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Communication

A Unique Phenotype in Heterozygotes of the Auxin-Insensitive Mutant of Tomato, *diageotropica*[1]

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ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) plants heterozygous for the *diageotropica* (*dgt*) mutation exhibit a unique phenotype, termed '*mottled*.' Unlike *dgt*, *mottled* individuals grow upright, exhibit normal root branching, and produce normal levels of ethylene in response to applied auxin. Leaves of *mottled* plants are deformed and reduced in size and are characterized by a mottled appearance on their surfaces with small dark-green islands clustered along the leaf veins. The lack of phenotypic overlap between *dgt* and *mottled* may represent interallelic interaction at a locus which influences auxin sensitivity or action in the tomato.

The *dgt*[3] mutant of the tomato was first described by Zobel (14) and was considered to be completely recessive. *Dgt* plants are characterized by diagravitropic (horizontal) shoot and root growth, thin stems, hyponastic leaves, altered phyllotaxy, and a lack of lateral roots (15). The geotropic orientation and root branching of *dgt* can be normalized by treatment with exogenous ethylene, but horizontal growth is resumed when the ethylene is removed (7, 15). Stems, roots, hypocotyls, and petioles of *dgt* produce reduced levels of ethylene compared to normal tissue after treatment with exogenous auxin; however, stresses such as wounding and anaerobiosis or treatment with the fungal toxin, fusicoccin, will induce ethylene production in *dgt* (1, 7, 8). Moreover, auxin has greatly reduced activity in promoting hypocotyl elongation (8) and petiole epinasty in *dgt* (1, 2). Based on these observations, it has been proposed that physiological insensitivity to auxin is the primary lesion resulting in the characteristic *dgt* phenotype (8).

Progeny from crosses between *dgt* and VFN8 exhibit normal phenotypes with respect to the major abnormalities present in *dgt* (14). However, we have observed plants with an unusual and distinct phenotype, termed '*mottled*,' in F_1 progeny from *dgt* × VFN8 crosses. The appearance of *mottled* individuals in segregating populations coincided with heterozygocity at the *dgt* locus. We report here on the morphology, genetics, and auxin sensitivity of the *dgt* heterozygote.

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[3] Abbreviations: *dgt*, *diageotropica*; TMV, tobacco mosaic virus; Adh_1, alcohol dehydrogenase.

MATERIALS AND METHODS

Plant Material and Genetics

Seeds of VFN8 tomato (*Lycopersicon esculentum* Mill.), the isogenic mutant line, *dgt*, and a nonisogenic line, VF36, were obtained from Dr. C. M. Rick, Department of Vegetable Crops, University of California, Davis, and have been maintained by self-pollination in greenhouse culture. A second nonisogenic line, T5, was obtained from Dr. R. A. Jones of the same department. Crosses were made by emasculation and hand pollination. Populations (F_1, F_2, and F_3) were grown in the greenhouse in flats and scored at approximately 4 weeks for the *dgt* characteristics, and at 6 to 8 weeks for the mottled features. Deviations from expected segregation ratios for a partially dominant locus were tested by χ^2.

Auxin Induction of Ethylene Synthesis

Ethylene production in response to 2,4-D was determined by placing moistened 1-g leaf tissue samples into 50-mL flasks, which were sealed with serum caps and kept at 28°C in the light. Ethylene production was measured in 1-mL samples of the gas phase by gas chromatography (Carle 211 gas chromatograph with flame ionization detector) at approximately 4-h intervals over a 20-h period prior to auxin treatment. After each measurement, flasks were purged with air and recapped. Tissues were treated with a solution of 100 μM 2,4-D (pH 5.5) by filling flasks with the auxin solution for 1 h, then decanting. Ethylene production was determined over the subsequent 8-h period.

Petiole Epinasty Assay

Stem sections with attached petioles were excised from the third, fourth, and fifth nodes above the cotyledonary node, approximately 6 weeks after sowing. Petioles were debladed 1 cm from the axil, and the stems were trimmed so that 1 cm of stem remained above the axil and 1 to 2 cm of stem remained below. Excised sections were incubated on a low-speed shaker in a volume of distilled water that kept petioles wet but not submerged for at least 1 h prior to use in the epinasty assay. After the incubation period, the explants were supported in an upright position in inverted serum caps inside an aerated humidity chamber. Agar blocks (0.4%) containing 50 μM IAA were placed on the cut petiole surfaces. Petiole angles were measured using a transparent protractor just prior

to treatment, and again 8 h after treatment. Epinasty was indicated by the increase in the angle from the stem of the adaxial portion of the first cm of the petiole.

RESULTS AND DISCUSSION

Plants heterozygous for *dgt* are normal with respect to most characteristics of the *dgt* phenotype, but exhibit a distinctive mottling of light and dark green patches on the leaf surface. When viewed with back-lighting, small dark-green islands can be seen clustered along the leaf veins (Fig. 1). The mottling of the leaves of the heterozygote may be due to interspersed regions of the dark green pigmentation, characteristic of *dgt*, and the lighter green pigmentation of the wild type phenotype (Fig. 1). Faint mottling is usually apparent in the second or third fully expanded leaf, and mottling, deformity, and reduction in size of leaves increase in severity as the plants develop. Flowering in *mottled* is severely reduced due to a high incidence of flower abscission before anthesis. Flowers that do develop appear normal, but fruits contain few seeds. Normal shoot and root gravitropism and lateral root formation were observed in *mottled* plants, but decreased shoot apical dominance was evident. The *mottled* phenotype resembles symptoms of TMV infection in tomato; however, plants exhibiting the *mottled* phenotype tested negative for the presence of this virus. In addition, F_1 progeny of *dgt* with T5, a tomato line homozygous for a dominant resistance to TMV (RA Jones, personal communication), also exhibited the mottled phenotype.

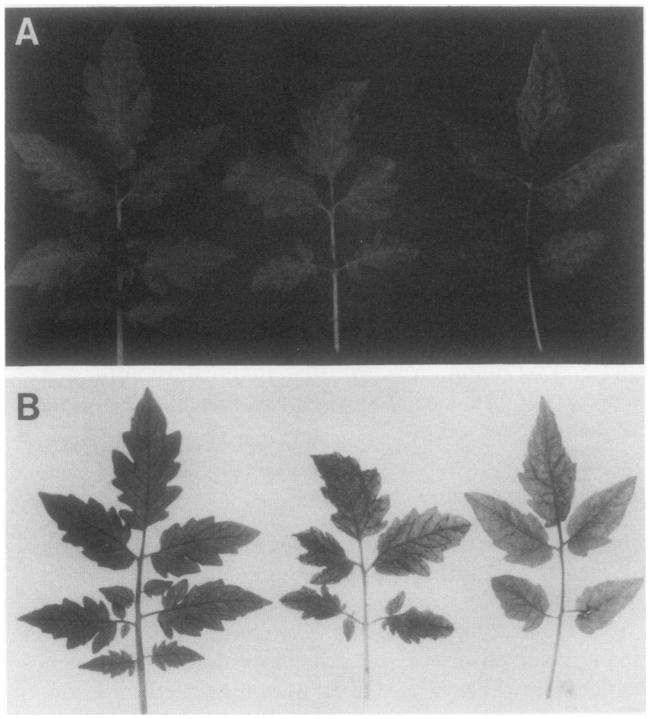

Figure 1. A, Adaxial surfaces of leaf blades of VFN8 (left), *mottled* (center), and *dgt* (right) from the sixth node of 4-week-old plants. Note the misshapen leaflets in *mottled*, particularly along the veins. B, View of abaxial surfaces of the same leaves with back-lighting to show dark green, opaque patches in *mottled* leaf (center). The abnormal features of *mottled* leaves tend to increase in severity as the plants mature.

Table I. *F_1, F_2, and F_3 Segregation Analysis of VFN8 × dgt and VF36 × dgt*

Population	Total	wt[a]	*mottled*	*dgt*	χ^{2b}	P[c]
			frequency			
VFN8 × *dgt* F_1	49	0	49	0		
VF36 × *dgt* F_1	50	0	50	0		
(VFN8 × *dgt*) F_2	44	12	20	12	0.3	0.9
F_3 from selfed *mottled* F_2 plants	199	59	103	37	5.1	0.2

[a] Wild type. [b] χ^2 for expected ratio (1:2:1) for a single, partially dominant locus. [c] Probability that deviation from expected ratio is random.

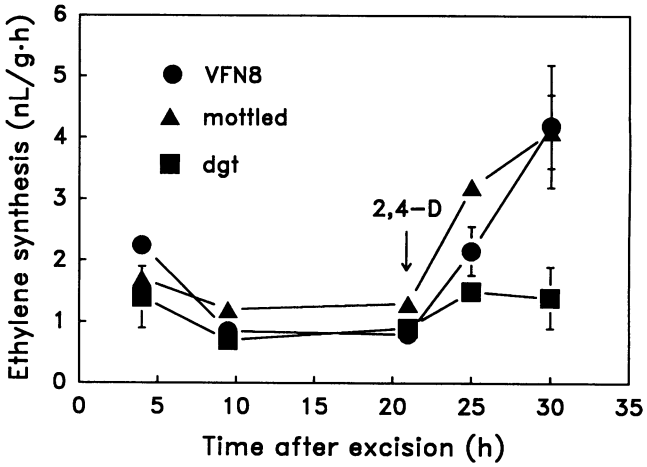

Figure 2. Time courses of ethylene synthesis by petiole and leaf sections from VFN8, *mottled*, and *dgt*, before and after treatment with 100 μM 2,4-D. Error bars indicate ± SE (n = 3).

The genetic basis of *mottled* was confirmed through segregation analysis. All progeny of crosses between *dgt* and the isogenic parent line, VFN8, or the non-isogenic lines, VF36 and T5, exhibited the *mottled* phenotype. F_2 and F_3 progeny of selfed *mottled* plants (from *dgt* × VFN8) were tested for goodness of fit to a 1:2:1 segregation of wild type (+/+) to mottled (+/*dgt*) to *dgt* (*dgt*/*dgt*), respectively (Table I). No significant deviation from this hypothesis was observed, confirming the genotype of *mottled* as +/*dgt*. Although we observe 100% penetrance of the *mottled* phenotype under our growth conditions, heterozygous individuals may be practically indistinguishable from wild type individuals under other environmental conditions (RW Zobel, personal communication). An environmental influence on the phenotypic expression of *mottled* is perhaps not surprising, since phenotypic variability has also been noted in *dgt* plants grown under different light and temperature conditions (unpublished observations).

Wound-induced and basal levels of ethylene production, determined during 20 h after excision of leaf and petiole tissue from the upper nodes of 30-d-old plants, did not differ significantly between VFN8, *dgt*, and *mottled* (Fig. 2). After exposure to 100 μM 2,4-D, ethylene synthesis rates increased only

slightly in *dgt* but increased nearly fourfold in VFN8 and *mottled* (Fig. 2). Similar results were obtained using IAA (data not shown). With respect to the induction of ethylene synthesis by auxin, *mottled* did not differ significantly from the wild type.

Sensitivity to auxin was also tested in *mottled* plants by measuring IAA-induced epinastic growth. Epinastic growth of petioles, which occurs in response to elevated ethylene levels in the tomato, is a sensitive and rapid assay for both ethylene and auxin sensitivity (12). In VFN8, auxin induces petiole epinasty by stimulating ethylene production, while *dgt* petioles are relatively insensitive to auxin (1, 12). The epinastic responses of VFN8, mottled, and *dgt* petioles to 50 μM IAA were 25 ± 2, 24 ± 3, and 13 ± 1 degrees, respectively (means ± SE, $n = 6$). The epinastic response of *mottled* to auxin did not differ significantly from that of VFN8, while both were almost double that of *dgt*, indicating that the *mottled* plants are normal with respect to auxin and ethylene sensitivity in petiole epinasty.

Despite a difference of only a single allele, there is not only little phenotypic overlap between *dgt* homozygotes and heterozygotes, but heterozygocity at the *dgt* locus results in a more extreme and disrupted phenotype in some respects than does homozygocity. Major abnormalities in *mottled* are observed in leaf and flower development and apical dominance, but the phenotype is normal with respect to several of the primary aberrations of *dgt*: geotropism, root branching, and induction of ethylene production and epinasty by auxin. Functional genetic interactions in which relatively weak, unlinked alleles combine to produce a more extreme phenotype have been reported in *Drosophila* (6). Such interactions indicate that the mutant gene products associate to form the functional product. 'Synthetic lethality' has been used to describe the interaction between loci where double heterozygocity (at two nonlethal mutant loci) results in lethality (4). If a gene product functions as a homodimer or multimer, then interallelic interactions of a similar nature are also possible, as mutant subunits may combine with normal subunits, resulting in proteins with different subunit compositions and hence different properties. At the maize Adh_1 locus, positive complementation between normal and mutant subunits results in a heterodimer with wild type activity (10). At the adenylosuccinase locus in *Aspergillus nigulans*, negative complementation between mutant subunits of the multimeric protein results in diminished enzyme activity in heterozygotes (5). It has been proposed that negative complementation is indicative of a multimeric enzyme (2). The occurrence of the *mottled* phenotype of +/*dgt* individuals provides genetic evidence that the *dgt* gene product may function as a dimeric or multimeric protein.

While it is possible that the *dgt* gene product is an auxin receptor (1, 8), there is no direct evidence for this. The kinetics of IAA uptake and polar transport are identical in *dgt* and VFN8 hypocotyls, but *dgt* sections have a slightly greater capacity for IAA transport (3). This makes it unlikely that the mutation has altered IAA uptake or efflux carriers. It is intriguing that some auxin-binding proteins are dimeric molecules (9, 13), and it has recently been shown that the photoaffinity auxin analogue ^{3}H-5N$_3$-IAA specifically labels a polypeptide doublet of 40 and 42 kD in membrane preparations from stems of the parental variety VFN8, but not from stems of *dgt* (G.R. Hicks, D.L. Rayle, and T.L. Lomax, personal communication). The lack of overlap in the phenotypes of *dgt* and *mottled* and the apparent normalization of some auxin responses in *mottled* may indicate that the *dgt* gene product is involved in various aspects of auxin action, *e.g.* regulation of ethylene production (altering gravitropism and root branching), leaf expansion, pigmentation, flower development, and apical dominance, which are differentially influences by the number of *dgt* alleles present. Specific auxin-regulated genes have been identified from several species (11). The relationship between the *dgt* alleles present and the expression of auxin-regulated genes in tomato may provide molecular clues to the function of the *dgt* gene product(s).

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