraping and transferred into a scintilation vial for counting radioactivity.

RESULTS

Sensitivity change of auxin transport system. Auxin transport capacity in corn coleoptile segments rapidly decreased with time following excision from the intact seedling (Yoon and Kang, 1992). Susceptability of the tissue to transport inhibitors, however, was found to be rather increased under these conditions.

Fig. 1 illustrates that transport inhibition by unlabelled IAA was more pronounced in tissue preincubated for 3 h

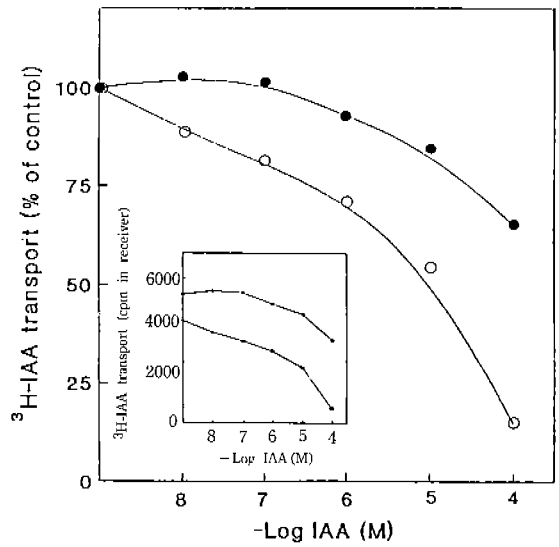

Fig. 1. Inhibition of ^{3}H-IAA transport by various concentrations of cold IAA in 5 mm coleoptile segments freshly excised (●) or preincubated for 3 h (○). The data were replotted as percent of the control (minus cold IAA) from the figure shown in the inset.

following excision compared with freshly excised tissue. Polar transport of labelled IAA dissapears at high concentrations of cold auxin because unlabelled IAA saturates the transport sites (Goldsmith, 1982). Since both uptake and efflux carriers could be saturated, unlabelled IAA may have dual effects, i.e. a stimulation or inhibition, depending on its concentration, of net uptake of labelled auxin (Edwards and Goldsmith, 1980). In our experiments, however, because unlabelled IAA was added to the receiver block attached to the basal cut surface of the tissue, it is likely to act at the efflux carrier. Increased sensitivity of the efflux carrier to IAA in 'aged' tissue was reported by Vesper (1989).

Phytotropins such as 3,4,5-TIBA or NPA were also found to inhibit polar transport more effectively in 'aged' tissues. As a weak inhibitor, 3,4,5-TIBA has only partial inhibitory effect on auxin transport at saturating concentrations (Depta *et al.*, 1983). However, more than 60% inhibition of the transport by 3,4,5-TIBA at high concentrations was observed when the tissues were preincubated for 3 h following excision (Fig. 2).

Data illustrated in Fig. 3 indicate that the inhibitory activity of NPA was significantly increased following excision, especially within the concentrations ranging from

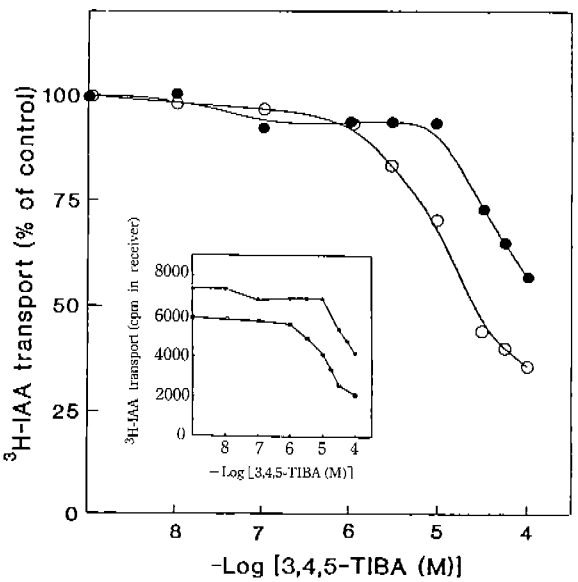

Fig. 2. Inhibition of ^{3}H-IAA transport by various concentrations of 2,3,4-TIBA in 5 mm coleoptile segments freshly excised (●) or preincubated for 3 h (○). The data were replotted as percent of the control (minus TIBA) from the figure shown in the inset.

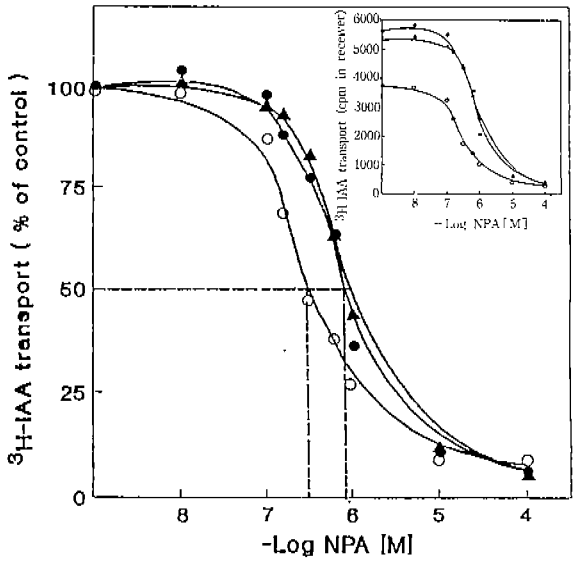

Fig. 3. Effect of IAA pretreatment on subsequent NPA inhibition of auxin transport. Subapical, 10 mm coleoptile segments were preincubated for 3 h in the presence (▲) or absence (○) of 3 μM IAA and then cut into 5 mm segments for subsequent transport test. Freshly excised coleoptile segments were also used (●). The data were replotted as percent of the control (minus NPA) from the figure shown in the inset. The intercepts on X axis indicate NPA concentrations for a half maximal inhibition (I_{50}).

0.1 to 1 μM. The discrepancy between this result and that of Vesper and Hale (1991) could probably be due to the narrow concentration ranges in which the changes could be detected. The shift of dose-response curve to the left for NPA in tissues preincubated for 3 h following excision indicate that the I_{50} value for NPA was decreased by about 3 fold when compared with that of freshly excised tissue (Fig. 3). The decreased I_{50} value could be interpreted as an increased sensitivity of the tissue to NPA (Firn, 1986). It is also indicated in Fig. 3 that presence of 3 μM IAA in the preincubation medium completely prevent both time-dependent transport dacay and increased sensitivity to NPA. It is suggested that they are closely related processes and are influenced by the same endogenous factor, i.e. auxin.

Temperature dependency of, and calcium involvement in, the sensitivity shift. When the coleoptile segments were preincubated at 4℃, sensitivity of the tissue to NPA was found to remain unchanged (Fig. 4). The temperature dependency of the sensitivity change implies metabolic

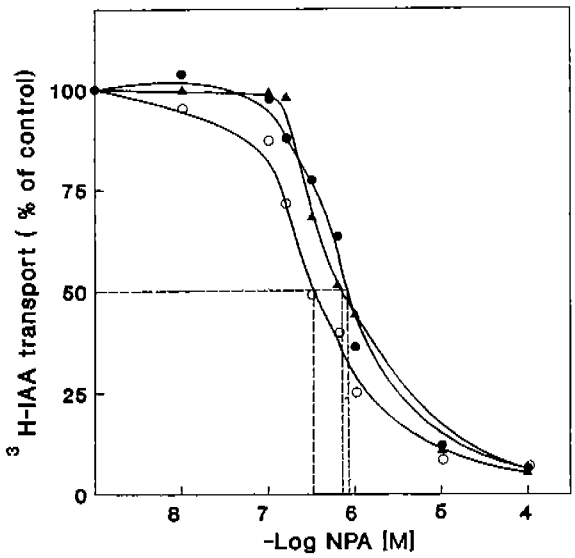

Fig. 4. Comparision of NPA inhibition curves of auxin transport. Subapical, 10 mm coleoptile segments were preincubated for 3 h at 4℃ (▲) or 28℃ (○) of and then cut into 5 mm segments for subsequent transport test. Freshly excised coleoptile segments were also used (●). Data are plotted as percent of the control (minus NPA).

Table 1. ^{3}H-IAA transport capacity and transport inhibition by NPA in coleoptile segments with low (LC) or high (HC) calcium level

Tissue type[a]	^{3}H-IAA transport (cpm)[b]		
	(−) NPA	(+) NPA	Percent inhibition
LC	9,429	5,470	42
HC	12,619	8,925	29

[a]Seeds were sown and seedlings grown for 4 days with 0.1 mM EDTA (LC) or 10 mM $CaCl_2$ (HC). [b]Auxin transport test was carried out at 28℃ for 2 h in the presence or absence of 0.3 μM NPA in the receivers. Representative data from four experiments with triplicates.

nature of these processes. Moreover, NPA action appears to depend on the tissue calcium level (Table 1). Using coleoptile seedlings grown with 0.1 mM EDTA or 10 mM $CaCl_2$, calcium-related changes in auxin transport system was investigated. The growth rate of primary root was slightly inhibited in EDTA-treated tissue (data not shown). We used 0.3 μM NPA to check the sensitivity because this concentration represents a range of NPA dose where a small change in the inhibitor level results

Table 2. Effect of EGTA pretreatment on subsequent NPA inhibition of auxin transport and its partial recovery by calcium

Pre-pretreatment[a] (First 4 h)	Pretreatment[b] (Another 2 h)	^{3}H-IAA transport (cpm)[c]		
		(−) NPA	(+) NPA	Percent inhibition
None	None	8,829	4,742	46
EGTA	EGTA	6,745	2,820	58
EGTA	$CaCl_2$	8,268	4,632	43

[a,b]Subapical coleoptile segments were preincubated for 4 h with or without 0.3 mM EGTA. Half of the segments were transferred to a medium containing 0.5 mM $CaCl_2$ and incubated for another 2 h. [c]Auxin transport test was carried out at 28℃ for 2 h in the presence or absence of 0.3 μM NPA in the receivers. Representative data from four experiments with triplicates.

in the most pronounced effect on auxin transport (see Fig. 3). Reduced transport capacity with increased NPA activity was found in coleoptile tissue with low calcium level (LC) compared with auxin transport system in high calcum (HC) tissue (Table 1). This may be relevant to what is characterized for the auxin transport system in 'aged' tissue (Fig. 3). The calcium-dependent change as shown in Table 1 did not result from possible damage of the tissue by calcium difficiency because shor-term calcium depletion by EGTA also gave similar results (Table 2). Data in Table 2 also indicated that changes in both transport capacity and NPA activity induced by EGTA could be partially reversed by application of exogenous calcium.

Auxin action and sensitivity to NPA. We found that NPA applied to coleoptile segments led to a strong promotion of auxin action. NPA alone had neglegible effect on the basal level of ethylene production and growth (data not shown), indicating that the NPA action was auxin dependent. This was possibly due to an elevated intracellular auxin level in the tissue (Vesper, 1991). Data in Table 3 indicating a two-fold increase in the amount of the radiolabelled free IAA level by NPA application was in accordance with the idea. Interestingly, it was noticed that different states of sensitivity to NPA were also seen in auxin action as well. Does-response curves for NPA-stimulation of growth at a low IAA concentration (0.3 μM) indicated an increased sensitivity to NPA following excision. NPA strongly stimulated IAA-induced cell elongation in 3 h aged tissue while it had a little effect in freshly excised tissues (Fig. 5). Sensitive state to NPA

Table 3. Free IAA levels in coleptile tissues incubated with labelled auxin in the presence or absence of NPA

Treatment	Free IAA (cpm/g·fr wt)[a]
(−) NPA	38,624
(+) NPA	73,329

[a]Subapical coleoptile segments were incubated for 6 h with 10 nM ^{3}H-IAA plus and minus 10 μM NPA. Radiolabelled free IAA were analyzed by TLC as described in Materials and Methods. Representative data from three experiments with duplicates.

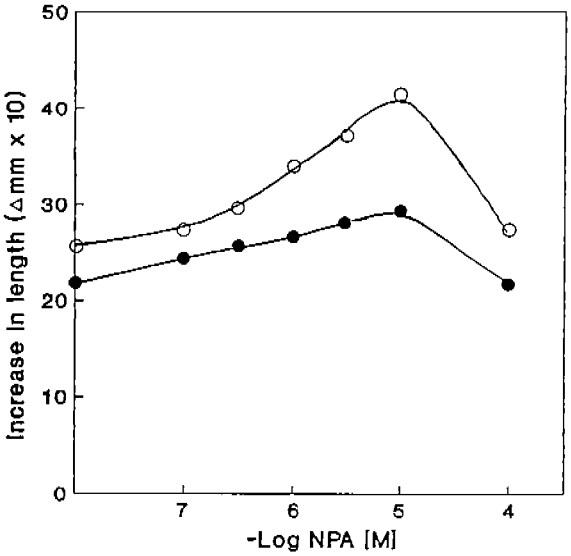

Fig. 5. Effect of NPA on IAA-induced cell elongation in freshly excised (●) or 3 h aged tissue (○). The segments were incubated with IAA (0.3 μM) for 18 h in the presence of NPA at indicated concentrations. Values are average of three experiments with duplicates.

in 'aged' tissue was also seen in NPA effect on IAA-induced ethylene production (Fig. 6A). However, the time-dependent change in sensitivity to NPA in the auxin response system was not seen at a high IAA concentration (10 μM, Fig. 6B). Data in Figs. 5 and 6 also indicate that the sensitivity of the auxin response system to IAA itself (the values at zero NPA) was also increased with time following excision.

DISCUSSION

Our present work deals with time-dependent changes in the sensitivity of auxin transport system. Evidence is presented here to indicate that polar transport could be a factor limiting auxin action. The NPA-stimulation of auxin action and tissues having the differential sensitivity to NPA in both auxin transport and action (Figs. 3 and 5) suggest a regulatory role of auxin efflux carriers on auxin action especially under conditions of limitted auxin supply (Fig. 6), which is likely to represent the physiological state of plant tissues. Relationship between elevated intracellular level of auxin by NPA and increased growth rate was well interpreted by Vesper and Kuss (1990). These appears to be seemingly two different but interrelated aspects of the sensitivity change of the transport system. With regard to the concentration dependency of NPA inhibition of auxin transport, aged tissues tended to have a lowered I_{50} value (i.e. incsreased sensitivity to NPA). The same aged tissue, on the other hand, responded to NPA in auxin-induced ethylene production with a greater magnitude compared with freshly excised tissues (Fig. 6). Since the rate of ethylene production is known to be directly related to the intracellular auxin levvel (Kang *et al.*, 1971), the increased magnitude of NPA effect on ethylene production in aged tissue could be explained by the following rationale. When tissues become more sensitive to NPA, the promotive effect of the phytotropin at a given concentration on net uptake of auxin in the sensitive tissue should have been exerted to a greater extent. Since intracellular auxin level represents a net result of relative activities at both uptake and exit sites, and since NPA is known to block the efflux process almost completely without affecting the uptake mechanism, it also follows that relative effect of NPA on the net uptake of auxin greately depends on the amount of auxin in the transport system. These are verified by the findings presented in the present work (see Fig. 6). However, when auxin efflux was completely inhibited by saturating concentrations of NPA which abolish the difference in net uptake between aged and freshed tissue (Yoon, 1991), ethylene production from the aged tissue in response to exogenous IAA was further enhanced compared to the fresh tissue. It is possible that the tissue might have undergone sensitivity changes of some factor other than auxin transport system following excision as indicated by Vesper and Evans (1979).

Mechanisms underlying the sensitivity shift of auxin transport system to its ligands are unknown. Allosteric, modulation of the carrier complex is likely to occur. However models of auxin efflux carrier depict a complex mode of regulation involving three different, but interac-

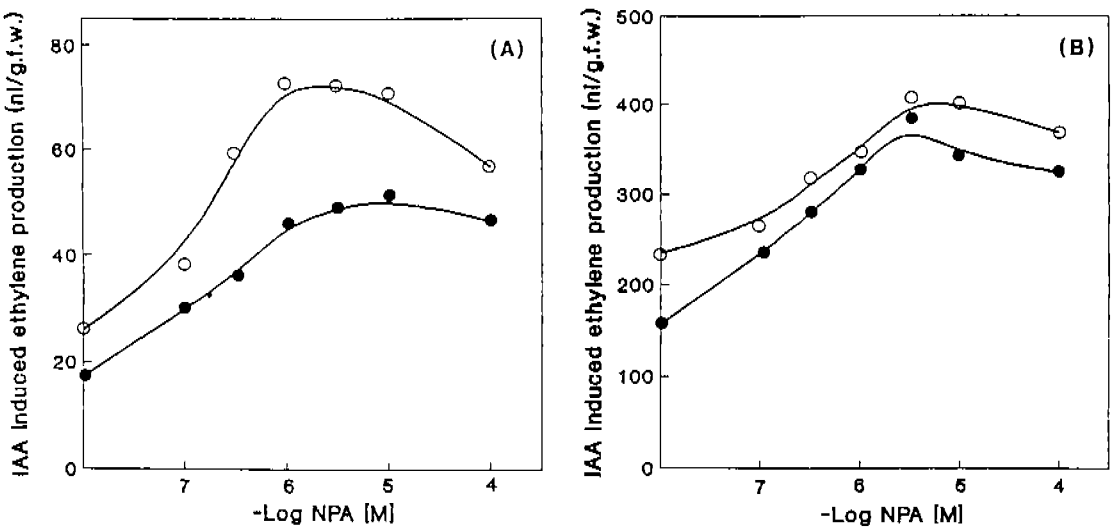

Fig. 6. Effect of NPA on IAA-induced ethylene production in freshly excised (●) or 3 h aged tissue (○). The segments were incubated for 18 h with IAA at two different concentrations, i.e. 0.3 μM (A) and 10 μM (B), respectively. Values are average of seven experiments with duplicates.

ting binding sites (Depta *et al.*, 1983; Rubery, 1985). Since the number of high affinity NPA binding site was found to be rather decreased without changing its affinity in "aged' tissue where sensitivity to NPA increased (Yoon and Kang, 1992), it seems probable that binding site having intermediate affinity for both NPA and TIBA (Depta *et al.*, 1983) would be responsible for the changed sensitivity. Function of the latter site in auxin transport inhibition was implicated by Michalke *et al.* (1992).

Endogenous auxin should play an important role in the sensitivity change of the tissue to NPA since presence of exogenous IAA in the preincubation medium completely prevent the change (Fig. 3). Rapid decrease in free IAA content in coleoptile tissue following excision (Yoon and Kang, 1992) support the idea. The nature of the sensitivity change is largely unknown at the present. What is apparent from the results obtained in this work is that it is likely to involve cellular metabolic progress (see Fig. 4). Calcium stimulated protein phosphorylation may be involved in the reversible conformational change of efflux carrer. The result indicating that both transport capacity and sensitivity to NPA were affected by tissue calcium level (Tables 1 and 2) is in line with the idea. Control by phosphorylation has been implicated for the fusicoccin receptor (Adduci *et al.*, 1984). Auxin depletion in the plant tissue could decrease the intracellular calcium level via stimulus-response coupling (Poovaiah and Reddy, 1990) or auxin/calcium antiport postulated by Hertel (1983). Otherwise, if auxin is assumed to suppress endogenous level of the natural ligands for the NPA receptor by a mechanism involved positive cooperativity, auxin depletion could result in increased sensitivity of the system to NPA. Certain natural flavonoids are suggested as an NPA agonist in plants (Rubery and Jacobs, 1990).

적 요

옥수수 (*Zea mays* L.) 자엽초 조직 절편의 옥신 이동능이 조직 절단 후 시간에 따라 감소하는 특징을 보인 반면, NPA, TIBA, 고농도 IAA 등 옥신 이동 억제제에 대한 반응은 오히려 더 증가한다는 것을 알았다. NPA 농도에 따른 옥신 이동 억제 곡선을 비교한 결과는 절단 후 3시간 된 조직에서 NPA가 옥신 이동을 50% 억제하는 농도(KI_{50})가 절단직후의 조직에서보다 약 3배 낮아졌음을 보여주며, 이는 NPA에 대한 감수성의 증가를 시사한다. 한편 조직 절편을 3 μM의 옥신을 첨가한 용액에서 배양함으로써 조직절단 후 시간에 따른 NPA에 대한 감수성의 증가가 억제되었는데, 이는 내재적 옥신의 양적 감소가 이런 현상의 원인이라는 것을 시사한다. 또한 조직 절편을 저온에서 처리하면 마찬가지로 NPA에 대한 감수성의 변화가 억제되었으므로 이 과정이 세포내 물질대사에 의존적임을 보여준다. 조직내 칼슘농도를 변화시킨 실험 결과는 가역적인 감수성 변화를 시사한다. 본 실험 결과에서 옥신 이동능의

감소와 NPA에 대한 감수성의 증가가 서로 연관되어 나타남을 알 수 있었다. 이런 현상은 옥신의 efflux를 조절함으로써 세포내 옥신의 양을 일정하게 유지시키는 기작으로 추측되며 특히 옥신 공급이 제한된 조건에서 옥신에 대한 반응을 제한하는 중요 요인으로 간주된다. 이는 NPA에 대한 감수성이 다른 두 조직에서 저농도 옥신의 작용이 현저한 차이를 보이는 사실로써 확인된다.

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