

Communication

Auxin Physiology of the Tomato Mutant *diageotropica*¹

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ABSTRACT

The tomato (*Lycopersicon esculentum*, Mill.) mutant *diageotropica* (*dgt*) exhibits biochemical, physiological, and morphological abnormalities that suggest the mutation may have affected a primary site of auxin perception or action. We have compared two aspects of the auxin physiology of *dgt* and wild-type (VFN8) seedlings: auxin transport and cellular growth parameters. The rates of basipetal indole-3-acetic acid (IAA) polar transport are identical in hypocotyl sections of the two genotypes, but *dgt* sections have a slightly greater capacity for IAA transport. 2,3,5-Triiodobenzoic acid and ethylene reduce transport in both mutant and wild-type sections. The kinetics of auxin uptake into VFN8 and *dgt* sections are nearly identical. These results make it unlikely that an altered IAA efflux carrier or IAA uptake symport are responsible for the pleiotropic effects resulting from the *dgt* mutation. The lack of auxin-induced cell elongation in *dgt* plants is not due to insufficient turgor, as the osmotic potential of *dgt* cell sap is less (more negative) than that of VFN8. An auxin-induced increase in wall extensibility, as measured by the Instron technique, only occurs in the VFN8 plants. These data suggest *dgt* hypocotyls suffer a defect in the sequence of events culminating in auxin-induced cell wall loosening.

The analysis of mutants is a classical approach to determining the physiological relevance of certain proteins and elucidating biochemical pathways. This tactic can be used to understand better the mechanism of auxin action. A tomato (*Lycopersicon esculentum*, Mill.) mutant known as *diageotropica* (*dgt*) is a particularly promising candidate for such work. *dgt* is a spontaneous single gene recessive mutant of the parental variety VFN8 (20). The mutant is characterized by diageotropic shoot growth, abnormal vascular tissue, altered leaf morphology, and lack of lateral root branching (21, 22). Although endogenous levels of IAA are the same in the shoot tips of *dgt* and VFN8 (8), *dgt* mutants are nearly insensitive to exogenously applied auxin with respect to hypocotyl elongation and ethylene production (2, 13). The suite of morphological abnormalities exhibited by *dgt* plants as well as their inability to elongate in response to IAA suggests that these

plants may have a defect associated with a primary site of auxin perception or action.

Additional evidence supporting the notion that *dgt* and its isogenic parent, VFN8, provide a unique system to study auxin action stems from recent work (11) utilizing the photo-affinity auxin analog, ³H-5N₃-IAA (azido-IAA). Azido-IAA specifically labels a polypeptide doublet at 40 and 42 kD in membrane preparations from shoots of VFN8, but fails to label polypeptides from *dgt* shoots. In *dgt* roots, however, these polypeptides are present and label with equal intensity when compared to VFN8 roots (11). These data suggest that this 40 to 42 kD polypeptide doublet is an auxin receptor which displays an altered pattern of tissue-specific expression in the mutant. At least three auxin-specific receptor proteins are thought to be present in the membranes of seedling shoots: (a) an auxin uptake carrier (1, 10, 15, 18), (b) an asymmetrically distributed auxin efflux carrier (9, 12, 19), and (c) an auxin receptor which influences an outwardly directed proton pump thought to be involved in elongation growth (7, 17, 19). The experiments described herein are designed to provide baseline information on the auxin physiology and growth parameters of *dgt* and VFN8 hypocotyls. Such information may help us understand the nature of the *dgt* lesion, its relationship to the 40 to 42 kD azido-IAA-binding polypeptides, and their relationship to putative auxin-specific receptor proteins.

MATERIALS AND METHODS

Plant Material

Seeds of VFN8 and *dgt* tomato (*Lycopersicon esculentum*, Mill.) isolines were obtained from Dr. K. J. Bradford of the University of California at Davis. Seeds were surface-sterilized in 10% Clorox for 10 min, sown on wet vermiculite, and germinated in a climate controlled room on a cycle of 14 h light (100 μ E m⁻² s⁻¹, 25°C) 10 h dark (20°C). Unless otherwise noted, experiments were performed with 6- or 10-mm sections cut 2 mm below the cotyledonary node of 2 to 3 week old hypocotyls. All experiments were initiated at least 1.5 h after cutting to eliminate any contribution from ethylene release associated with the initial wounding event (13). Ethylene pretreatment of plants consisted of transferring approximately 30 intact plants and surrounding vermiculite from their original containers to 50-mL beakers which were then placed in 800-mL canning jars and sealed with lids specifically modified with rubber injection ports for ethylene additions. Ethylene

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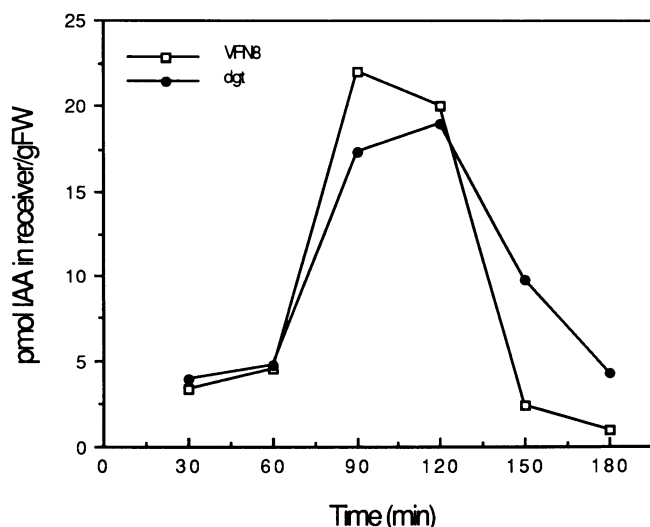


Figure 1. Basipetal transport rate of a 15 min [¹⁴C]IAA pulse in *dgt* and VFN8 hypocotyl sections. Each point represents the radioactivity collected over 30 min in agar receiver blocks from 10, 1 cm sections. Blocks were removed at the indicated times.

was added in all experiments to give a final concentration of 1 μL/L and treatment periods were 18 h.

Auxin Transport Profiles

Ten randomly selected 6-mm sections of VFN8 or *dgt* hypocotyls were placed on 1% aqueous agar receiver blocks (1.5 × 1.5 × 0.2 cm on glass microscope slides) oriented with their physiological tips up (for basipetal transport) or down (for acropetal transport). Donor blocks containing 10 nM [¹⁴C] IAA (55.6 μCi/mol, Amersham) were then placed on the segments with the aid of clay support pillars and the system inverted to prevent drainage of [¹⁴C]IAA onto the receiver blocks. The donor-receiver block units were then placed in a high humidity container (6 × 20 × 40 cm plastic planting trays lined and covered with water saturated blotter paper) and maintained at 27°C. Transport rate profiles were determined by pulsing the hypocotyl sections for 15 min and immediately replacing the radioactive donor blocks with blocks containing nonradioactive IAA at 1 μM. Sections were moved to new receiver blocks at 30 min intervals. Transport capacity profiles were determined as above, but donor blocks were left in place for the duration of the transport period (3.5 h). The effect of TIBA³ on transport was determined by adding TIBA to the receiver blocks at a final concentration of 0.1 mM and allowing a 30 min diffusion period before application of the donor blocks to the hypocotyl sections.

Auxin Uptake and Efflux Determination

One hundred randomly selected hypocotyl sections of *dgt* or VFN8 hypocotyls (6 mm) were weighed then floated on 5 nM [¹⁴C]IAA in 5 mM Mes buffer (pH 5.8). Random samples consisting of four replicates of two hypocotyl sections were removed from the incubation medium every 15 min for 3 h, briefly (5 s) rinsed in distilled water, and radioactivity deter-

³Abbreviation: TIBA: 2,3,5-triiodobenzoic acid.

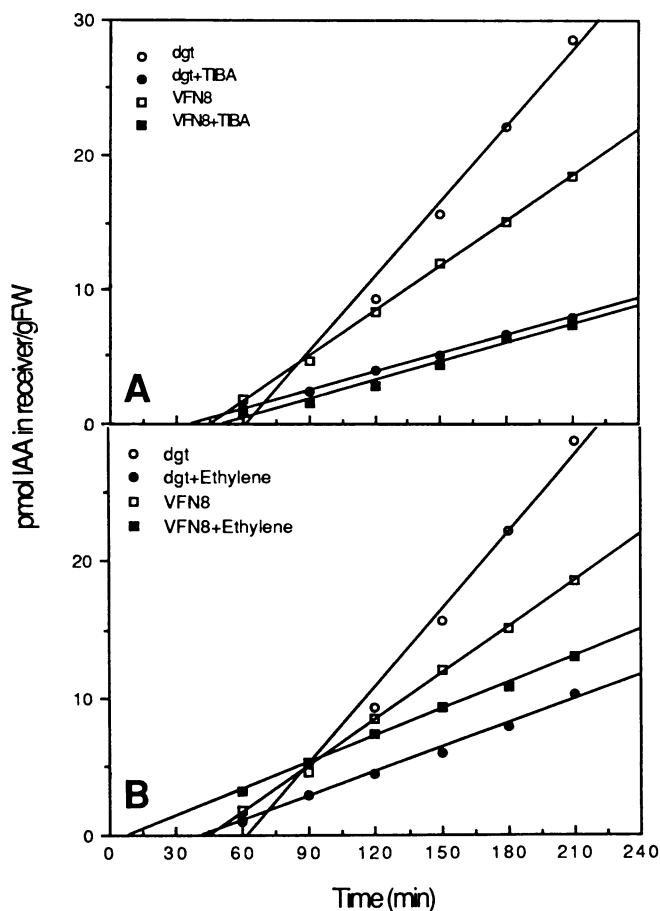


Figure 2. A, Basipetal auxin transport capacity of 1 cm *dgt* and VFN8 hypocotyl sections ±0.1 mM TIBA in receiver blocks. B, Effect of 18 h, 1 μL/L ethylene pretreatment on basipetal [¹⁴C]IAA transport capacity of *dgt* and VFN8 hypocotyl sections. Each point represents the radioactivity collected in agar receiver blocks from 10, 1 cm sections at the indicated time.

mined directly by scintillation spectroscopy after an overnight extraction in Aquasol (DuPont). Scintillation data were converted to pmol IAA/g fresh weight of tissue. For measurement of auxin efflux, *dgt* and VFN8 sections were allowed to take up [¹⁴C]IAA (in the presence of 5 nM unlabeled IAA in 5 mM Na-citrate buffer, pH 5.0) for 2.5 h, after which the tissue was rinsed briefly (5 s) in distilled water and transferred to 5 mM Mes (pH 5.8). Four 20 μL samples were removed at 30 min intervals and radioactivity determined in Aquasol as described above. Data are expressed as pmol IAA effluxed/g fresh weight (normalization of the efflux and uptake data to fresh weight were necessary because VFN8 hypocotyl sections are consistently larger in diameter than *dgt* sections at 2 to 3 weeks of age).

Osmotic Potential of Cell Sap

The osmotic potential of expressed cell sap was determined for hypocotyl sections from 3-week-old *dgt* and VFN8 plants. The 1 cm of hypocotyl just below the cotyledons were removed from 25 *dgt* and VFN8 plants and incubated for 6 h in a medium containing 5 mM KCl, 5 mM sucrose, and 1 mM Mes (pH 6.5) with or without 0.01 mM IAA. After the incu-

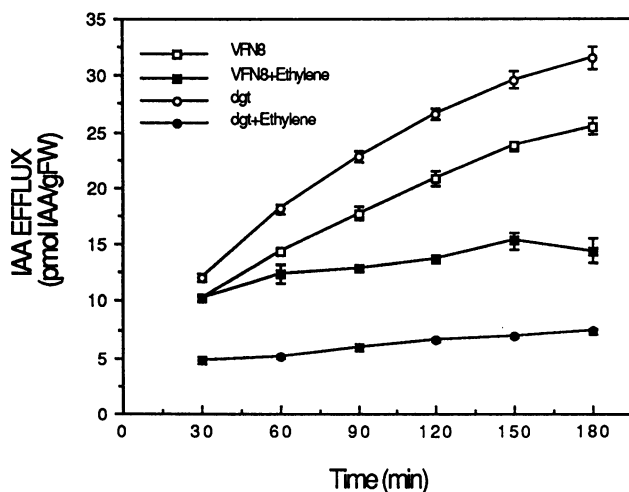


Figure 3. Net efflux of [^{14}C]IAA from preloaded (2.5 h) *dgt* and VFN8 hypocotyl sections. Each point represents the average radioactivity found in four, 20 μL samples of the efflux medium \pm SE. Ethylene treatments as in Figure 2.

bation period, the hypocotyl sections were blotted dry with tissue, wrapped in foil and frozen for 10 min on dry ice. The tissue sections were then allowed to thaw for 10 min and were cut into approximately 5 mm sections using a razor blade. The osmolarity of the expressed sap from approximately ten 5 mm sections (enough to give a 10 μL sample) was determined using a Wescor vapor pressure osmometer, converted to bars and subsequently expressed as the average osmotic potential of five replicates \pm the standard error of the mean.

Instron Measured Wall Extensibility

Hypocotyl sections (1.5 cm) were incubated for 6 h with or without 0.01 mM IAA in 1 mM Mes (pH 6.5) after which they were boiled 5 min in methanol. Upon rehydration (30–60 min) the sections were subjected to Instron analysis (5, 6). From the slopes of the first and second extensions, total elastic and plastic extensibility values were determined. Data are expressed as percent plastic extension/100 g load.

RESULTS AND DISCUSSION

The rate of basipetal IAA transport (*i.e.* polar transport) is similar in *dgt* and VFN8 hypocotyl sections (Fig. 1). Acropetal transport is nil in both varieties (data not shown). Although the rate of basipetal transport is equal in both plants, the capacity (*i.e.* CPM transported) of *dgt* hypocotyls for basipetal auxin transport exceeds that of VFN8 (Fig. 2, A and B). TIBA, a noncompetitive inhibitor of polar transport (10, 19), reduces basipetal auxin transport in *dgt* and VFN8 (Fig. 2A). Ethylene also reduces polar transport in both mutant and wild-type sections (Fig. 2B) although the percent inhibition by ethylene is considerably greater in *dgt* sections. It is interesting to note that ethylene has been reported to normalize some of the morphological abnormalities of *dgt* (21, 22). At the present time we have no data that would explain the apparent increase in polar transport capacity of *dgt* sections relative to VFN8; however, several possibilities exist. For example, the altered morphology of *dgt* stems (22) may include more cells capable

of polar transport. Alternatively, VFN8 sections may mobilize more IAA as it moves through sections by selectively shunting label into an organellar compartment, forming nontransportable metabolites or auxin conjugates or by the binding of IAA to macromolecules.

In spite of the observed difference in transport capacity, the fact remains that the rate of auxin polar transport in VFN8 and *dgt* is quite similar. Further, in both mutant and wild-type sections transport is down-regulated by TIBA and ethylene. Collectively, these data make it unlikely that an altered IAA efflux carrier is responsible for the pleiotropic effects resulting from the *dgt* mutation.

It seems equally improbable that the *dgt* lesion results in an altered IAA uptake carrier or symport. Auxin uptake kinetics into VFN8 and *dgt* sections are nearly identical (data not shown). Ethylene treatment increases uptake in both varieties, but is somewhat more effective in *dgt* (50 versus 38% after 3 h).

The rate of IAA efflux for hypocotyl sections would, in theory, reflect the combined activities of the putative efflux carrier and the putative uptake symport as well as any differences in the amount of label which was immobilized. As shown in Figure 3, the efflux of IAA from *dgt* tissue exceeds efflux from VFN8 tissue. The explanation for this result may be related to the above data (Fig. 2, A and B) showing that *dgt* has a greater capacity for auxin transport than VFN8. That is, enhanced efflux (like the enhanced capacity for transport) may result from more cells capable of auxin export in *dgt* or indicate increased immobilization in VFN8 sections. In any case, the efflux of IAA from *dgt* and VFN8, like direct measurements of polar transport and uptake, fail to provide compelling evidence for an altered efflux or uptake carrier in *dgt*.

Since alterations in auxin uptake or transport seem unlikely explanations for the combination of phenotypes exhibited by *dgt*, we felt it might be constructive to focus more finely on one particular manifestation of this lesion, the inability of *dgt* hypocotyl sections to elongate in response to exogenously applied IAA. A large body of literature (see for example 3–6, 14, 16, 17) suggests that two of the parameters that regulate

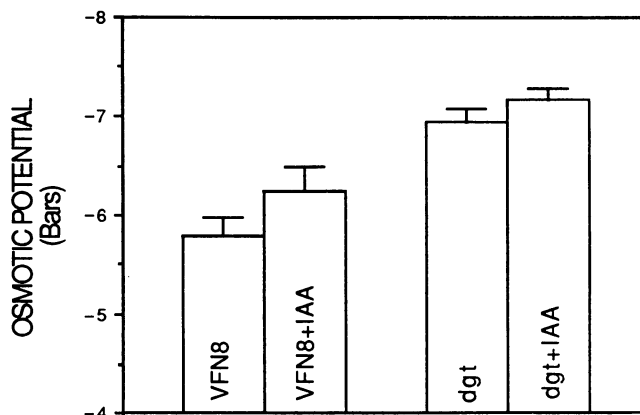


Figure 4. Osmotic potential of expressed cell sap from *dgt* and VFN8 hypocotyls. Sections (1 cm) were incubated for 6 h in 1 mM Mes (pH 6.5) \pm 10 μM IAA. The tissue was then quickly rinsed and blotted and the OP of expressed cell sap determined using a Wescor vapor pressure osmometer.

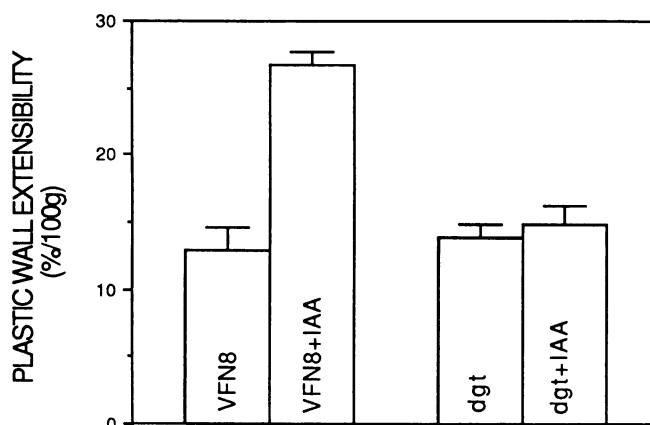


Figure 5. Effect of auxin on wall extensibility in *dgt* and VFN8. Hypocotyl sections were incubated as described in Figure 4 after which they were boiled 5 min in methanol, rehydrated, and their plastic extensibility determined using the Instron technique.

plant cell water potential and thus turgor-driven cell extension are: (a) the osmotic potential of the cell sap and (b) the extensibility of the cell wall. In Figure 4 we present data showing the osmotic potential of *dgt* cell sap is more negative (*i.e.* contains more solutes/unit volume) than similar fluid expressed from wild-type hypocotyls. These data suggest that turgor in *dgt* cells is adequate to drive cell extension. Such data were not unexpected, since Kelly and Bradford (13) previously reported that fusicoccin (FC) stimulates growth to an equal extent in both *dgt* and VFN8.

Data in Figure 5 focus on the extensibility of *dgt* and VFN8 hypocotyl cell walls. Wall extensibility was assessed using the Instron technique. In the absence of exogenous auxin, both wild-type and mutant walls exhibit similar plastic extensibilities. Auxin treatment causes an increase in wall extensibility (*i.e.* wall loosening) in VFN8 sections, but has no effect on walls of *dgt* hypocotyls. These results indicate that in wild-type tomato, as is the case with other species (6, 17), auxin treatment results in the cleavage of load-bearing cell wall bonds and thus produces an enhanced rate of cell extension. Conversely, the inability of auxin to increase wall extensibility and stimulate cell extension in *dgt* suggests this mutant lacks the ability to initiate a key event (*e.g.* auxin-induced proton secretion) which culminates in auxin-induced cell wall loosening. In addition, these results indicate a clear separation between the sites for auxin transport and those for physiological action including cell extension and induction of ethylene synthesis. It is possible the altered polypeptides in *dgt* hypocotyls that lack azido-IAA binding (11) represent the molecular basis of this hypothesized defect.

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