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Isolation and identification of lateral bud growth inhibitor, indole-3-aldehyde, involved in apical dominance of pea seedlings

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Abstract

A lateral bud growth inhibitor was isolated from etiolated pea seedlings and identified as indole-3-aldehyde. The indole-3-aldehyde content was significantly higher in the diffusates from explants with apical bud and indole-3-acetic acid treated decapitated explants, in which apical dominance is maintained, than in those from decapitated ones releasing apical dominance. When the indole-3-aldehyde was applied to the cut surface of etiolated decapitated plants or directly to the lateral buds, it inhibited outgrowth of the latter. These results suggest that indole-3-aldehyde plays an important role as a lateral bud growth inhibitor in apical dominance of pea seedlings.

Keywords: *Pisum sativum*; Leguminosae; Apical dominance; Lateral bud growth inhibitor; Indole-3-aldehyde; Indole-3-acetic acid (IAA)

1. Introduction

When the apical bud of a plant seedling is actively growing, the outgrowth of lateral buds is suppressed. This phenomenon is called "apical dominance". Apical dominance is released by excision of the apical bud or by application of auxin transport inhibitors, such as 1-naphthylphthalamic acid (NPA) and 2,3,4-triiodobenzoic acid (TIBA) to the apical bud or the internode of seedlings (Michell et al., 1965; Parigrahi and Audus, 1966; Nakajima et al., 2001). Conversely, apical dominance is maintained by the application of auxin to the cut surface of decapitated seedlings (Thimann and Skoog, 1934). It therefore appears that auxin is synthesized in the apical bud and translocated basipetally by polar transport from the apical bud towards the lateral buds, resulting in the suppression of outgrowth of lateral buds. However, direct application of auxin to the lateral buds of decapitated seedlings does not maintain apical dominance (Phillips, 1975). Moreover, we recently demonstrated that application of natural auxin-inhibiting substances, raphanusanin B {(3*R**,6*S**)-3-[methoxy(methylthio)-methyl]-2-pyrrolidinethione} and 6-methoxy-2-benzoxazolinone (MBOA) not only to the apical bud or the internode but also directly to the lateral buds of pea seedlings released apical dominance in either intact or IAA-treated, decapitated seedlings (Nakajima et al., 2001). These results suggest that antagonist(s) of the auxin-inhibiting substances, raphanusanin B or MBOA are synthesized during auxin translocation from the apical bud towards the lateral buds, exerting apical dominance. In this paper, we aimed to isolate and identify lateral bud growth inhibitor(s), which transport from the apical bud towards the lateral buds, from pea seedlings.

2. Results and discussion

Lateral bud growth inhibitor was isolated from etiolated pea seedlings. The mass spectrum of isolated lateral bud growth inhibitor gave *m*/*z* (relative intensity) 146 ($[M^+H]^+$, 100) and 118 (75). The ^{1}H NMR spectrum (Table 1) showed signals for five methine protons (δ 7.28, 7.33, 7.52, 8.17, 8.20) and one proton of CHO (δ 9.92). These spectrometric data exactly coincide with those of indole-3-aldehyde (Fig. 1). Although indole-3-

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aldehyde was identified in *Pinus sylvestris* (Ernstsen and Sandberg, 1986), its role as a growth inhibitor has not been reported. This is the first time that indole-3-aldehyde has been implicated as a lateral bud growth inhibitor.

The content of indole-3-aldehyde in the diffusates from explants with apical bud or IAA-treated decapitated explants, in which apical dominance is maintained, was 1.8 or 1.68 times larger than that in the diffusates of decapitated ones releasing apical dominance, respectively (Table 2). In addition, the content of IAA in the diffusates from decapitated explants was smaller than that in the diffusates from apical ones in a similar respect to indole-3-aldehyde. It is thought that the high content of IAA in the diffusates from IAAH treated decapitated explants is due to treatment with IAA. These results indicate that the changes in indole-3-aldehyde and IAA levels of diffusates from explants correlates with apical dominance.

Isolated and synthetic indole-3-aldehyde or their relative substances was applied to the cut surface of decapitated plants or directly to the lateral buds and their effects on lateral bud growth was tested (Table 3). Isolated indole-3-aldehyde completely suppressed the outgrowth of lateral buds in both assays and its activity was identical to activity of synthetic indole-3-aldehyde. IAA applied to the cut surface of decapitated plants showed inhibitory activity on lateral bud growth, whereas IAA applied directly to the lateral buds showed no activity. 4-Chloroindole-3-acetic acid (4-Cl-IAA), which has been isolated from *Pisum sativum* (Magnus et al., 1997), suppressed growth to a limited extent in both assays. Indole-3-pyruvic acid and tryptophan applied to the cut surface of decapitated plants showed some inhibitory activity on lateral bud growth, but when applied directly to the lateral buds they showed no activity. These results suggest that the regulatory substance of elongation of lateral buds is not IAA, indole-3-pyruvic acid and tryptophan, but indole-3-aldehyde.

All of the results suggest that indole-3-aldehyde, as lateral bud growth inhibitor, plays an important role in apical dominance of pea seedlings. Whether indole-3-aldehyde is metabolized from IAA transported from apical bud or is synthesized during IAA translocation, needs to be studied.

Table 1
^{1}H NMR chemical shifts (δ values from TMS) and multiplicities (*J* value in Hz) of isolated indole-3-aldehyde (recorded in 500 MHz in CD_3OD)

Position	
1	–
2	8.17 (1H, *s*)
3	–
4	–
5	8.20 (1H, *d*, *J* = 7.1 Hz)
6	7.28 (1H, *dd*, *J* = 8.3,7.1 Hz)
7	7.33 (1H, *dd*, *J* = 8.3,7.9 Hz)
8	7.52 (1H, *d*, *J* = 7.9 Hz)
9	–
10	9.92 (1H, *s*)

3. Experimental

3.1. Isolation of lateral bud growth inhibitor

Pea (*Pisum sativum* L. cv. Alaska) seeds were soaked in running tap water for 1 day, sown on two sheets of kimtowel moistened with distilled water and incubated for 1 day at 25 °C in the dark. Germinated seeds were transplanted under dim green light into large trays containing vermiculite moistened with distilled water, and were kept in the dark at 25 °C for 6 days. Lateral bud growth at all nodes was completely inhibited.

Above-ground parts of etiolated seedlings (1 kg, with length of the third internode between 4 or 5 cm) were harvested, rinsed with distilled water and frozen at −40 °C. The frozen materials were homogenized in of 60% aq. cold acetone (2 l) with a homogenizer. The filtered extract was concentrated to dryness in vacuo at 35 °C. The concentrate was separated into four

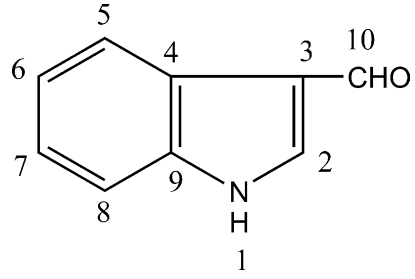

Fig. 1. The chemical structure of indole-3-aldehyde.

Table 2
Amounts of indole-3-aldehyde, IAA and 4-CI-IAA in diffusates from explants with apical bud decapitated explants and IAA-treated decapitated explants

	Indole-3-aldehyde	IAA	4-CI-IAA (ng/explant)
Explants with apical bud	3.10±0.19	2.50±0.43	7.74±0.15
Decapitated explants	1.72±0.07	1.43±0.15	6.35±0.20
IAA-treated, decapitated explants	2.89±0.18	10.1±0.37	7.78±0.13

Each value is the mean of ten explants±SE. This experiment was repeated three times.

fractions of 10, 30, 60 and 100% MeOH in water by Sep-Pak C18 cartridge chromatography (Waters). The of active fraction (60% MeOH eluate) was concentrated and then separated into three fractions of 20, 50 and 100% acetonitrile in water by Sep-Pak C18 cartridge chromatography (Waters). The active fraction (50% acetonitrile eluate) was evaporated to dryness in vacuo at 35 °C. The concentrate (361 mg) was further purified by HPLC (Tosoh, ODS-80Ts, 4.6×40 mm, H_2O: acetonitrile = 7: 3 v/v, 0.8 ml/min, detected at 300 nm). The active fraction with a retention time of 13–14 min was dried, yielding indole-3-aldehyde (0.5 mg).

3.2. Spectrometric analyses

APCI-MS, and ^1H NMR spectrum was taken on a platform LC (Waters) and AVANCE500 NMR spectrometer (Bruker), respectively.

3.3. Determination of the contents of endogenous indole-3-aldehyde, IAA and 4-Cl-IAA in the diffusates from explants with apical bud, decapitated explants or IAA-treated, decapitated explants

The third internodes of 3 or 4 cm length with or without apical buds were cut from 8-day-old etiolated seedlings. They were lined up vertically in contact with 0.8% agar blocks in 4.5 cm dishes, respectively. Decapitated explants were divided into two groups, one being left untreated and the other being treated with IAA (2 μg in 1 μl of 50% EtOH) on the top of the internodes. The explants were incubated in the dark at 25 °C; the dishes were placed in moist, transparent trays to prevent drying out. All manipulations were immediately carried out under dim green light. After a 3-h diffusion, the explants were removed and the agar blocks were immediately immersed in acetone (200 ml) for the determination of indole-3-aldehyde diffused from each internode. The extracts were filtered through Toyo filter paper No.1. The filtrates were evaporated to dryness in vacuo at 35 °C. The samples were dissolved in distilled water and subjected to HPLC (Tosoh, ODS-80Ts, 4.6×140 mm, H_2O (pH 2.5): acetonitrile = 6: 4 v/v, 0.8 ml/min, detected at 280 nm). Endogenous indole-3-aldehyde, IAA and 4-Cl-IAA were determined by measurement of the peak area. The recoveries of indole-3-aldehyde, IAA and 4-Cl-IAA during purification were about 60%.

3.4. Bioassay

Two-day-old germinated pea seeds were transplanted under dim green light to test tubes (1.5×8.5 cm) containing wet cotton wool and incubated for 6 days at 25 °C in the dark. Uniform seedlings (length of the third internode was between 4 or 5 cm) were selected for bioassay and decapitated at the top of the third internode using a razor blade. The cut surface of decapitated seedlings was treated with 1 μl of test solution. The length of the lateral bud (the larger one of two buds) at the second node was measured every 30 min, by using a binocular dissecting microscope.

Table 3
Effects of indole-3-aldehyde and its relative substances on outgrowth of lateral bud of decapitated seedlings

	30 min	60 min
		(mm)
Intact	0.48±0.02	0.49±0.04
Decapitated seedlings	0.90±0.02	1.12±0.02
(A) applied to cut surface of decapitated seedlings		
Isolated indole-3-aldehyde	0.48±0.04	0.49±0.03
Synthetic indole-3-aldehyde	0.52±0.03	0.50±0.02
IAA	0.51±0.01	0.49±0.03
Indole-3-pyruvic acid	0.57±0.01	0.62±0.04
4-Cl-IAA	0.72±0.01	0.90±0.02
Tryptophan	0.56±0.04	0.60±0.05
(B) applied directly to lateral buds of decapitated seedlings		
Isolated indole-3-aldehyde	0.49±0.03	0.52±0.03
Synthetic indole-3-aldehyde	0.50±0.03	0.51±0.02
IAA	0.67±0.01	0.89±0.03
Indole-3-pyruvic acid	0.69±0.03	0.84±0.05
4-Cl-IAA	0.70±0.04	0.90±0.02
Tryptophan	0.72±0.02	0.91±0.04

Thirty nanograms of each substance was applied to the cut surface or to the lateral buds of decapitated seedlings. Initial length of lateral bud was 0.48±0.03 mm. Each value is the mean of five explants±S.E. This experiment was repeated twice. The results were similar.

References

Ernstsen, A., Sandberg, G., 1986. Identification of 4-chloroindole-3-acetic acid and indole-3-aldehyde in seeds of *Pinus sylvestris*. Physiol. Plantarum 68, 511–518.

Magnus, V., Ozga, J.A., Reinecke, D.M., Pierson, G.L., Larue, T.A., Cohen, J.D., Brenner, M.L., 1997. 4-Chloroindole-3-acetic and indole-3-acetic acids in *Pisum sativum*. Phytochemistry 46, 675–681.

Michell, J.W., Marth, P.C., Freeman, G.D., 1965. Apical dominance in bean plants controlled with phthalamic acids. J. Agr. Food Chem. 13, 326–329.

Nakajima, E., Yamada, K., Kosemura, S., Yamamura, S., Hasegawa, K., 2001. Effects of the auxin-inhibiting substances raphanusanin and benzoxazolinone on apical dominance of pea seedlings. Plant Growth Reg. 35, 11–15.

Panigrahi, B.M., Audus, L.J., 1966. Apical dominance in *Vicia faba*. Ann. Bot 30, 457–473.

Phillips, I.D.J., 1975. Apical dominance. Annu. Rev. Plant Physiol. 26, 341–367.

Thimann, K.V., Skoog, F., 1934. On the inhibition of bud development and other functions of growth substance in *Vicia faba*. Proc. Roy. Soc. B 114, 317–339.