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Ethylene-Induced Auxin Sensitivity Changes in Petiole Epinasty of Tomato Mutant *dgt*

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The tomato (*Lycopersicon esculentum* Mill.) mutant *diageotropica* (*dgt*) lacking normal gravitropic response is known to be less sensitive to auxin compared with its isogenic parent VFN8. Straight growth as well as ethylene production in response to added auxin in hypocotyl segments of *dgt* was negligible. However, there was no significant difference between the two genotypes in auxin transport in petiole segments and its inhibition by the phytotropin *N*-1-naphthylphthalamic acid (NPA). Kinetic parameters of NPA binding to microsomal membranes were also non-distinguishable between the two. Its petiolar explants treated with ethylene developed epinastic curvature with the magnitude of response increased about 3 folds over non-mutant wild type. Ethylene-induced epinasty in both *dgt* and VFN8 was nullified by treatment of explants with the ethylene antagonist 2,5-norbonadiene. Lateral transport of ^{3}H-IAA toward the upper side of ethylene-treated petioles in *dgt*, however, was not significantly more pronounced than in VFN8, the implications being that auxin sensitivity in the mutant was restored, or even rised above the wild type, by ethylene.

Keywords: Tomato (*Lycopersicon esculentum*) mutant *diageotropica*, auxin sensitivity, ethylene

The *diageotropica* (*dgt*) mutant of tomato is a spontaneous, single gene recessive mutant of the parental variety VFN8 (Zobel, 1972). The mutant is phenotypically characterized by horizontal growth of shoots and roots, lack of lateral roots, thin stems, and hyponastic leaves (Zobel, 1973, 1974). The mutant is insensitive to auxin (Kelly and Bradford, 1986), but its endogenous auxin level as determined by gas chromatography-mass spectrometry is not significantly different from that of non-mutant VFN8 (Fujino *et al.*, 1988).

The most interesting characteristic of the mutant phenotype is its requirement of exogenous ethylene for normal growth and development (Zobel, 1973). Ethylene modulation of cellular sensitivity to ciher auxin or gravity was postulated to account for ethylene promotion of cell elongation (Kang *et al.*, 1992), epinasty (Kang, 1979), and other ethylene-mediated cellular processes (Burg and Kang, 1993). Leaf petiole epinasty is a growth response involving concerted action of both auxin and ethylene. An auxin-insensitive, and ethylene-requiring mutant should provide a suitable tool for studies on ethylene mediation of auxin sensitivity, and we therefore investigated petiole epinasty in the two genotypes of tomato.

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MATERIALS AND METHODS

Plant material

Seeds of both mutant (*dgt*) and wild type (VFN8) of *Lycopersicon esculentum* Mill. originally provided by C. M. Rick of University of California at Davis were propagated to obtain sizable quantity of seeds, which were used in the present work. These seeds were sowed on a wet filter paper in a petri-dish in total darkness at 27℃ for 5 d. One-cm segments excised from the subapical hypocotyl were used for auxin responsive growth and ethylene biosynthesis

experiments. For epinasty-related experiments, petiolar explants isolated from the second through the fourth petioles from the apex of 4 to 6-wk old, greenhouse grown tomato plants were employed.

Chemicals and radiochemicals

[^{3}H]-*N*-1-naphthylphthalamic acid (55 Ci/mmol) and [^{3}H]-indole-3-acetic acid (28 Ci/mmol) were perchased from Cen Saclay of France. *N*-1-naphthylphthalamic acid (NPA) was a gift from R. Hertel of Freiburg University, Germany and other fine chemicals were Sigma products.

Growth and auxin transport

Straight growth of the hypocotyl segments was measured by incubating 1 cm segments in 50 mM K phosphate buffer (pH 6.8) for 20 h at 28℃. Epinastic growth was measured with petiolar explants. The basal end of the explant was inserted upright in 1% agar bed, and placed in a desiccator for 24 h at 28℃ with various concentrations of applied ethylene and/or 3000 ppm of 2,5-norbonadiene (NDE). At the end of this period, epinastic curvature was measured on shadowgraphs.

Lateral auxin transport was tested by applying ^{3}H-IAA in lanolin paste (2 μCi/g) on the apical cut end of the petiole with the entire explant inserted upright on agar bed as in the epinasty experiments. At the end of a 24 h period, the basal 2 cm of 3 cm petiole was excised, the upper and lower quarters were longitudinally split with a razor blade with the middle half discarded, and radioactivity of the upper and lower flanks were counted, respectively. For polar auxin transport, labelled auxin (7.14×10^{-8} M ^{3}H-IAA) was applied in agar blocks which were placed on the apical cut end of 1 cm petiole segments, and at the end of a 3 h transport period, radioactivity in receiver agar blocks attached to the basal end of the segment was counted. Receiver blocks contained various concentrations of NPA. All growth and transport experiments employed 10 tissue segments for each treatment.

NPA binding

In vitro binding of NPA to microsomal membranes from the petiole tissue was carried out according to the method descibed elsewhere (Yoon and Kang, 1991).

RESULTS AND DISCUSSION

Ethylene strongly induces petiolar epinasty through modification of lateral auxin transport culminating in accumulation of auxin in the upper flank of the petiole, which in turn leads to differential growth of downward curvature (Kang, 1979). We examined epinastic curvature induced by applied ethylene at various concentrations in *dgt* and its isogenic parent VFN8, and the results are shown in Fig. 1. It is clearly evident that the mutant displayed magnified response to ethylene in curvature development compared with the non-mutant control. To the contrary, epinastic curvature developed in the presence of applied auxin is more pronounced in VFN8 compared with the mutant (Ursin and Bradford, 1989) obviously because the mutant is insensitive to auxin. The magnitude of epinastic response to auxin alone is, however, negligible, and cannot be compared with that induced by exogenous ethylene.

The data in Table 1 show that treatment of the petiole with the ethylene antagonist NDE completely abolished the ethylene effects in both VFN8 and *dgt* indicating that the epinasty developed under these conditions were entirely attributable to ethylene action. Like tropistic responses, epinastic curvature results from auxin redistribution, and actually might

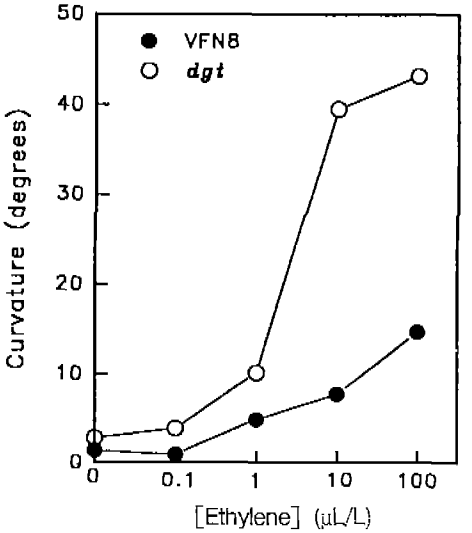

Fig. 1. Epinastic curvature of petiolar explants in VFN8 and *dgt* induced by applied ethylene at various concentrations.

Table 1. Nulification of petiolar epinasty induced by exogenous ethylene in VFN8 and *dgt*

Treatment	Curvature (degrees)	
	VFN8	*dgt*
None	1.5	2.4
NDE	1.2	−4.4
C_2H_4	17.2	47.4
C_2H_4+NDE	6.6	6.6

Table 2. Epinastic curvature and lateral transport of ^{3}H-IAA in petiole explants in the presence or absence of applied ethylene

Genotypes	C_2H_4	cpm/g fr wt		Ratio	Curvature
		upper (U)	lower (L)	(U/L)	(degrees)
VFN8	−	1322	959	1.38	1.4
	+	2299	1345	1.64	14.7
dgt	−	1710	1285	1.33	2.8
	+	2044	1258	1.75	43.2

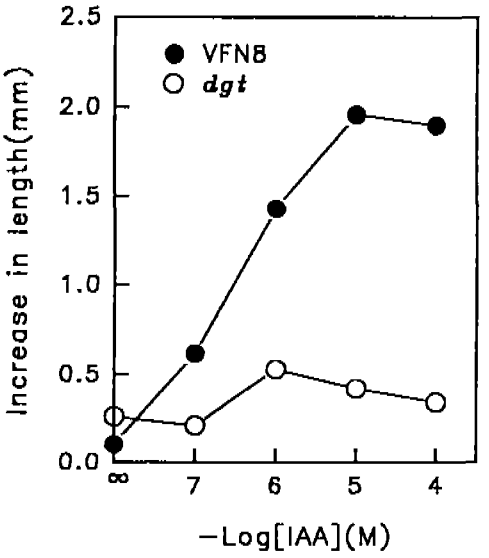

Fig. 2. Growth of hypocotyl segments with various concentrations of auxin in VFN8 and *dgt*.

be regarded as gravitropism in reverse (Burg and Kang, 1993). The ratio in cpm of the upper to the lower flanks of the petiole with labelled IAA applied on the apical cut end, reflecting upward auxin migration, did not significantly differ between VFN8 and *dgt* either in the presence or absence of exogenous ethylene (Table 2). Curvature development was, however, about three times more pronounced in the mutant in the presence of ethylene compared with the non-mutant control. Assuming that there is a close correlation between the degrees of curvature and those in auxin assymetry, an explanation is needed to accomodate these data.

The discrepancy could be resolved if it is assumed that growth of ethylene-treated petiolar cells in *dgt* is more sensitive to auxin compared with VFN8. Indeed, the auxin-insensitive mutant was originally characterized by requirement of exogenous ethylene for normal growth and development (Zobel, 1973). Modulation of auxin sensitivity by ethylene is also known to account for ethylene promotion of cellular growth in *Ranunculus* petioles (Kang *et al.*, 1992) and other ethylene-mediated processes (Burg and Kang, 1993).

Hypocotyl segments of the mutant tomato did not respond to added auxin in growth response, whereas auxin treatment resulted in a usual growth promotion in the wild type hypocotyl as illustrated in Fig. 2. These results are in accordance with findings previously reported for this *dgt* mutant (Kelly and Bradford, 1986).

Insensitivity to auxin could result from a defect in any of the elements associated with the perception of auxin molecules, the transduction of auxin signal, or the effector system of auxin response. The recognition of auxin is realized through specific binding of the ligand to a respective site on the receptor proteins. Recent studies have accumulated information on the identity and nature of auxin binding proteins in plant tissues (*e.g.* Prasad and Jones, 1991; Feldwisch *et al.*, 1992), but information on biological functions of these putative receptors is still largely elusive (Jones and Prasad, 1992).

Using the photoaffinity auxin analog, ^{3}H-5N_3-IAA (azido-IAA), Hicks *et al.* (1989) were able to demonstrate binding of auxin to a polypeptiide doublet at 40 and 42 kD in membrane preparations from shoots of VFN8, but failed to obtain labelled polypeptides from *dgt* shoots. The data strongly imply that altered polyptides in *dgt* tissue is responsible for the *dgt* lesion through interference at the site of auxin perception. The fact that action of auxin to induce ethylene biosynthesis is also impaired in *dgt* (Kelly and Bradford, 1986) support the notion that the mutant carries a defect at or near the site where auxin molecules are recognized. The data in Fig. 3 confirm these findings. That the impairment of

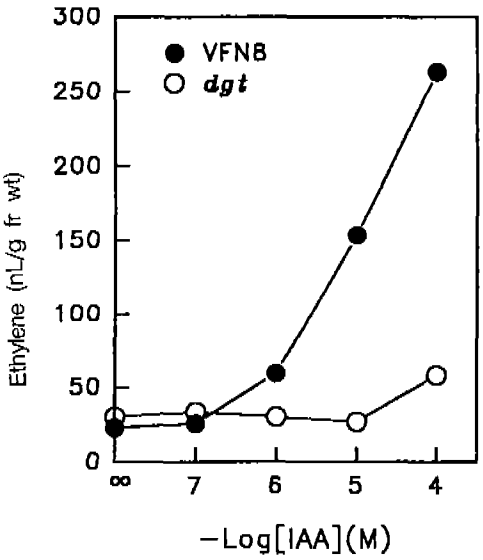

Fig. 3. Ethylene production from hypocotyl segments with various concentrations of auxin in VFN8 and *dgt*.

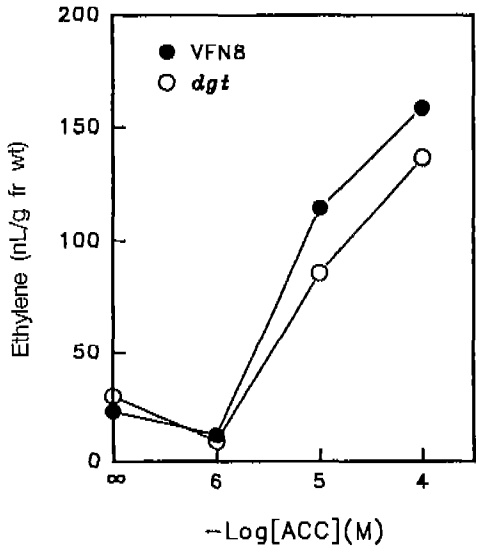

Fig. 4. ACC-based ethylene production from hypocotyl segments in VFN8 and *dgt*.

ethylene production is specific to auxin is demonstrated in Fig. 4, where ethylene production from tissues fed with 1-aminocyclopropane-1-carboxylic acid (ACC), the enzyme product of auxin-induced ACC synthase and the immediate precursor of ethylene (Adams and Yang, 1979), in *dgt* was about the same as that in VFN8.

In a molecular model for transport and action of auxin proposed by Hertel (1983), it was inferred that auxin action is functionally linked to auxin transport through a common site presumably localized on the plasma membrane. This notion was supported by the finding that growth of *Ranunculus* petioles is inhibited by the phytotropin NPA. NPA specifically inhibits auxin transport by binding to the putative auxin efflux carrier on the plasma membrane, and treatment with NPA results in an elevated intracellular auxin level by specific inhibition of auxin efflux with cellular entry of auxin unaffected.

In view of the finding that the mutant lacks an auxin binding polypeptide doublet of 40 and 42 kD that is present in the microsomal membranes of VFN8 (Hicks *et al.*, 1989), we tested NPA binding activities of microsomal membranes from *dgt* and

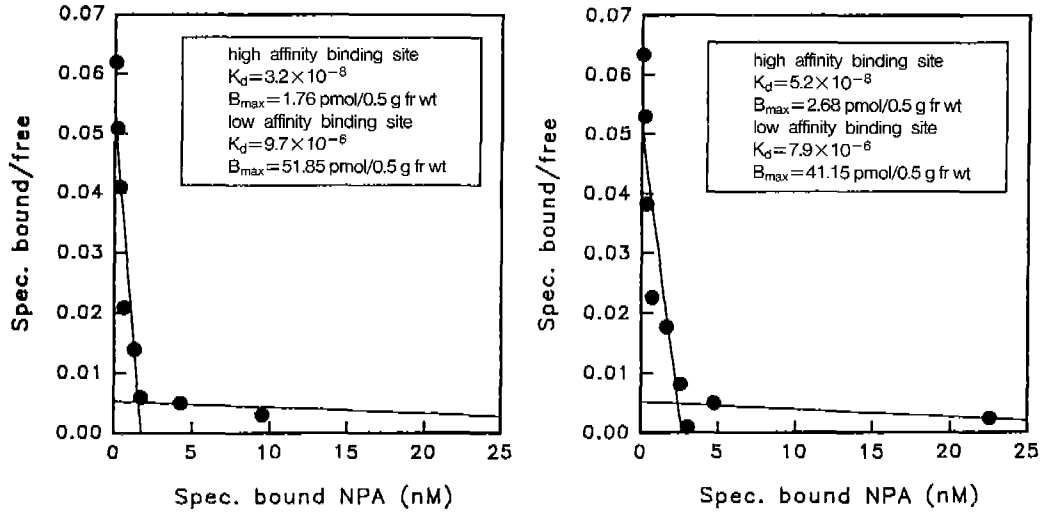

Fig. 5. Scatchard plots of NPA binding to microsomal membranes from VFN8 (left) and *dgt* (right) petioles.

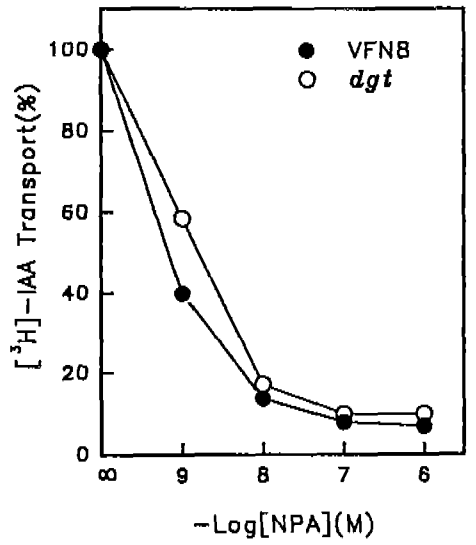

Fig. 6. Inhibition of auxin transport by NPA in VFN8 and *dgt* petiole segments.

VFN8, respectively. As shown in Fig. 5, the kinetic parameters of NPA binding in the mutant were not significantly different from those of non-mutant VFN8. Inhibition of auxin transport by NPA in *dgt* petiole segments was as effective as in VFN8 petioles (Fig. 6). Daniel *et al.* (1989) also compared several parameters of growth and auxin transport in *dgt* and VFN8, and found no significant difference between the two in auxin transport activities, but auxin-induced increase in cell wall extensibility was absent in *dgt*. From these results they concluded that the *dgt* lesion may be related to a defect in the sequence of events leading to auxin-induced wall loosening.

ACKNOWLEDGMENTS

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토마토 *dgt* 突然變異體의 葉柄 上便生長에서 에틸렌에 의한 옥신 感受性의 變化

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적 요

정상적인 굴중성 반응을 보이지 않는 토마토(*Lycopersicon esculentum* Mill.) 돌연변이체인 *dgt*는 자신의 isogenic parent인 VFN8에 비하여 옥신에 대한 반응에 있어서 둔감한 것으로 알려져 있다. 예를 들어 하배축 조직에서 옥신 처리에 의한 길이생장이나 에틸렌 합성의 증가가 매우 미미하였다. 그러나 엽병조직에서 *dgt*와 VFN8는 옥신의 극성 이동과 *N*-1-naphthylphthalamic acid(NPA)에 의한 옥신의 극성 이동 억제 정도에서 차이를 뚜렷이 보이지 않았다. 또한 미크로좀 소낭을 얻어 NPA 결합실험을 한 결과 *dgt*와 VFN8은 거의 비슷한 수치의 K_d와 B_{max}값을 나타냈다. 그런데 상편생장의 경우, 에틸렌 처리에 따른 상편생장 증가 정도가 *dgt*의 경우 VFN8의 3배에 해당하였다. 이러한 상편생장의 증가는 *dgt*와 VFN8 모두에서 에틸렌의 작용억제제인 2,5-norbonadiene에 의하여 소멸되었다. 에틸렌의 작용과 옥신의 관계를 밝히기 위하여 에틸렌에 의한 생체내에서의 옥신 측면이동 양상을 ^{3}H-IAA를 사용하여 조사하였다. 그 결과 *dgt*와 VFN8 사이에서 에틸렌에 의한 ^{3}H-IAA의 측면이동 정도의 차이가 나타나지 않았다. 따라서 *dgt*의 경우 에틸렌은 옥신이 엽병의 상편생장을 유도할 때 조직의 옥신에 대한 감수성을 회복하거나 야생형 이상으로 증가시키는 역할을 하는 것으로 보인다.

주요어 : 토마토(*Lycopersicon esculentum* Mill.) *diageotropica* 돌연변이체, 옥신 감수성, 에틸렌

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