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Some Physiological Characteristics of the Ethylene-requiring Tomato Mutant Diageotropica[1]

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ABSTRACT

The diageotropica mutant of tomato (*Lycopersicon esculentum* Mill.) is shown to require exogenous ethylene for normal growth and development. This single gene mutant is characterized by unsupported horizontal growth of shoots and roots, dark green hyponastic leaf segments, thin rigid stems, and primary and adventitious roots which lack lateral roots. Experiments with growth regulators indicate that the mutant does not produce normal amounts of ethylene in response to auxin treatment. Tests with ethylene-producing compounds or ethylene precursors demonstrate that the mutant requires ethylene for normality. Ethylene concentrations as low as 0.005 microliters per liter are capable of completely normalizing mutant characteristics. This mutant with its isogenic parent variety, cv. VFN8, should be a suitable tool for investigating auxin-stimulated ethylene production and their interrelationship in the control of plant morphology and physiology.

The diageotropica mutant of tomato (*Lycopersicon esculentum* Mill.) is characterized by diageotropic growth (unsupported horizontal growth) of the shoot (Fig. 1) and roots, dark green hyponastic leaf segments, thin rigid stems, and roots which lack lateral root primordia (4). The horizontal growth habit is not the result of a lack of response to gravity, but a preferential response whereby a vertically reoriented shoot reassumes a horizontal posture as its apex elongates. The thin stem is able to support the shoot and several small immature fruit in a horizontal attitude until the apex is over 50 cm from physical support. Primary and adventitious roots branch only when the root apex has been severely damaged or destroyed. Despite the absence of lateral root primordia, adventitious rooting appears normal. Mutant plants set fruit which ripen normally and have many seeds (4).

The use of mutants in higher plant research has met with limited success. Although complex developmental patterns are partially responsible, the complex or inadequately understood inheritance of the mutant or its parent lines is often the major handicap. Genetic studies of this mutant have shown it to be conditioned by a point mutation (3). The accurate genetic information about dgt[2] and the presence of an isogenic parent variety make this mutant suitable for studying biochemical, physiological, and morphological characteristics modified by the mutation.

A detailed discussion of the mutant's morphology and some other aspects of its utility will be published elsewhere. Results of initial investigations to determine the physiological basis for the many pleotropic characteristics of this mutant are presented in this paper.

MATERIALS AND METHODS

Plant material consisted of greenhouse grown dgt plants and the isogenic parental variety cv. VFN8 as control.

Chemical Treatment of Whole Plants. Solutions of growth regulators and other chemicals were applied after dark by spraying leaves of 2-month-old plants until runoff with a deVilbis atomizer. A spreading agent was not required since adequate amounts remain on leaf and stem surfaces. Responses were noted 7 days after treatment.

Ethylene Treatments. Fixed concentrations of ethylene were supplied to whole plants by means of a gas flow system similar to the one described by Goeschl (2). With this system, it was possible to provide accurate continuous flow ethylene atmospheres down to 0.005 μl/l depending upon the ethylene concentration in the air.

Whole plants were placed in 30-liter containers with an ethylene-air flow rate of 7.5 liters/hr through the container. Concentrations of 10, 1, 0.1, 0.05, 0.005 and 0.0 μl/l ethylene were applied to plants over a 12-hr period. Lower concentrations could not be accurately measured with available equipment. Treatments, at each concentration, except 10 and 1 μl/l, were repeated three times with two mutant plants at each concentration. Fresh, untreated plants were used in each replicate. No light was provided during treatment. For controls, untreated mutant and normal plants were placed in sealed containers and left on the bench top with the treated material during the 12-hr experiment.

Chemical Treatment of Stem Sections and Leaf Segments. Whole plants were brought into the laboratory from the greenhouse. Leaves were immediately cut off the main stem and side branches at the base of the petiole, and leaf segments in turn were cut off the petiole. These were weighed and 1 to 2 g of the leaf material placed in a 50-ml Erlenmeyer flask. The remaining stem internode regions were cut into pieces 20 to 30 mm long, weighed, and placed in 50-ml Erlenmeyer flasks at 10 g/flask.

In an initial experiment, 13-ml vials were used with 0.5 to 1 g of stem or leaf tissue in each. One milliliter of water was added to each vial, and one-fifth of the vials were immediately sealed with serum caps. These first vials were sampled for ethylene content after 2 hr, air was blown into each vial to flush out ethylene, and they were then recapped along with

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[2] Abbreviation: dgt: diageotropica.

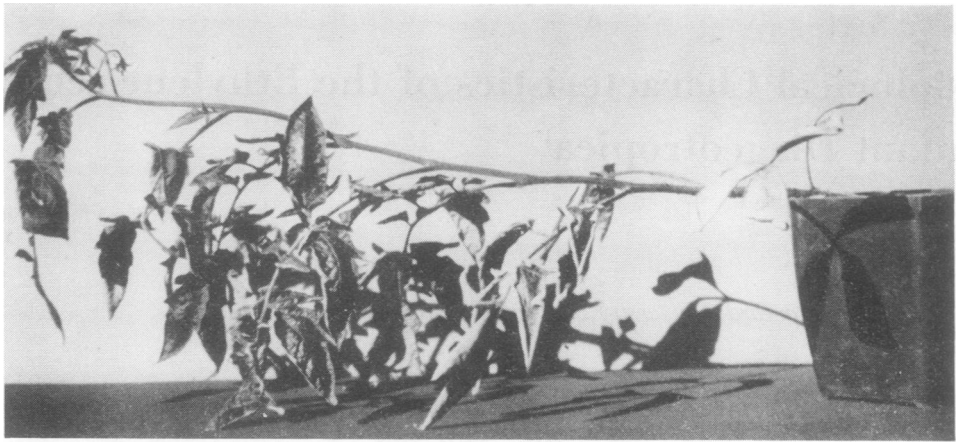

FIG. 1. A 2-month-old plant of dgt exhibiting a diageotropic habit and hyponastic leaves. (The slight negative geotropy and flat character of very young portions of this plant are atypical. Normally, the mutant apex is perfectly diageotropic and leaf segments become hyponastic as the leaf expands.) X one-half. (Reprinted from ref. 3 by permission of the Journal of Heredity.)

another one-fifth of the vials. This procedure was repeated three more times, each time an additional group of vials was capped. After the last vial was capped, they were left for 12 hr in the dark and then sampled a final time for ethylene content. Sampling consisted of extracting 1 ml of air from a vial and injecting this into a gas chromatograph to determine the ethylene concentration, compared to an 0.88 μl/l standard.

To examine the response of these tissues to chemicals which produce ethylene or stimulate ethylene production, 5 ml of 5 μM IAA, or methionine, or propanal solution was added to the flasks after a 6-hr delay period to avoid "wound ethylene." After adding the solutions, the flasks were shaken thoroughly, and any excess liquid was poured out. Flasks were immediately capped with serum caps and placed in an incubator in the dark at 25 C for 12 hr. Ethylene concentrations were determined with a gas chromatograph, and the rate of production was calculated.

RESULTS

A cursory consideration of the development and morphology of the mutant—diageotropy, lack of lateral root primordia, thin stems, and hyponastic leaf segments—suggests an auxin-related morphology, perhaps conditioned by a breakdown in some auxin-mediated process. To investigate this possibility, the following chemicals were sprayed on young plants in three inch pots: catechol, caffeic acid, tryptophane, GA, kinetin, riboflavin, riboflavin monophosphate, and IAA. The responses are summarized in Table I.

Mutant plants gave no response to 10 μM IAA, while strong epinasty was observed in the isogenic control. Riboflavin stimulated a few lateral roots in dgt; however, the root systems were not normal. Application of gibberellin caused comparable increases in internode length in both normal and dgt plants, indicating no significant difference in their responses. Catechol and caffeic acid had no observable effect on the mutant or the control; indicating that the effect which conditions the dgt morphology may not be one of auxin degradation or conjugation. The lack of response with tryptophane and kinetin indicates that neither of these are active in modifying the mutant, and most likely are not involved in conditioning its morphology.

On the basis of the previous results, a second experiment to test IAA at 10 μM and 100 μM was initiated. Since IAA-induced epinasty is known to be caused by ethylene production, a parallel experiment treating plants with 10 and 100 μM Ethephon (Ethrel) was also initiated. The chemicals were applied as foliar sprays to young nonflowering plants in 3-inch pots. Mutant sensitivity to IAA was approximately 10-fold

Table I. *Response of Nonflowering dgt and Normal Plants to Growth Regulators*

Five plants of each line were treated with each compound; dgt = mutant phenotype; + = normal phenotype.

Compound	Concn	dgt		Normal	
		Roots	Shoots	Roots	Shoots
H_2O		dgt	dgt	+	+
IAA	10^{-5} M	dgt	dgt	+	+[1]
GA	10^{-4} M	dgt	dgt[2]	+	+[2]
Kinetin	10^{-6} M	dgt	dgt	+	+
Trytophan	10^{-5} M	dgt	dgt	+	+
Riboflavin	10^{-5} M	dgt[3]	dgt	+	+
Riboflavin-mono-phosphate	10^{-5} M	dgt	dgt	+	+
Catechol	10^{-5} M	dgt	dgt	+	+
Caffeic acid	10^{-5} M	dgt	dgt	+	+

[1] Very strong epinasty observed in normals only.
[2] Internodes elongated.
[3] A few lateral roots were formed.

Table II. *Responses of Nonflowering dgt and Normal Plants to IAA and Ethrel*

Five plants of each were used for each treatment. dgt = mutant phenotype; + = normal phenotype.

Treatment	Concn	dgt		Normal	
		Roots	Shoots	Roots	Shoots
IAA spray	10^{-4} M	+[1]	+[2]	+	+[3]
IAA spray	10^{-5} M	dgt	dgt	+	+[2]
Ethrel spray	10^{-4} M	+	+[3]	+	+[3]
Ethrel spray	10^{-5} M	+	+[2]	+	+[2]

[1] Increase in branching although not normal.
[2] Strong epinasty and normal geotropic response.
[3] Extreme epinasty and normal geotropic response.

lower than that demonstrated by the normal (Table II). Both IAA concentrations produced near maximum epinasty in the normal, while only the high concentration produced epinasty in dgt. Both foliar Ethrel applications, on the other hand, produced near maximum epinasty in both dgt and normal plants. Only Ethrel and high IAA applications normalized the mutants geotropy and root development. This normalization consisted of the apical 3 to 7 inches bending from a horizontal orientation into a vertical orientation, and primary and adventitious roots began developing lateral roots where no primordia had previously existed. This was in addition to strong epinasty in the shoot.

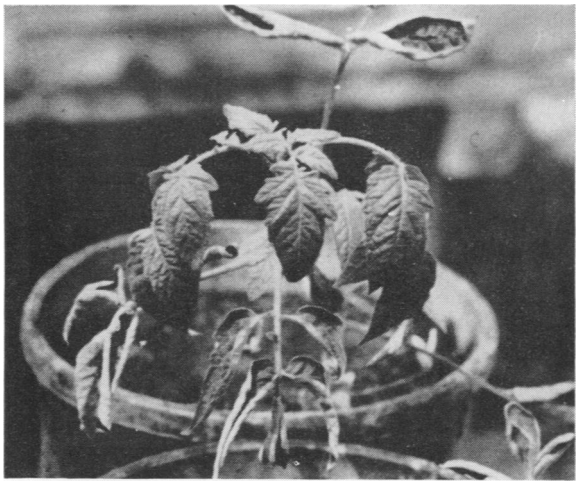

FIG. 2. An ethylene-treated dgt shoot demonstrating the "normalized" young leaves and hyponastic older leaves. × 1

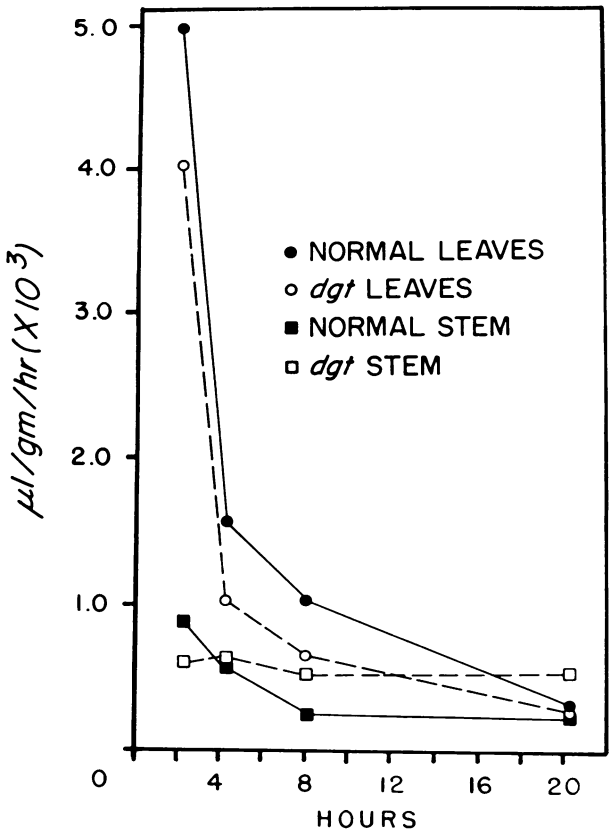

FIG. 3. Ethylene production expressed in μl/gm·hr by dgt and VFN8 stem section and leaf tissue for the first 20 hr after excision.

Ethylene Treatments. After this interesting result, the next obvious move was to test ethylene as a possible "normalizer" of dgt. To establish that Ethrel effects are caused by ethylene, several young nonflowering mutant plants were treated with several different concentrations of the gas for 12 hr. Ethylene at all concentrations tested including 0.005 μl/l, elicited morphological normalization of mutant plants. Young expanding leaf segments became normal in shape while mature, fully expanded leaves were not modified and maintained their hyponastic leaf segments (Fig. 2). The elongating portion of the stem became normally geotropic (this consists of the apical 7 cm in tomato), demonstrating a normal response to gravity only on ethylene treatment. Lateral roots were initiated on unbranched primary and adventitious roots previously devoid of lateral primordia. Concentrations in excess of 0.1 μl/l also induced epinasty. Mutant plants in sealed containers maintained their mutant morphology while similarly treated normal plants became epinastic over periods in excess of 24 hr.

Experiments with Excised Tissue. Experiments were initiated in an attempt to determine the extent to which dgt produces ethylene naturally. The first experiment was designed to determine the relative rates of ethylene production in stem and leaf tissue, and, if possible, to differentiate between dgt and normal tissues. In this experiment, duration of ethylene production in response to cutting the tissue ("wound" ethylene) was also studied. The results (Fig. 3) brought out two very interesting points: leaves, although producing more ethylene initially than stem tissue, return to a level similar to normal stem tissue; in contrast, mutant stem tissue starts out at a lower rate than normal stem tissue and continues at about the same rate for at least 20 hr. The final rate of ethylene production for dgt stem tissue is about 0.006 μl/g·hr, and for normal tissue about 0.002 μl/g·hr. Normal leaf and stem tissues and dgt leaf tissues have a typical wound ethylene production curve, while dgt stem tissue gives a constant rate without an initial peak. It is therefore impossible to differentiate between wound and normal endogenous ethylene production in dgt stem tissue.

A subsequent experiment examined the effect of several compounds on the production of ethylene by excised stem tissue. Stem tissue pieces treated with IAA, methionine, or propanal were compared with water-treated stem tissue pieces for ethylene production (Fig. 4). Methionine and propanal treatments resulted in ethylene production rates in dgt and normal tissue not significantly higher than the water control. The IAA treatment however caused relatively extensive ethylene production in normal tissue with considerably less ethylene production in dgt stem tissue (Fig. 4). These results indicate that IAA treatment, at concentrations at or below optimum for stem tissue (0.5 μM), induces ethylene production at a rate in normal tissue which is six times that in dgt (Fig. 5). The obvious conclusion is, under these conditions dgt produces significantly less ethylene in response to IAA treatment than its isogenic parent.

DISCUSSION

The dgt mutant in tomato has been shown to be a point mutation and to be isogenic with cv. VFN8 (3). Briefly, the dgt morphology is therefore conditioned by a change in its DNA of one nucleotide pair. This results in one enzyme or another protein having a single amino acid changed from that found in cv. VFN8. The only difference between dgt and its isogenic control variety is therefore, a single DNA nucleotide pair and its resultant single amino acid in a single enzyme. Any re-

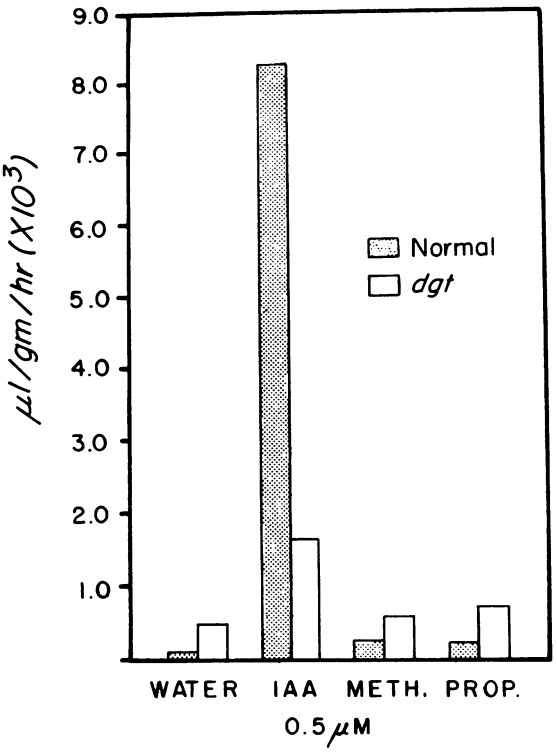

FIG. 4. Ethylene production expressed in μl/gm·hr by stem sections in response to ethylene-stimulating or ethylene-producing compounds. Chemicals added at 0.5 μM 6 hr after excision.

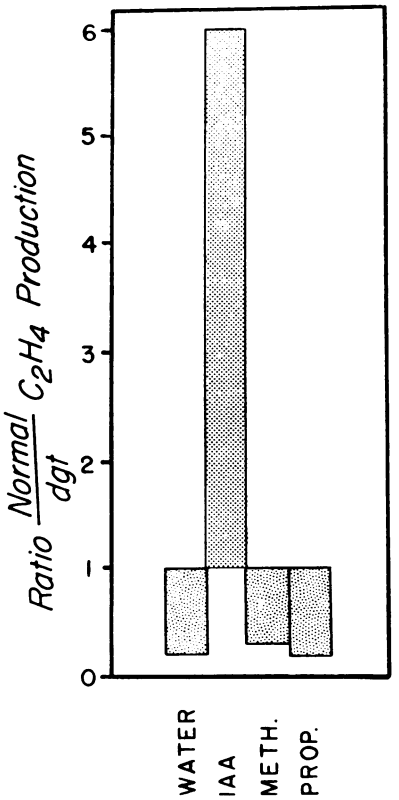

FIG. 5. Ratio of rates of ethylene production for VFN8 *versus* dgt stem sections treated with ethylene-stimulating or ethylene-producing compounds.

sponse in VFN8 plants not paralleled by an identical response in dgt must, therefore, be due to this single difference between them. The converse, a response in dgt not paralleled by one in VFN8, is also due to the single difference.

In the initial experiment to test the hypothesis that dgt morphology was conditioned by a disruption in normal auxin-mediated responses, none of the tested chemicals, except IAA, gave a significant response at the concentrations applied. Epinasty in normal plants but not in dgt in response to IAA indicates a significant difference between the two plants. The lack of a response to catechol or caffeic acid by dgt argues against the obvious possibility of increased IAA decarboxylation or conjugation as the cause of dgt morphology and reduced sensitivity to IAA. The results in Table II indicate a reduction in dgt sensitivity to IAA of about 10-fold. These results were based upon induction of epinasty which is known to be the result of ethylene synthesis in response to IAA treatment. Epinasty in normal plants but not in dgt indicates that auxin-induced ethylene synthesis either has not occurred at normal rates in dgt, or that dgt is not as sensitive to ethylene as its isogenic control. The response to Ethrel in both dgt and normal plants indicates that it is a lack of, or reduction in, auxin-induced ethylene synthesis, at IAA concentrations of 10 μM rather than reduced sensitivity to ethylene. Production of a geotropic response and lateral root initiation in dgt by treatment with IAA or Ethrel concentrations sufficient to cause epinasty leads to the possibility that ethylene may have a role in these processes.

Normalization of dgt by ethylene concentrations at or below 0.005 μl/l indicates that internal concentrations must be below that value if there is any endogenous ethylene. The isogenic control, on the other hand, shows no response to ethylene until epinasty appears at concentrations around 0.1 μl/l. On several occasions, ambient ethylene concentrations have been sufficiently high (up to 8 nl/l) to cause dgt normalization. The level of ethylene active in this normalization of dgt is at or below previously estimated threshold values for the responses involved, and also at or below previously estimated internal concentrations for most plants (1). Since dgt is isogenic with VFN8 and 0.005 μl/l of ethylene is sufficient to cause (*a*) induction of lateral root primordia and subsequent lateral root development, (*b*) initiation of a normal geotropic response in actively elongating stem tissue, and (*c*) expanding leaf segments to become normal in appearance rather than hyponastic, it can be assumed that dgt requires exogenous ethylene for normal development. It can also be assumed that in tomato these morphogenetic processes require at least extremely low concentrations of ethylene for "normality."

Results from treatment of excised tissues with IAA, methionine, propanal, and water provide a further insight into the nature of the ethylene requirement for dgt. As previously discussed, dgt has an approximately 10-fold reduction in sensitivity to IAA as measured by epinasty, *i.e.* auxin-induced ethylene production. The data from excised tissue treatments show that there is a 6-fold difference in dgt sensitivity to IAA as measured by ethylene production. This lower sensitivity is apparently not due to increased destruction or conjugation of IAA, since catechol and caffeic acid have little effect and other IAA-mediated morphogenetic systems are not modified: stem elongation, root elongation, leaf elongation, adventitious rooting, flowering, and fruit development. Optimum concentrations of active IAA for these later characters are in the range of those used in the excised tissue experiments. It appears that the effect of the mutation which conditions the morphology of dgt is to cause a reduction in or elimination of auxin-induced ethylene synthesis.

The abnormal response of dgt stem tissue to wounding, as measured by ethylene production, is difficult to explain. From all appearances this response tends to controvert the idea of a lack of ethylene production or even reduced ethylene production in dgt stem tissue. The lack of a peak in production, then a tapering off to "normal" production is suspicious. The climb to a plateau and then continuous production at that rate for up to 20 hr can best be explained by assuming the presence of a substance in higher than normal concentrations which may be slowly degraded to ethylene and other compounds by the many destructive enzymes present. This is, however, pure conjecture and is supported only by unpublished results (Zobel, unpublished). The abnormal nature of the "wound" ethylene peak of dgt stem tissue is itself the best argument against the idea that it indicates a "normal" high rate of ethylene production.

Fruit develop normally, implying a normal climacteric, and the established precursor to ethylene in fruit, methionine, is readily converted to ethylene by dgt flowers (4). Light acting via phytochrome has been shown to control ethylene production in dgt floral tissues, but not in stem or leaf tissues (4). Normal development of tomato fruit and production of ethylene by flowers, in contrast to a general need for exogenous ethylene by stem, root, and leaf tissues for normal development, indicates a possible divergence of mechanisms controlling ethylene synthesis in different tissues.

Without time course experiments, the characteristics of ethylene control over the processes stated here, as requiring at least minute amounts of ethylene for normality, cannot be determined. Although the precise role of ethylene control over these characters cannot be determined from the data presented, it can be concluded that it does not act directly, since exogenous application of ethylene would result in equal action on all parts of the plant. It would be logical to speculate that ethylene control is mediated through its modification of auxin transport. Although this hypothesis is supported by research with greater than threshold concentrations of ethylene, it is only hinted at by the characteristic modifications of this single gene mutant and must await further extensive investigation.

Indeed, the response of this single gene mutant to such extremely low concentrations of ethylene, while its isogenic control does not respond to such low levels, questions the conclusions arrived at by previous research on ethylene-plant interactions. The lowest level of ethylene reported as normalizing dgt is able to completely normalize the mutant and is therefore at least optimal for those responses in the mutant and its isogenic control. Much of the previous research on effects of ethylene on plants has been carried out at concentrations in excess of 0.05 μl/l. These concentrations are at least 10-fold greater than those necessary to normalize dgt. It appears therefore that in tomato, at least, concentrations of ethylene in excess of 0.005 μl/l may be supraoptimal, and results derived from studies with such concentrations may not be applicable to normal tomato responses to ethylene. Although many of the responses to ethylene noted for dgt can be explained by reference to previous theories based on supraoptimal ethylene concentrations, assessments of their validity must await further study.

The only definite conclusion which may be validly arrived at based upon the data presented is that the dgt mutant of tomato requires extremely low concentrations of ethylene for normal morphological development, and that this appears to be due to a reduction in or elimination of auxin-induced ethylene synthesis. The results clearly show a requirement by several different tomato plant organs for extremely low concentrations of ethylene for normal development. This mutant and its isogenic control variety offer an unusually simple system for the characterization of ethylene effects at threshold concentrations. In its sensitivity to ethylene and its rather general need for exogenous ethylene of extremely low concentrations, dgt should prove a very useful tool for further characterization of the control of plant development which auxin and ethylene so dramatically mediate.

Seeds of the mutant and its control variety are available from the author.

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