The *diageotropica* Mutation and Synthetic Auxins Differentially Affect the Expression of Auxin-Regulated Genes in Tomato

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The effect of a tomato (*Lycopersicon esculentum*) mutation, *diageotropica* (*dgt*), on the accumulation of mRNA corresponding to tobacco homologs of three auxin-regulated genes, LeAux, LeSAUR, and Lepar, was examined. The *dgt* mutation inhibited the induction of LeAux and LeSAUR mRNA accumulation by naphthalene acetic acid (NAA) but had no effect on NAA-induced Lepar mRNA accumulation. The effect of two synthetic auxins, NAA and 3,7-dichloro-8-quinoline carboxylic acid (quinclorac), on the accumulation of LeAux, LeSAUR, and Lepar mRNA was also examined. Quinclorac induced the expression of each of the auxin-regulated genes, confirming its proposed mode of herbicidal action as an auxin-type herbicide. Concentrations of quinclorac at least 100-fold higher than NAA were required to induce LeAux and LeSAUR mRNA accumulation to similar levels, whereas Lepar mRNA accumulation was induced by similar concentrations of NAA and quinclorac. Collectively, these data suggest the presence of two auxin-dependent signal transduction pathways: one that regulates LeSAUR and LeAux mRNA accumulation and is interrupted by the *dgt* mutation and a second that regulates Lepar mRNA accumulation and is not defective in *dgt* tomato hypocotyls. These two auxin-regulated signal transduction pathways can be further discriminated by the action of two synthetic auxins, NAA and quinclorac.

Auxins are a class of plant growth regulators that play a major role in many aspects of plant development, including cell elongation, cell division, and cell differentiation (Went and Thimann, 1937). Recently, considerable progress has been made in elucidating the mechanisms of auxin action at the molecular level (Estelle, 1992). For instance, a number of genes that are induced very rapidly by exogenous auxin have been isolated, and the induction of these genes is postulated to be one of the initial plant cell responses to auxin (reviewed by Guilfoyle, 1986; Theologis, 1986; Key, 1989). Although these auxin-regulated genes are thought to be the targets of auxin-mediated signal transduction events and to be potentially responsible for auxin-mediated cellular responses, their precise biochemical or physiological function is not known.

A powerful approach to the study of hormone-mediated signal transduction pathways and hormone-mediated physiological responses has been the characterization of mutants altered in specific hormone responses. The tomato (*Lycopersicon esculentum*) mutant *dgt* is a good example of this. *dgt* mutants are characterized by diagravitropic shoot growth (Zobel, 1973) and have been shown to be auxin insensitive (Kelly and Bradford, 1986). Hicks et al. (1989) suggested that the *dgt* mutant may have a defect associated with a primary site of auxin signal perception and, using [3H]NAA, showed that the *dgt* shoot was deficient in a 40- to 42-kD auxin-binding protein that may function as an auxin receptor.

Although the tomato *dgt* mutant has been characterized both physiologically and biochemically and the lesion has been suggested to be associated with a primary site of auxin perception (Kelly and Bradford, 1986; Hicks et al., 1989), relatively little attention has focused on the relationship between the *dgt* lesion and known molecular responses to auxin (Zurek et al., 1994). Because *dgt* is defective in some auxin-mediated responses, such as auxin-induced ethylene production, and is deficient in a shoot-localized auxin-binding protein, it was of interest to investigate the effect of the *dgt* mutation on the expression of several auxin-regulated genes. These experiments established the relationship among the activity of specific auxin-regulated genes, the 40-kD putative auxin receptor, and the auxin-regulated physiological responses that are defective in the tomato *dgt* mutant.

**MATERIALS AND METHODS**

**Plant Materials**

Wild-type tomato (*Lycopersicon esculentum* VFN8) seeds were obtained from Dr. K.J. Bradford (University of California, Davis), and mutant *dgt* seeds in a VFN8 background were obtained from Dr. Terry Lomax (Oregon State University, Corvallis). Etiolated tomato seedlings were grown hydroponically in aerated 1X Hoagland solution in the dark for 4 d at 29°C. Hypocotyl length was 4 to 5 cm at harvest.

**Auxin Treatment of Hypocotyls**

Auxin treatment of seedlings was carried out by excising etiolated hypocotyls into 6-mm sections. The sections were preincubated for 2 h in 1 mM KH2PO4 (pH 7) buffer to

Abbreviations: *dgt*, *diageotropica*; NAA, naphthalene acetic acid; quinclorac, 3,7-dichloro-8-quinoline carboxylic acid.
deplete endogenous auxin and then transferred to fresh 1 mM KH$_2$PO$_4$ containing NAA or quinclorac (synthesized and provided as a gift from Sumitomo Chemical Co., Hyogo-Ken, Japan) at the indicated concentration. Incubations were carried out at 25°C in the light with gentle agitation on a rotary shaker. After 2 h of NAA treatment, sections were frozen in liquid nitrogen for RNA isolation. Measurement of ethylene production and hypocotyl elongation was conducted as described by Kelly and Bradford (1986).

**PCR Amplification of Tomato Auxin-Regulated Genes**

Total RNA was isolated from auxin-treated tomato hypocotyls by the method of Guilliano et al. (1993). Tomato auxin-regulated genes were amplified by reverse transcription and PCR. Briefly, 1 µg of total RNA was heated to 65°C for 5 min, placed on ice, and incubated with Moloney murine leukemia virus reverse transcriptase (100 units; BRL) for 30 min at 42°C in a total volume of 10 µL, containing 1 X first-strand buffer (50 mM Tris-HCl [pH 8.3], 37.5 mM KCl, 1.5 mM MgCl$_2$), 0.5 mM each deoxynucleotide triphosphate, 10 mM DTT, 10 units of RNasin (Promega), and 100 pmol of random hexamer primers (Promega). Following the reverse transcription reaction, the mixture was heated at 94°C for 5 min to denature reverse transcriptase and used for the PCR amplification. Degenerate primers were designed from known auxin-regulated genes as described in Figure 1. Amplifications were carried out in a final volume of 50 µL containing 1 X PCR buffer (50 mM KCl, 10 mM Tris-HCl [pH 9], 1.5 mM MgCl$_2$, 0.01% gelatin [w/v], 0.1% Triton X-100 [v/v]), 0.2 mM each deoxynucleotide triphosphate, 25 pmol of each primer, and 0.25 unit of AmpliTaq DNA polymerase (Perkin-Elmer Cetus) and 10 mM DTT, 10 units of RNasin (Promega), and 100 pmol of random hexamer primers (Promega). Following the reverse transcription reaction, the mixture was heated at 94°C for 5 min to denature reverse transcriptase and used for the PCR amplification. Degenerate primers were designed from known auxin-regulated genes as described in Figure 1. Amplifications were carried out in a final volume of 50 µL containing 1 X PCR buffer (50 mM KCl, 10 mM Tris-HCl [pH 9], 1.5 mM MgCl$_2$, 0.01% gelatin [w/v], 0.1% Triton X-100 [v/v]), 0.2 mM each deoxynucleotide triphosphate, 25 pmol of each primer, and 0.25 unit of AmpliTaq DNA polymerase (Perkin-Elmer Cetus) and overlaid with 50 µL of mineral oil. PCR was performed in an automated thermal cycler (Perkin-Elmer Cetus) for 30 cycles with the denaturation for 1 min at 94°C, annealing for 2 min at 55°C, and extension for 2 min at 72°C. The PCR products were subcloned into the PCR-II vector (Invitrogen, San Diego, CA), and the sequence of the cloned products was determined by double-stranded sequencing by the chain termination method using dideoxynucleotides and Sequenase II (United States Biochemical).

**RNA Gel Blot Hybridization**

Total RNA was isolated as described above, and the amount of RNA was quantified spectrophotometrically. Total RNA (20–30 µg) was electrophoresed on a 1.2% agarose formaldehyde denaturing gel, transferred to a nylon membrane (Hybond N, Amersham), and cross-linked to the membrane by UV illumination. Prehybridization was carried out in 50% formamide, 6X SSC, 5X Denhardt’s solution, 0.5% SDS, and 100 µg/mL base-denatured salmon sperm DNA at 37°C. Hybridization was carried out in the same solution using a $^{32}$P-labeled probe. $^{32}$P-labeled probes for LeAux and Lepar mRNA were prepared by random priming of the cDNAs isolated by PCR cloning described above (United States Biochemical). Because of the relatively lower level of LeSAUR mRNA, $^{32}$P-labeled antisense RNA probes for LeSAUR were made using SP6 RNA polymerase to transcribe the cDNA insert in the PCR-II TM vector following the supplier’s instructions (Promega). The samples were washed twice in 1X SSC, 0.1% SDS at room temperature and three times in 0.1X SSC, 0.1% SDS at 65°C. Autoradiography was carried out at −80°C using preflashed Kodak XAR-5 film with an intensifying screen. Hybridization signals were quantified by densitometric scanning using the BioImage analysis system (Millipore).

**RESULTS**

**Cloning of Partial-Length Tomato Auxin-Regulated cDNA**

Tomato auxin-regulated genes were PCR amplified using degenerate oligonucleotide primers corresponding to the highly conserved regions of soybean SAUR (McClure et al., 1989) Arabidopsis AtAux2–11 (Conner et al., 1990), and tobacco parA (Takahashi and Nagata, 1992). The conserved domains and the corresponding degenerate PCR primers used are indicated in boldface in Figure 1. The PCR products of the anticipated size were cloned into a plasmid vector and several inserts from each amplification reaction were completely sequenced. Figure 1 shows the deduced
Amino acid sequence of LeSAUR (Lycopersicon esculentum SAUR), LeAux, and Lepar, which show strong sequence similarity to SAUR, AtAux2-11, and parA, respectively, and presumably encode the corresponding tomato homologs. The deduced amino acid sequences of the tomato homologs were aligned with SAUR, AtAux2-11, and parA homologs from different species. LeSAUR is 70 to 82% identical with the corresponding genes from several other plant species. LeAux and Lepar were 45 to 62% and 59 to 80% identical with the corresponding genes, respectively (Table I).

Activity of Auxin and an Auxin Herbicide in Tomato Auxin Responses

Quinclorac is a herbicide with structural similarities to known auxins that induces auxin-like effects in cucumber (Berghaus and Wuerzer, 1987). Although the herbicidal activity of quinclorac is strong, its auxin-like activity, such as the induction of ethylene production or hypocotyl elongation, is weaker than that of other auxin-type herbicides (Berghaus and Wuerzer, 1987). We tested the effectiveness of quinclorac on ethylene production and hypocotyl elongation in tomato. Quinclorac was not effective in inducing ethylene production (Fig. 2) and is only partially effective in promoting hypocotyl elongation, with the differences between treatments with NAA and quinclorac being significant at the 0.99 confidence level. In contrast, NAA induced both ethylene production and hypocotyl elongation (Fig. 2).

Auxin Regulation of LeAux, LeSAUR, and Lepar mRNA Accumulation in the dgt Mutant

The accumulation of LeAux, LeSAUR, and Lepar mRNA was investigated in the tomato dgt mutant (Fig. 3). In wild-type tomato, LeAux and LeSAUR mRNA accumulation was induced at an NAA concentration as low as 1 μM, whereas 100-fold higher concentrations of NAA did not significantly induce LeAux or LeSAUR in the dgt mutant. In contrast, although induction of Lepar mRNA accumulation in wild-type plants required 100 μM NAA, no difference was observed in its induction by NAA in wild-type and dgt mutant tomato hypocotyls.

Induction of LeAux, LeSAUR, and Lepar mRNA Accumulation by NAA and Quinclorac

Induction of LeAux, LeSAUR, and Lepar mRNA accumulation by the synthetic auxins NAA and quinclorac was also investigated (Fig. 4). Quinclorac induced the expression of each of the auxin-regulated genes, confirming its proposed mode of herbicidal action as an auxin-type herbicide. Concentrations of quinclorac at least 100-fold higher than NAA were required to induce LeAux and LeSAUR mRNA accumulation to similar levels. In contrast, Lepar mRNA accumulation was induced by similar concentrations of NAA and quinclorac (Fig. 4).

Table I. Comparison of the amino acid sequences of auxin-regulated genes

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<thead>
<tr>
<th>Gene from Other Species</th>
<th>Tomato Gene</th>
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<tr>
<td></td>
<td>LeAux</td>
</tr>
<tr>
<td>AtAux2-11</td>
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</tr>
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LeAux, LeSAUR, and Lepar are the tomato homologs of known auxin-regulated genes. The tomato dgt mutation had a severe effect on the induction of LeAux and LeSAUR mRNA accumulation by auxin but did not affect Lepar mRNA accumulation. dgt mutants have been previously shown to be insensitive to auxin with respect to hypocotyl elongation and ethylene production (Kelly and Bradford, 1986). Thus, the induction of LeAux and LeSAUR mRNA accumulation correlated with auxin regulation of ethylene production and hypocotyl elongation in this comparative genetic system. In contrast, Lepar mRNA accumulation in wild-type and dgt mutant tomatoes did not correlate with auxin regulation of these processes, in agreement with the hypothesis that the Lepar gene product is not involved in regulating these auxin-mediated physiological events.

To elucidate the role of these auxin-regulated genes, we investigated the effect of two different auxins, NAA and quinclorac, on the accumulation of LeAux, LeSAUR, and Lepar mRNA. NAA is a synthetic auxin with strong auxin activity, and it promoted typical auxin-regulated responses, such as ethylene production and hypocotyl elongation in tomato. On the other hand, quinclorac, which induces symptoms similar to those induced by other auxin-type herbicides in cucumber, exhibited no auxin activity with respect to ethylene production and only weak promotion of hypocotyl elongation (Berghaus and Wuerzer, 1987). These compounds also exhibited a differential effect in inducing the accumulation of auxin-regulated mRNAs in tomato. Lepar mRNA accumulation was induced by similar concentrations of both NAA and quinclorac (100 μM). However, NAA induced the accumulation of LeAux and LeSAUR strongly at a concentration of 1 μM, whereas induction of mRNA accumulation by quinclorac required much higher concentrations. In addition to the differential sensitivity of these auxin-regulated genes to NAA and quinclorac, it is interesting to note that, in spite of its reduced auxin activity in promoting ethylene production and hypocotyl extension at a concentration of 100 μM, quinclorac induced the expression of LeAux, LeSAUR, and Lepar at this same concentration. Thus, the accumulation of LeAux, LeSAUR, or Lepar mRNA did not correlate with the induction of ethylene production or hypocotyl elongation.

The tobacco parA gene, to which Lepar has high sequence similarity, has been reported to be expressed during the transition from the G0 to S phase in tobacco mesophyll protoplasts (Takahashi et al., 1989). Expression of parA and its homolog parC is regulated by auxin as well as other stimuli, including heavy metals and heat shock (Takahashi and Nagata, 1992). It is possible that parA-like gene products are involved in the control of the cell cycle by auxin but do not contribute to auxin-regulated ethylene production or cell elongation. Quinclorac at 100 μM showed strong phytotoxic activity on tomato grown on agar (data not shown), and the phytotoxic activity of auxin-

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**Figure 3.** Induction of LeAux, LeSAUR, and Lepar mRNA accumulation in wild-type VFN8 and dgt mutant tomato hypocotyls. Total RNA was isolated from VFN8 and VFN8-dgt tomato hypocotyls following the indicated NAA treatment and separated by formaldehyde agarose gel electrophoresis. RNA was transferred to a nylon membrane and the blot was hybridized with 32P-labeled probe and exposed to x-ray film overnight. The graph shows the quantification of the band on x-ray film. CON, Control.

**Figure 4.** Induction of LeAux, LeSAUR, and Lepar mRNA accumulation by NAA and quinclorac. Total RNA was isolated from VFN8 tomato hypocotyls following the indicated NAA or quinclorac treatment and separated by formaldehyde agarose gel electrophoresis. mRNA levels were quantified as described in the legend to Figure 3. CON, Control.
type herbicides is thought to be due to their activity in disturbing cell division (Ashton and Monaco, 1991).

Results obtained in this study suggest the presence of multiple auxin-dependent signal transduction pathways that lead to diverse physiological responses. Our data indicate that NAA induction of LeSAUR and LeAux mRNA accumulation are regulated by a common signal transduction pathway and that this pathway is interrupted by the dgt mutation. The accumulation of Lepar mRNA appears to be regulated by a different pathway, which is not defective in the dgt mutant. The data indicate that these two auxin-regulated signal transduction pathways can also be discriminated by the action of two synthetic auxins, NAA and quinclorac. Gee et al. (1991) demonstrated the differential expression of two auxin-regulated genes, SAUR and GH3, in different organs and suggested that this may implicate distinct auxin receptors and/or signal transduction pathways that are organ specific.

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