Roles of Auxin and Ethylene in Adventitious Root Formation by a Flood-resistant Tomato Genotype

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Abstract. The relative contributions of auxin and ethylene (C_2H_4) in stimulating the initiation of adventitious root primordia (ARP) and their subsequent development into adventitious roots (ARs) by flooded tomato (*Lycopersicon esculentum* Mill. PI 406966) seedlings were evaluated using TIBA and STS. Flooded plants treated with STS (F + STS) produced $\approx 40\%$ as many emerged ARs as plants that were flooded only (F). Only 7% of the ARP initiated by F + STS plants developed enough to emerge through the epidermis by 120 hours of treatment compared with 95% emerged for F plants. A band of TIBA applied below the lowest leaves of flooded plants (F + TIBA) virtually eliminated AR formation. Plants with two or four leaves below the TIBA band produced 16- and 35-fold more ARs, respectively, than those with no leaves below the TIBA band. Relative to nonflooded (NF) plants, F + STS plants exhibited a nearly 40-fold increase in C_2H_4 evolution, while F and F + TIBA plants exhibited about a 5-fold increase in C_2H_4 production. These results suggest that auxin accumulation at or above the floodline is essential for ARP initiation and that auxin action is not mediated through C_2H_4 . Ethylene may be required for elongation of flood-induced ARP leading to their emergence as ARs. Chemical names used: 2,3,5-triiodobenzoic acid (TIBA); silver thiosulphate (STS).

Auxin and C_2H_4 have been implicated in the regulation of adventitious root (AR) formation by flooded plants (Jackson and Campbell, 1975; Kramer, 1951), but no studies have established specific roles for these hormones under flooded conditions (Jackson, 1985). Chemical inhibition of polar auxin transport to the lower stem prevented AR initiation by flooded *Helianthus annuus* (Wample and Reid, 1979) and *Acer negundo* seedlings (Yamamoto and Kozlowski, 1987), indicating that auxin accumulation at or above the floodline is essential for AR initiation and/or development. However, apparent auxin effects actually might be mediated by C_2H_4 action, since auxin accumulation stimulates C_2H_4 biosynthesis (Yang and Hoffman, 1984). Application of C_2H_4 -releasing compounds stimulated AR emergence from stems of non-flooded tomato seedlings (Kuo and Chen, 1980; Massei and Valio, 1983). Ethylene also blocks polar auxin transport (Goldsmith, 1977). Thus, high concentrations of C_2H_4 in the lower stem of flooded plants may cause auxin to accumulate above the floodline and induce AR formation there.

The objective of this study was to deter-

mine the roles of auxin and C_2H_4 in stimulating the appearance of ARs on lower stems of waterlogged tomato plants by use of TIBA, an inhibitor of polar auxin transport (Niedergang and Skoog, 1956), and STS, an inhibitor of C_2H_4 action (Veen, 1983).

Seeds of PI 406966 tomato, a flood-resistant genotype (McNamara and Mitchell, 1989), were sown in 10-cm-diameter (0.44 liters) plastic pots containing a 1 soil :2 peat :2 perlite (by volume) medium amended as follows (in g·m⁻³): 597 KNO₃, 597 MgSO₄, 391 P (as P₂O₅), and 75 Peter's standard trace element mix (Peter's, Allentown, Pa.). Seedlings were watered once daily with nutrient solution containing (in mg·liter⁻¹) 517 KNO₃, 368 NH₄NO₃, and 101 H₃PO₄; pH was 6.3. Seedling culture and experimentation were conducted in a growth chamber under the following conditions: photosynthetic photon flux (400-700 nm) of 500

$\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$, photoperiod of 16 h (beginning at 0600 HR) on a 24-h cycle, and air at $26 \pm 2^\circ\text{C}$ days/ $22 \pm 2^\circ\text{C}$ nights.

Cotyledons were excised 1 week before initiation of treatment. Flood treatment was imposed by placing each seedling pot inside a 1.2-liter container filled with nutrient solution. Flooded plants (F) were immersed to 1 cm above the soil surface. Solution was changed after 60 h of treatment. Nonflooded control plants (NF) were placed inside similar containers with bottom drainage holes and were irrigated to field capacity once daily with nutrient solution.

For inhibition of auxin transport, 0.2 ml of 1% (w/w) TIBA in anhydrous lanolin was applied as a band encircling the stem immediately below the lowest true leaf. For inhibition of C_2H_4 action, the entire shoot was sprayed to runoff with STS solution (2 mM AgNO₃, 8 mM Na₂S₂O₃). Both TIBA and STS applications were made 1 h before initiation of flood treatment.

Leaf epinasty, an C_2H_4 -induced response (Bradford and Dille, 1978), was measured after 24 h of treatment to check the effectiveness of STS inhibition of C_2H_4 action. Epinasty was measured as the angle between stem and adaxial side of the petiole of the third-oldest leaf.

For C_2H_4 measurement, 2.5-cm hypocotyl sections were excised after 48 h of treatment, weighed, and enclosed in 5-ml vials. After 1 h, a 1-ml gas sample was withdrawn and injected into a gas chromatography (Varian Model 3300; Varian Instrument Group, Palo Alto, Cal if.) equipped with a flame-ionization detector and a 2-mm-diameter (i.d.) $\times$ 182-cm-long column packed with activated alumina. Chromatograph oven temperatures were column 80°C, injector 120°C, and detector 150°C. Flow rate of the nitrogen carrier gas was 40 ml·min⁻¹.

The number of emerged and unemerged ARs present on the hypocotyl and first internode after 120 h of treatment were determined by the method of Aung (1976).

A second approach was used to further elucidate the importance of endogenous auxin accumulation at the floodline to initiate adventitious root primordia (ARP). A band of 1% (w/w) TIBA in lanolin was applied directly below either the oldest, third-oldest, or fifth-oldest leaf of 25-day-old seedlings to

Table 1. Stress responses by PI 406966 tomato seedlings to 120 h of root-zone flood treatment. Angle of the adaxial side of the petiole from the stem, number of nonemerged and emerged adventitious roots on the lower stem, and C_2H_4 evolution rate by excised hypocotyls were measured as a function of flood-stress treatment and/or treatment with auxin transport (TIBA) or ethylene-action inhibitors (STS).^{2,3}

Treatment	Petiole angle (degrees)	Adventitious roots (no.)			C_2H_4 (nl·g ⁻¹ fresh wt/h)
		Nonemerged	Emerged	Total	
Nonflood (NF)	53 d	0.0 b	0.0 b	0.0 c	0.29 b
NF + TIBA	68 c	0.0 b	0.0 b	0.0 c	0.67 b
NF + STS	56 d	0.0 b	0.0 b	0.0 c	0.48 b
Flood (F)	78 b	6.4 b	128.8 a	135.2 a	1.68 b
F + TIBA	85 a	0.4 b	0.0 b	0.4 c	1.51 b
F + STS	54 d	46.5 a	3.5 b	50.0 b	13.96 a

¹Each value represents the mean of measurements from six plants within a treatment.

²Mean separation within columns by Student-Newman-Keuls test, $P = 0.05$.

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vary the amount of endogenous auxin available to the lower stem during flooding. The number of roots visible on the hypocotyl and first internode were counted after 12 days of flooding.

A completely random design was used in both experiments with six plants per treatment. Data were subjected to analysis of variance procedures.

Leaves of F plants were significantly more epinastic than leaves of NF plants (Table 1). The greater leaf epinasty of NF + TIBA and F + TIBA plants relative to that of F and NF plants, respectively, indicates enhanced C_2H_4 production in response to auxin accumulation above the TIBA band. Petiole angle of both NF + STS plants and F + STS plants was similar to that of NF plants, indicating that STS effectively blocked the epinasty-inducing action of C_2H_4 produced in response to flood stress.

Adventitious root formation by flooded tomato plants generally is believed to involve stimulation of preformed root initials (Jackson, 1985). Stems of PI 406966 lack preformed ARP, but root-zone flooding stimulates their initiation and subsequent development into ARs (McNamara and Mitchell, 1990). The microtechniques used were adequate to determine the absence of preformed root primordia in PI 406966, but were insufficient to determine whether root initials consisting of clusters of several undifferentiated cells were preformed or induced.

No ARs developed on NF plants of PI 406966, regardless of chemical treatment. However, flooding stimulated the initiation of many primordia (McNamara and Mitchell, 1990), most of which emerged as ARs by 120 h of treatment (Table 1). Placement of TIBA around the stem below the lowermost leaves prevented initiation of primordia by F plants, suggesting that auxin accumulating at the floodline is essential for initiation of AR primordia. These results agree with those of previous studies conducted with other species (Wample and Reid, 1979; Yamamoto and Kozłowski, 1987). Additional evidence for an auxin requirement comes from observations that, after 12 days of flooding, PI 406966 seedlings with zero, two, or four leaves below the TIBA band had produced an average of 4, 65, and 143 visible ARs, respectively (means significantly different at $P = 0.05$). This observation confirmed that the TIBA band provided effective blockage to basipetal auxin transport when applied below leaves, which are excellent sources of endogenous auxin (Wareing and Phillips, 1981).

The auxin requirement for ARP initiation does not appear to be mediated by C_2H_4 , since preventing auxin accumulation with TIBA had little effect on C_2H_4 evolution from excised hypocotyls of F plants, but totally negated AR formation. However, this finding does not discount the possibility that a threshold level of C_2H_4 is required for auxin-stimulated AR initiation (Robbins et al., 1983). The elevated level of C_2H_4 occurring

normally in stems of F tomato plants may render additional stimulation of C_2H_4 biosynthesis by auxin accumulation inconsequential.

Flooded plants sprayed with STS had a 40-fold greater rate of C_2H_4 evolution than did F plants not sprayed with STS (Table 1). Stimulation of C_2H_4 biosynthesis by STS has been reported for other systems as well (Aharoni et al., 1979; Reid et al., 1981) and was hypothesized to result from attachment of Ag^+ ions to C_2H_4 receptor sites, thereby preventing binding of C_2H_4 essential for feedback repression of additional C_2H_4 biosynthesis (Aharoni et al., 1979). However, the absence of elevated C_2H_4 evolution from NF + STS plants indicates that this C_2H_4 action inhibitor did not stimulate 1-aminocyclopropane-1-carboxylic acid (ACC) synthesis. The large quantity of C_2H_4 produced by F + STS plants, therefore, likely was derived from ACC synthesized by roots in response to hypoxia. The considerably lower C_2H_4 level measured for F plants suggests that tomato seedlings possess mechanisms for regulating endogenous levels of ACC and C_2H_4 during flood stress. One such mechanism may be conversion of excess ACC to 1-(malonylamino)-cyclopropane-1-carboxylic acid (MACC) (Yang and Hoffman, 1984). Amrhein et al. (1982) reported that levels of MACC in roots, stems, and leaves of tomato increased with duration of flooding. Conversion of ACC to MACC may have been greatly reduced in the presence of STS, leading to greater production of C_2H_4 .

Adventitious root emergence from the hypocotyls of F + STS plants was only 37% of that by F plants. Neither NF + STS nor F + STS plants exhibited visible toxicity symptoms, indicating that STS was not phytotoxic. The few ARs produced by F + STS plants likely is due to the C_2H_4 -inhibiting action of STS. Elevated levels of C_2H_4 in stem tissues near the floodline may indirectly induce ARP formation by inhibiting auxin transport (Goldsmith, 1977), thereby causing auxin accumulation above the waterline. Inhibition of C_2H_4 action by STS would prevent auxin accumulation in the hypocotyl of F plants and negate initiation of ARP.

Adventitious roots formed by F + STS plants also did not develop to the same extent as roots of F plants. Ninety-five percent of roots produced by F plants emerged through the epidermis by 120 h of treatment, compared with only 7% for F + STS plants. Zimmerman and Hitchcock (1933) demonstrated that C_2H_4 stimulated development of preformed root primordia in tomato. Thus, suppressed development of ARP in F + STS plants may be a direct consequence of inhibited C_2H_4 action.

Results of the present study and a previous study (McNamara and Mitchell, 1990) together indicate that auxin is essential for stimulation of AR initiation on stems of a flood-resistant tomato accession during root zone inundation. Stress C_2H_4 promotes auxin accumulation at the floodline and stimulates

elongation of flood-induced root primordia.

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