

Insensitivity of the *ageotropum* Pea Mutant Roots to Gravity

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완두 돌연변이체 *ageotropum* 뿌리의 중력불감성

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Root gravitropism was investigated in the pea (*Pisum sativum* L.) mutant *ageotropum* lacking normal gravitropic response. Exogenous ethylene treatment inhibited gravitropic response in the normal (wild type) pea roots, but had no significant effect to restore the unresponsiveness in the mutant. Neither inhibitors of ethylene biosynthesis nor antagonists of ethylene action were able to bring about the development of gravitropic curvature in the *ageotropum* roots. Auxin action seems to be normal since asymmetric application of agar blocks containing auxin to the mutant roots caused normal gravitropic response to occur. Endogenous as well as auxin-induced ethylene production in tissue segments of the mutant root was about equal to that of the wild type. However, no appreciable lateral transport of labeled auxin was observed in gravistimulated mutant roots whereas typical auxin asymmetry was apparent in the wild type roots under the same conditions. It is concluded that the mutant has a defect in either gravity perception or its transduction, but not in the effector system involving auxin action.

Key words: pea mutant *ageotropum*, root gravitropism

Plants have evolved to cope with everlasting changes in the environment by exhibiting appropriate responses mediated through the hormonal systems. The ability of plants to respond to gravitational stimulus provides means by which plants acquire the characteristic posture in relation to gravity. Gravitropic curvature develops in horizontally placed stems and roots as a result of asymmetric auxin distribution between the two opposing sides of the organ in reference to the gravitational vector, the notion being commonly known as Cholodny-Went theory (Cholodny, 1926; Went and Thimann, 1926). Time-dependent, gravity-induced changes in hormone sensitivity as well as changes in sensitivity to gravity, however, were also suggested to play important roles in the intricacies of the gravitropic response (Evans, 1991). Gravitropic response involves a series of reactions starting from the perception of gravitational stimulus followed by signal transduction, which lead to a modulation in the effector system involving auxin transport. The resulting asymmetric

redistribution of auxin gives rise to differential growth. Gravitropic mutants make a powerful tool to dissect these processes, and to elucidate underlying mechanisms by which the response is brought about.

The pea mutant *ageotropum* was obtained by treatment with X-rays (Blixt et al., 1958). Early work with the mutant reported insensitivity of the roots to centrifugal force of 2.7 g (Scholdeen and Burstrom, 1960). Shoots of the mutant also exhibit agravitropic growth and respond to externally applied ethylene to a lesser extent in the plumular hook formation compared with the wild type (Takahashi et al., 1991). In the present work, gravitropism of the *ageotropum* mutant roots were compared with the wild type pea in auxin transport and ethylene status in an effort to localize a lesion for the mutant.

Plant Materials

Seeds of the *ageotropum* mutant of *Pisum sativum* L. were kindly provided by Dr. H. Suge of Tohoku University. They were propagated in a field to obtain quantities of seeds for use. Seeds of *Pisum sativum* cv. Alaska were used as the normal, wild type control, but they were not the isogenic parent of the mutant. Presoaked seeds were germinated on wet paper towel supported on a glass plate. The setup was placed vertically in a desiccator having 100 % R.H. and seedlings were grown for 2-3 days in complete darkness at 27 °C.

Chemicals

(5-³H)-indole-3-acetic acid (³H-IAA, 28 Ci/mmol) was purchased from CEA, France, Gif-sur-Yvette. Aminoxyacetic acid (AOA), indole-3-acetic acid (IAA) and other fine chemicals were from various commercial sources.

Gravitropic Stimulation and Curvature Measurements

When the emerging roots reach 2-3 cm in length, the seedlings were placed horizontally by turning around the glass plate. Curvature development was determined on a transparency film at appropriate times after gravitropic induction. Data presented are average values of at least 3 experiments repeated, values from each experiment representing an average of 10 individual measurements. Ranges of the standard deviation for each value were less than ±10 degrees. Ethylene was applied at appropriate concentrations to the seedlings in a sealed desiccator. Other test chemicals were applied by having the roots immersed in buffer (5 mM potassium phosphate, pH 6.8) containing appropriate chemical(s) in a small vial aerated with oxygen for an appropriate period. Following the pretreatment, the roots were subjected to gravistimulation. For asymmetric application of auxin, agar (1.5%) blocks (1.5 mm × 1.5 mm × 1.5 mm) containing 0.1 mM indole-3-acetic acid (IAA) were attached to one side of the horizontal root in reference to gravity vector about at the elongation zone and about 1 mm from the tip while plain agar blocks were attached to the opposite side.

Lateral Auxin Transport

Buffered (5 mM K-phosphate, pH 5.0) agar blocks

containing 0.5 μCi/mL ³H-IAA were designated a "donor block", and plain buffered blocks a "receiver block". A donor block was attached to one side of horizontally placed roots in reference to gravity about 1 mm from the tip, and a receiver block was attached to the opposite side. Ten roots were employed for each measurement. At the end of a 120 min period, radioactivity in the receiver block was counted with a liquid scintillation spectrometer (Packard Tricarb 300). Results are presented as average cpm values from at least 3 experiments repeated.

Measurements of Ethylene Production

Twenty root segments excised 1 cm from the tip were incubated with 2 ml of buffer (5 mM K-phosphate, pH 6.8) plus 2% sucrose in the presence or absence of added auxin (100 mM) in a 25 mL Earlen-Meyer flask sealed with a silicon cap for 18 hr in the dark at 27°C. Ethylene accumulated in the sealed flask was measured at the end of an incubation period from a gas sample withdrawn from the flask with a gas chromatograph (Shimadzu, GC-3BF). Results are presented as average values from at least 3 experiments repeated.

RESULTS AND DISCUSSION

Development of gravitropic curvature sets in rapidly following displacement of roots in a horizontal position. As illustrated in Fig. 1a, roots of pea seedlings underwent curvature within 30 min of gravistimulation, proceeded to bend for about 2 hr, and remained thereafter with no further bending. In the presence of externally applied ethylene at 10 ppm, curvature development was greatly suppressed. Gravitropic response of etiolated pea stems is likewise inhibited by exogenously applied ethylene (Kang and Burg, 1972). On the contrary, roots of *ageotropum* exhibited essentially no gravitropic response (Fig. 1b) either with or without ethylene.

Reduced level of endogenous ethylene through inhibition of its biosynthesis by red illumination to etiolated pea seedlings results in increased gravitropic curvature in third internodes implying that the endogenous concentration of the gas is in a rate-limiting range (Kang and Burg, 1971). To check if this applies also to the root tissue, the roots were pretreated with AOA which inhibits ethylene production by blocking the conversion of S-adenosylmethionine (SAM) to 1-

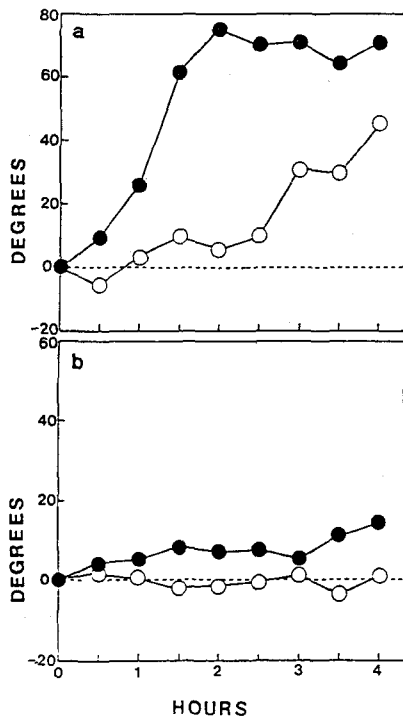


Figure 1. Time course for gravitropic curvature response in the normal (a) and *ageotropum* (b) roots in the presence (open symbols) or absence (closed symbols) of 10 ppm ethylene. The roots were displaced horizontally at time zero.

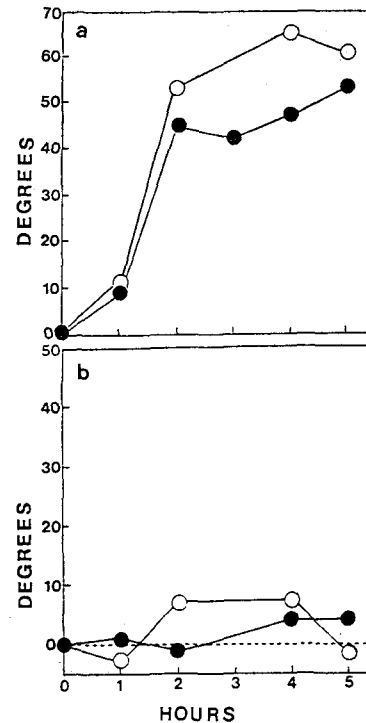


Figure 2. Time course for gravitropic curvature response in the normal (a) and *ageotropum* (b) roots in the presence (open symbols) or absence (closed symbols) of 10 uM AOA. The roots were displaced horizontally at time zero.

Table 1. Ethylene production from root segments of the normal and *ageotropum* peas incubated with or without 10 μ M IAA for 18 h.

Pea types	Ethylene production (nL/g.fr.wt.)	
	- IAA	+ IAA
Normal	12.47	20.82
Mutant	10.75	19.25

aminocyclopropane-1- carboxylic acid (ACC). The data presented in Fig. 2 indicate that AOA pretreatment caused a slight but significant increase in gravi-curvature in the normal pea, but not in the mutant. Agents which inhibit ethylene action such as AgNO₃ and 2,5-norbornadiene (NBD) had similar effects (data not shown).

The rate of ethylene production in the mutant, either basal or induced by added auxin, did not differ significantly from the normal pea as shown in Table 1. The data indicate that action of auxin to induce ethylene production is not impaired in the mutant. This is one of the features of the pea mutant, which is a contrast to the auxin-insensitive, gravitropic tomato mutant *diageotropica* (*dgt*), where auxin does not induce synthesis of ethylene (Kelly and Bradford, 1986).

Whether the pea mutant is in defect of auxin response for cell growth was tested by applying auxin asymmetrically on the root. Unilateral application of auxin to the lower side of

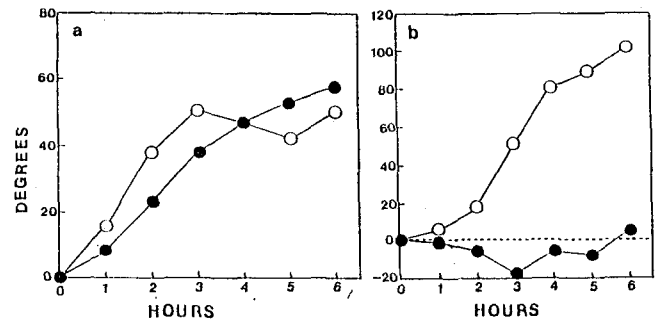


Figure 3. Time course for gravitropic curvature response in the normal (a) and *ageotropum* (b) roots with agar blocks containing 0.1 mM IAA (open symbols) or plain buffered agar blocks (closed symbols) attached to the lower side of horizontal roots at the zone of elongation and near the tip. Negative values in degrees denote negative gravitropic (upward) curvature. The roots were displaced horizontally at time zero.

horizontally displaced roots of the wild type accelerated downward gravitropic curvature in an early stage of bending upto 3 hrs of gravi-stimulation, after which a plateau was maintained (Fig. 3a). Under the same conditions of unilateral auxin application, however, the *ageotropum* roots otherwise exhibiting no gravitropic response underwent a strong downward bending (Fig. 3b), indicating that the normal, growth inhibiting action of auxin is operative in the mutant.

Reversed auxin asymmetry by means of unilateral auxin application to the upper side of horizontal roots resulted in a

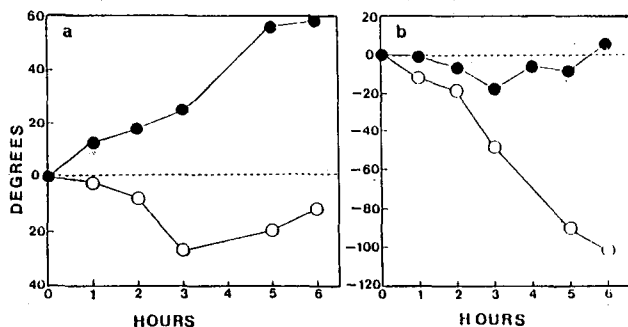


Figure 4. Time course for gravitropic curvature response in the normal (a) and *ageotropum* (b) roots with agar blocks containing 0.1 mM IAA (open symbols) or plain buffered agar blocks (closed symbols) attached to the upper side of horizontal roots at the zone of elongation and near the tip. Negative values in degrees denote negative gravitropic (upward) curvature. The roots were displaced horizontally at time zero.

slight upward curvature in the wild type roots (Fig. 4a). It is of interest that in the mutant, however, the upward curvature was even more pronounced than the wild type as shown in Fig. 4b. The data could be interpreted as a normal asymmetric distribution of endogenous auxin in the gravitational field in the wild type roots being partially compensated by reversed application of exogenous auxin at a high concentration (0.1 mM). If it is assumed that lateral auxin transport in gravistimulated root does not occur in the mutant *ageotropum*, the reversed curvature of upward bending should appear more intensified than the wild type. The data support this notion.

This is further supported by results of the following experiment. A donor agar block containing labeled auxin was placed on the upper side of horizontal roots near the tip, and auxin transported across the root in a downward direction in the gravitational field was collected into a plain receiver block attached on the opposite, lower side. Radioactivity collected in the receiver for this kind of arrangement appeared nearly three times that for a reversed arrangement where a donor block was applied on the lower side and an upward transport was measured by collecting labeled auxin in a receiver block attached on the upper side (Table 2). The data clearly indicate that in the mutant root, however, there was virtually no difference in auxin transport between the two opposing arrangements, suggesting that lateral auxin transport was not operative in the mutant under inductive conditions. It is inferred therefore that the mutant has a genetic defect whereby gravitational stimulus fails to bring about lateral auxin transport, and hence no gravitropic curvature. The lack of gravity-induced lateral auxin transport in *ageotropum*

Table 2. Lateral transport of ^3H -IAA across horizontally displaced roots of the normal and *ageotropum* peas.

Pea types	direction of transport ^a		Ratio (D/U) ^b
	Downward	Upward	
Normal	294	106	2.8
Mutant	179	181	1.0

^aFor downward transport, a donor agar block containing labeled auxin was placed on the upper side of horizontal roots near the cap and labeled auxin transported laterally across the root for 120 min was collected in a plain, receiver block attached to the opposite (lower) side of the root. The orientation of agar block application was reversed for upward transport. The data are presented as cpm values of the receiver blocks.

^bThe ratio denotes downward/upward transport.

epicotyl segments has previously been reported (Takahashi et al., 1991). However, since sites for the gravity perception and underlying mechanisms for the gravity sensing may be distinct between shoots and roots, the present finding that gravity-induced auxin asymmetry might also be absent in the roots of the mutant merits due attention.

In the auxin-insensitive, gravitropic tomato mutant *diageotropica* (*dgt*), pretreatment with ethylene, however, is able to restore the sensitivity to auxin and to normalize the tropic behavior in response to gravity. (Zobel, 1973; Kelly and Bradford, 1986; Munday et al., 1995). No significant difference in the endogenous concentration of IAA can be noted between the *dgt* and the parent variety, VFN8, of tomato (Fujino et al., 1988), but the mutant lacks high specific activity auxin binding sites, a polypeptide doublet of 40 and 42 kDa as visualized by azido-IAA photoaffinity labeling (Hicks et al., 1989). The mutant is insensitive to IAA in several physiological responses, and is thought to have a defect associated with or affecting a primary site of auxin perception or action (Munday et al., 1995). The genetic lesion is therefore located "downstream" in the effector system involving auxin action in the pathway toward gravity-induced differential growth.

There are indications from morphological studies for some structural distinction in the *ageotropum* mutant associated with processes following the perception of gravitational stimulus. Olsen and Iversen (1980) compared ultrastructure of, and movements of intracellular structures in the normal and *ageotropum* pea roots. The only major difference they noted was that the distribution of rough endoplasmic reticulum (ER) is mainly concentrated in the distal part at the bottom of the columella cells in the root cap of the normal pea,

whereas in the mutant it is evenly distributed throughout the cell. The displacement of ER is supposed to take place as an indirect result of the amyloplast redistribution (Sievers and Volkmann, 1972; Juniper, 1976). Further studies should be necessary to elucidate a possible causal relationship between the absence of gravity-induced auxin asymmetry and the lack of ER displacement in the *ageotropum* mutant.

적 요

완두 돌연변이체 *ageotropum* 뿌리의 굴중성 결여에 대하여 조사하였다. 정상 완두의 뿌리에서 에틸렌은 굴중성 반응을 억제하였다. 돌연변이체에서는 에틸렌이나 에틸렌 생합성 억제제 또는 작용 억제제 모두 뿌리의 굴중성 반응 결여를 회복시키지 못하였다. 옥신을 비대칭으로 처리한 뿌리에서 굴중반응이 일어나는 것으로 보아 옥신의 작용은 정상적으로 일어나는 것으로 판단되었다. 내생적 또는 옥신 유도에 의한 에틸렌 생성은 정상 완두나 돌연변이체에서 크게 차이가 없었다. 그러나 정상 완두에서와는 달리 돌연변이체의 뿌리에서는 굴중성 반응의 작동체계인 옥신작용의 결합이 아니고 중력의 인식 또는 전달과정에 결합이 있다는 결론을 얻었다.

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