

Root Formation in Ethylene-Insensitive Plants¹

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Experiments with ethylene-insensitive tomato (*Lycopersicon esculentum*) and petunia (*Petunia × hybrida*) plants were conducted to determine if normal or adventitious root formation is affected by ethylene insensitivity. Ethylene-insensitive *Never ripe* (NR) tomato plants produced more belowground root mass but fewer aboveground adventitious roots than wild-type Pearson plants. Applied auxin (indole-3-butyric acid) increased adventitious root formation on vegetative stem cuttings of wild-type plants but had little or no effect on rooting of NR plants. Reduced adventitious root formation was also observed in ethylene-insensitive transgenic petunia plants. Applied 1-aminocyclopropane-1-carboxylic acid increased adventitious root formation on vegetative stem cuttings from NR and wild-type plants, but NR cuttings produced fewer adventitious roots than wild-type cuttings. These data suggest that the promotive effect of auxin on adventitious rooting is influenced by ethylene responsiveness. Seedling root growth of tomato in response to mechanical impedance was also influenced by ethylene sensitivity. Ninety-six percent of wild-type seedlings germinated and grown on sand for 7 d grew normal roots into the medium, whereas 47% of NR seedlings displayed elongated taproots, shortened hypocotyls, and did not penetrate the medium. These data indicate that ethylene has a critical role in various responses of roots to environmental stimuli.

The plant hormone ethylene is known to play a critical role in many diverse physiological processes, such as leaf and flower senescence, abscission of organs, flower initiation, fruit ripening, and seed germination (for review, see Abeles et al., 1992). Since the initial discovery that ethylene has stimulatory effects on adventitious root formation in many plant species (Zimmerman and Hitchcock, 1933), a wide array of experiments have been conducted to determine its role in root initiation and development. Results of these studies have been highly variable. For example, in experiments using applied ethylene or ethylene-generating compounds to examine adventitious rooting in mung bean, researchers showed that ethylene stimulates rooting (Robbins et al., 1983), inhibits rooting (Geneve and Heuser, 1983), or has no effect (Mudge and Swanson, 1978). Similar experiments in tomato (*Lycopersicon esculentum*) have shown stimulatory effects (Hitchcock and Zimmerman, 1940; Orion and Minz, 1969; Phatak et al., 1981) and inhibitory effects (Roy et al., 1972; Coleman et al., 1980).

Since the initial report that auxin induces ethylene synthesis in many plant species and tissues (Zimmerman and Wilcox, 1935), there have been numerous attempts to determine if interactions exist between auxin and ethylene during adventitious root formation and development. In experiments designed to correlate the magnitude of rooting stimulation with the magnitude of auxin-induced ethylene synthesis, there has been no clear correlation between the two (Mullins, 1972; Batten and Mullins, 1978; Coleman et al., 1980; Geneve and Heuser, 1982). Mudge (1988) pointed out that the lack of correlation between auxin-induced ethylene synthesis could be misleading, and hypothesized that if a low saturating concentration for ethylene-stimulated rooting exists, then auxin-stimulated ethylene production above this level would have no additional effect on rooting.

Reports of the variable rooting response of many plant systems to ethylene compared with ubiquitous reports of auxin-stimulated rooting have suggested that ethylene is less often a limiting factor or is less directly involved in the rooting process than auxin (Mudge, 1988). With the development of chemicals designed to block ethylene synthesis and perception, ideas for the role of ethylene sensitivity in adventitious root formation have become clearer. Inhibitors of ethylene biosynthesis, such as aminoethoxyvinylglycine, have been shown to reduce the number of adventitious roots of mung bean cuttings (Robbins et al., 1983; Jusaitis, 1986). Inhibitors of ethylene perception such as silver thiosulfate and 2,5-norbornadiene have been shown to reduce root number on mung bean cuttings (Robbins et al., 1985) and to reduce the responsiveness of sunflower hypocotyls to endogenous and exogenous auxin (Liu and Reid, 1992). Support for this type of interaction between auxin and ethylene comes from experiments with waterlogged *Rumex palustris* plants (Visser et al., 1996), in which higher tissue ethylene concentrations increased the sensitivity of root-forming tissues to endogenous IAA.

Different sensitivity of plants to ethylene at different stages of development could account for much of the reported variability in the rooting response to ethylene. Maximal rooting of bean cuttings in response to auxin-induced ethylene synthesis occurred during the first 44 h after excision (Linkins et al., 1973). Conversely, maximal rooting response due to the apparent accumulation of ethylene sensitivity over time of mung bean (Robbins et al., 1985) and sunflower (Fabijan et al., 1981) has been observed. These contradictory results suggest that ethylene has a role in the adventitious rooting response of many different plants. However, it is likely that regulation of the rooting

¹ This work was supported in part by the Fred C. Gloeckner Foundation. This paper is University of Florida Journal Series no. R-06707.

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response may differ greatly from species to species depending on the developmental stage of the experimental tissue. Attempts to clarify the roles of auxin and ethylene in adventitious rooting will likely depend on newly characterized mutants that are insensitive to these hormones.

Experiments conducted with *Arabidopsis* wild-type and mutant seedlings to determine the roles of ethylene and auxin have offered clues to the complex regulation of these two hormones in root hair development. Treatment of wild-type seedlings with increased concentrations of ACC resulted in increased root hair formation (Tanimoto et al., 1995; Masucci and Schiefelbein, 1996). Seedlings treated with inhibitors of ethylene synthesis (aminoethoxyvinylglycine) or perception (silver thiosulfate) exhibited reduced root hair formation with increased inhibitor concentration (Tanimoto et al., 1995).

In a series of experiments conducted with auxin and ethylene response mutants, Masucci and Schiefelbein (1996) showed that neither the ethylene response mutations *etr1* and *ein2* nor the auxin response mutations *aux1* and *axr1* affected root hair initiation. Interestingly, the *aux1/etr1* double mutant was shown to produce fewer root hairs than wild-type seedlings or either single mutant. The reduced root hair phenotype in *aux1/etr1* could be reversed by treatment with IAA but not by treatment with ACC. These results led Masucci and Schiefelbein (1996) to propose that both AUX1 and ETR1 may normally act in separate redundant pathways to promote root hair formation. Furthermore, they proposed that ethylene could be involved in at least two, and possibly three, signal transduction pathways leading to root hair formation. To further expand the role of ethylene in root hair development, Pitts et al. (1999) reported that root hair elongation in *Arabidopsis* was positively regulated by both auxin and ethylene.

In recent years, a number of physiological and molecular experiments have been conducted with ethylene-insensitive *Arabidopsis* and tomato mutants to elucidate the mechanism of ethylene perception and action in plants (for review, see Kieber, 1997). The main physiological response systems used for much of this work have been the seedling triple response and fruit ripening: Ethylene-insensitive (*etr1-1*) *Arabidopsis* and the tomato mutant *Never ripe* (NR) produce seeds that do not display an ethylene-mediated triple response (Bleecker et al., 1988; Lanahan et al., 1994), and NR tomato plants produce fruit that never completely ripen even when treated with exogenous ethylene (Giovannoni, 1993). Isolation and characterization of the genes responsible for ethylene insensitivity in these plants led to the discovery that the *etr1-1* gene from *Arabidopsis* and the NR gene from tomato encode mutant ethylene receptor proteins (Schaller and Bleecker, 1995; Wilkinson et al., 1995). Evidence of the conserved role for the control of ethylene sensitivity was demonstrated using genetic transformation of the *etr1-1* mutant gene into the heterologous species tomato, petunia (*Petunia × hybrida*), and tobacco. Using the constitutive CaMV 35S promoter to drive expression of the dominant mutant *etr1-1* gene, Wilkinson et al. (1997) were able to transform all of these different plant species and obtain various ethylene-insensitive phenotypes: wild-type tomato plants trans-

formed with *etr1-1* displayed delayed flower senescence and a NR fruit phenotype, and *etr1-1* petunias produced flowers with delayed senescence after ethylene treatment and pollination (Wilkinson et al., 1997).

The purpose of this study was to examine the effects of genetically manipulated ethylene insensitivity on the adventitious rooting responses of tomato and petunia in response to applied auxin and ethylene. Using NR tomato and petunia plants engineered for constitutive ethylene insensitivity (CaMV 35S/*etr1-1*) we show that endogenous ethylene sensitivity is necessary for the formation of adventitious roots on vegetative stem cuttings. We also demonstrate that ethylene sensitivity is required for normal seedling root growth in response to mechanical impedance, but is not required for normal root growth of plants under greenhouse conditions.

MATERIALS AND METHODS

Plant Materials and Culture Methods

Inbred cv Mitchell Diploid (wild-type) and transgenic (line 44568; Wilkinson et al., 1997) petunia (*Petunia × hybrida*) plants were grown under greenhouse conditions with a day/night temperature regime of 25°C/18°C in commercial potting medium (Fafard 2B, Conrad Fafard, Agawam, MA) in 15-cm, 1.5-L pots, and were fertilized at each irrigation with 150 mg L⁻¹ N from 15:7:14.1 soluble fertilizer (Peter's Fertilizer Products, Fogelsville, PA). Experiments using inbred cv Pearson (wild type) and mutant *Never ripe* (NR) tomato (*Lycopersicon esculentum*) plants were grown with cultural and environmental conditions similar to those described for petunia. The only notable exception was that tomato plants used in experiments to determine the effects of indole-3-butyric acid (IBA) and ACC on adventitious root formation of vegetative stem cuttings received day/night temperature regimes of 28°C/21°C.

Growth Data of Tomato Plants

To determine if differences in growth exist between wild-type and NR tomato plants, roots from 5-week-old plants were excised at the soil level and washed in tap water. Data for root fresh weights and stem diameters of 50 35-d-old plants of each genotype were recorded, and the means and SE values were calculated. To quantify differences in above ground adventitious roots on plant stems between wild-type and NR tomato plants, 25-cm sections of stems from 20 stems of 77-d-old plants were excised at the soil level. Data for the number of root initials for each genotype were recorded, and the means and SE values were calculated.

Response of Tomato Seedlings to Mechanical Impedance

To determine the influence of mechanical impedance on growth of wild-type and NR seedlings, 96 seeds of each genotype were arranged in a completely randomized design on either silica sand or commercial potting mix and

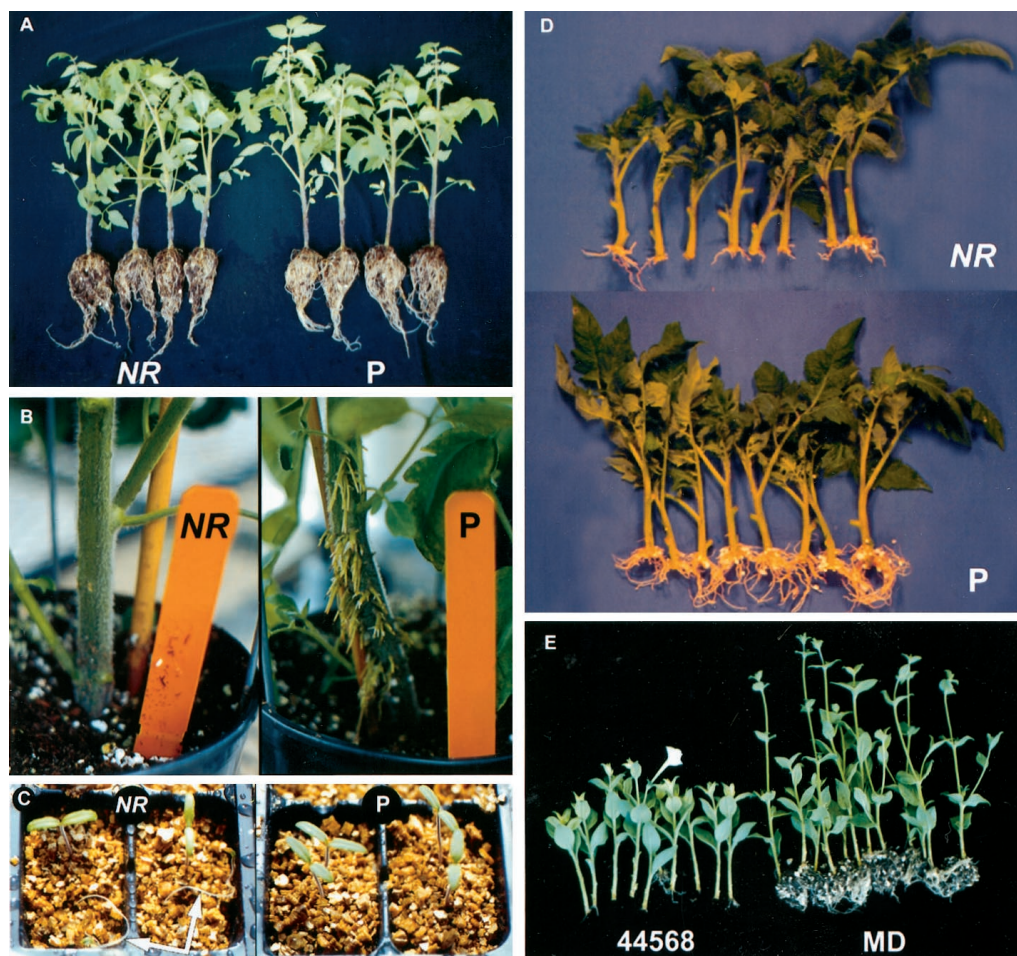


Figure 1. A, Thirty-five-day-old mutant NR and wild-type tomato plants. Whole-plant root morphologies are not visually distinguishable. B, Seventy-seven-day-old mutant NR tomato stems with reduced adventitious root formation compared with wild-type stems. C, Response of 7-d-old mutant NR and wild-type seedlings germinated and grown on sand. Approximately one-half of NR seedlings grew horizontally and had longer taproots and shorter hypocotyls than wild-type seedlings (denoted by arrows). D, Reduced adventitious root formation in mutant NR vegetative cuttings compared with wild-type cuttings. Stem cuttings were propagated for 21 d. E, Reduced adventitious root formation in transgenic 44568 petunia vegetative cuttings compared with wild-type cuttings. Stem cuttings were propagated for 21 d.

covered with 1 cm of vermiculite. During 7 d of growth, seedlings received intermittent mistings with tap water during daylight hours for 5 s every 30 min. Mist house temperatures were approximately 25°C during the day and 22°C at night. After 7 d, taproot and seedling hypocotyl lengths were measured for all seedlings, and means and \pm SE values were calculated. To observe gross differences in root hair growth of tomato seedlings, wild-type and NR seeds were plated on 2% agar plates and germinated in the dark for 5 d. Typical seedlings treated in this manner were documented graphically on a dissecting microscope (data not shown).

Adventitious Root Formation

Tomato

For all experiments using stem cuttings from tomato, vegetative cuttings were taken from 35-d-old wild-type

and NR plants at the developmental stage of six to seven true leaves. To determine the effect of auxin on adventitious root formation, the basal ends of cuttings that were approximately 10 cm in length were dipped in talcum powder containing 0, 500, 1,000, or 1,500 $\mu\text{g g}^{-1}$ IBA (Rhizopan AA#2, Hortus USA, New York). Ten cuttings were taken from plants of each genotype per treatment, and cuttings were propagated in four-pack cells (each cell = 100 mL) containing perlite and arranged in a completely randomized design in a mist house. Misting schedules and mist house temperatures were the same as described previously. After 3 weeks, the total number of root initials and root lengths for each cutting were measured.

To determine the effect of ethylene on adventitious root formation, the basal ends of cuttings that were approximately 10 cm in length were dipped in talcum powder containing 0, 1, 3, 10, 30, 100, 300, or 1,000 $\mu\text{g g}^{-1}$ ACC (Sigma). Eight cuttings were taken from plants of each

genotype per treatment, and cuttings were propagated as described for previous IBA treatments. After 3 weeks, the total number of root initials and root lengths for each cutting were measured, and the means and SE values were calculated.

Petunia

Vegetative stem cuttings were taken from approximately 8-week-old wild-type and T₁ generation petunia plants from 44568 (Wilkinson et al., 1997). The basal ends of cuttings that were approximately 5 cm in length and had two fully expanded leaves were dipped in talcum powder (0 $\mu\text{g g}^{-1}$; control) or 1,000 $\mu\text{g g}^{-1}$ IBA. Ten cuttings per genotype were propagated in six-pack cells (each cell = 70 mL) containing perlite, and were arranged randomly in a mist house. During 3 weeks of propagation, cuttings received intermittent mist with tap water during daylight hours for 5 s every 15 min for the first week, then for 5 s every 30 min for 2 subsequent weeks. Mist house temperatures for all rooting experiments were approximately 25°C during the day and 22°C at night. After 3 weeks, the total number of root initials and root lengths for each cutting were measured, and the means and SE values were calculated. There were no observed adventitious roots on intact stems of either wild-type or transgenic 44568 plants at any point in development from seed germination to seed production.

RESULTS

Growth of Tomato Plants

In an effort to characterize gross differences in root morphology and plant development between wild-type and NR tomato plants, root fresh weights of 35-d-old seedling plants were measured. Observations of belowground root growth indicated no obvious morphological differences between genotypes (Fig. 1A). However, upon closer investigation we observed that NR plants produced approximately 13% more root mass than wild-type plants (data not shown). We also noticed that there were significant differences between NR and wild-type plants for the number of

adventitious roots produced on the lower portion of plant stems (Fig. 1B). To quantify this observation, we counted the number of adventitious roots on the basal-most 25 cm of stems from 77-d-old NR and wild-type plants, and found that wild-type plants produced almost 20 times more adventitious roots on stems than NR plants (data not shown).

Response of Tomato Seedlings to Physical Impedance

When germinating and growing tomato seeds on sand, we observed a tendency for many of the NR seedlings to grow abnormally compared with wild-type seedlings. All of the wild-type seedlings and most of the NR seedlings grown on commercial potting medium had normal vertical stems and roots that penetrated the medium. On sand, most of the wild-type seedlings grew normally, but many of the NR seedlings grew horizontally and their roots did not penetrate the sand (Fig. 1C). To quantify this response, NR and wild-type seeds were germinated and grown for 7 d in a mist house on either sand or commercial potting medium covered with vermiculite to maintain consistent moisture. After 7 d, most of the NR seedlings (96%) and all of the wild-type seedlings germinated and grown on commercial potting medium produced a normal phenotype (Table I). Taproot and shoot lengths of NR and wild-type seedlings grown on potting soil and showing a normal phenotype did not differ greatly due to genotype and were visually indistinguishable. When germinated and grown on sand, 96% of wild-type seedlings grew normally, while only 53% of NR seedlings displayed a normal erect phenotype and roots penetrating the medium (Table I). The remaining 47% of the NR seedlings showed the abnormal phenotype, with significantly longer taproots and shorter hypocotyls than those with normal phenotypes (Table I; Fig. 1C).

The Effects of IBA, ACC, and Ethylene on Adventitious Root Formation in Tomato

To investigate the effects of ethylene insensitivity on adventitious rooting of tomato, a series of propagation experiments was conducted on vegetative cuttings taken

Table I. Seedling root formation of wild-type and NR tomato plants germinated and grown for 7 d on sand (S) or potting media (PM)

Genotype	Medium	Phenotype ^a		Root Length	Shoot Length
		Normal	Abnormal		
<i>mm</i>					
Wild type	S	81		28.3 ± 1.0	28.8 ± 0.5
	PM	90	3	48.7 ± 5.2	16.3 ± 0.3
NR			0	37.1 ± 1.2	36.7 ± 0.5
	S	42		—	—
	PM	74	37	32.0 ± 1.2	30.4 ± 0.8
			3	85.0 ± 4.6	15.2 ± 0.7
				37.0 ± 1.4	38.8 ± 0.6
				77.3 ± 15.2	18.0 ± 1.2

^a A seedling was deemed to have a normal phenotype if it grew vertically into the soil medium. An abnormal seedling did not penetrate the medium and instead grew horizontally across the top.

Table II. Number and length of adventitious roots of wild-type and NR vegetative tomato cuttings treated with various concentrations of IBA

Data were taken 3 weeks after the onset of propagation.

[IBA] $\mu\text{g/g}^{-1}$	No. of Adventitious Roots		Root Length	
	NR	Wild type	NR	Wild type
0	16.1 \pm 2.6	31.9 \pm 4.3	25.6 \pm 1.0	30.1 \pm 2.0
500	21.1 \pm 2.7	38.1 \pm 3.2	35.5 \pm 2.2	30.1 \pm 2.4
1,000	19.4 \pm 1.8	53.8 \pm 4.9	34.2 \pm 1.0	26.1 \pm 2.3
1,500	23.0 \pm 2.7	48.3 \pm 3.6	31.5 \pm 1.3	22.3 \pm 2.1

from 35-d-old wild-type and NR plants at the six to seven true leaf stage. In the first experiment, NR and wild-type cuttings were treated with a range of IBA concentrations to determine if applied auxin affected adventitious rooting. Ethylene insensitivity affected how cuttings responded to IBA concentration for the number of adventitious roots (Table II). With increased applied IBA concentration, wild-type cuttings were more prolific in rooting, producing approximately 50% more adventitious roots with 1,000 or 1,500 $\mu\text{g g}^{-1}$ IBA treatments than controls. NR cuttings did not produce more adventitious roots with increased applied IBA concentration, and never produced as many adventitious roots as wild-type cuttings rooted at any IBA concentration (Fig. 1D). Lengths of adventitious roots produced by wild-type cuttings were reduced with higher IBA concentrations, while lengths of NR roots were unchanged (Table II). Although significant, the magnitude of the difference in length of both wild-type and NR cuttings in response to IBA was slight, with observed growth differences of less than 1 cm due to any particular treatment.

In a second experiment, NR and wild-type cuttings were treated with a range of ACC concentrations to determine if applied ethylene affected adventitious rooting. ACC had a promotive effect on adventitious rooting for both genotypes (Table III). Wild-type cuttings treated with 30 $\mu\text{g g}^{-1}$ ACC produced approximately 22% more adventitious roots than control wild-type cuttings. NR cuttings treated with 10 $\mu\text{g g}^{-1}$ ACC produced approximately 41% more adventitious roots than control NR cuttings. NR cuttings never produced as many adventitious roots as wild-type cuttings at any applied ACC concentration (Table III).

Table III. Number of adventitious roots of wild-type and NR vegetative tomato cuttings treated with various concentrations of ACC

Data were taken 3 weeks after the onset of propagation.

[ACC] $\mu\text{g/g}^{-1}$	No. of Adventitious Roots	
	NR	Wild type
0	26.0 \pm 3.1	54.6 \pm 2.4
1	31.0 \pm 2.8	55.5 \pm 4.3
3	33.4 \pm 2.6	56.8 \pm 3.4
10	38.0 \pm 2.6	60.0 \pm 4.3
30	35.4 \pm 2.3	69.9 \pm 6.5
100	36.0 \pm 2.5	57.3 \pm 4.6
300	25.9 \pm 2.2	51.5 \pm 4.2
1,000	21.3 \pm 3.3	50.1 \pm 3.8

Adventitious Root Formation of Petunia after Treatment with IBA and Ethylene

In an effort to investigate the conserved nature of endogenous ethylene insensitivity on adventitious rooting, we conducted an experiment to determine the effects of applied auxin on adventitious rooting of wild-type and 44568 petunia (Wilkinson et al., 1997) cuttings. Based on preliminary adventitious rooting experiments, we chose to treat cuttings with 0 or 1,000 $\mu\text{g g}^{-1}$ IBA. As was observed in the previous experiment on tomato, 44568 petunia cuttings had consistently fewer adventitious roots than wild-type cuttings after both treatments (Fig. 1E). At 1,000 $\mu\text{g g}^{-1}$ IBA, wild-type cuttings were more prolific in rooting, producing almost twice as many adventitious roots than controls (Table IV); 44568 cuttings also produced slightly more adventitious roots with increased applied IBA concentration, but never produced as many adventitious roots as control wild-type cuttings. Lengths of adventitious roots produced by both wild-type and 44568 cuttings were unaffected by IBA (Table IV). Although the length of adventitious roots produced by 44568 cuttings was reduced compared with the wild type, the low number of roots on 44568 cuttings may not allow for a valid comparison of root length between the two genotypes.

DISCUSSION

Using ethylene-insensitive plants, we have shown that ethylene must be perceived by tomato plants to induce normal seedling root growth and normal levels of adventitious rooting on plant stems and vegetative cuttings. We have also shown that petunia plants genetically engineered

Table IV. Adventitious root formation of wild-type and transgenic T_1 generation 44568 petunia cuttings treated with 0 or 1,000 $\mu\text{g/g}^{-1}$ IBA

Data were taken 3 weeks after the onset of propagation.

Genotype	[IBA] $\mu\text{g/g}^{-1}$	No. of Adventitious Roots	Root Length
			mm
Wild type	0	26.6 \pm 4.3	12.4 \pm 0.6
	1,000	49.0 \pm 8.2	12.1 \pm 0.4
44568	0	0.2 \pm 0.1	3.2 \pm 1.4
	1,000	2.1 \pm 1.3	3.9 \pm 0.6

with constitutive ethylene insensitivity show reduced adventitious root formation similar to that of NR tomato.

In terms of normal plant growth, 35-d-old NR plants had more belowground root mass than wild-type tomato plants. Until now, this phenomenon has been unreported to our knowledge, but given that NR seedlings are insensitive to the ethylene-mediated seedling triple response (Lanahan et al., 1994), it is not surprising that plants could show differences in gross morphology during subsequent development as well. It is important that these plants were started and grown in potting soil under optimal greenhouse conditions that promote maximal growth. Future experiments designed to characterize the growth of NR and wild-type plants under less than optimal soil and environmental conditions may lead to a better understanding of the role of ethylene in belowground root development.

Adventitious rooting of vegetative cuttings from NR tomato and 44568 petunia plants was greatly inhibited. We also observed that 77-d-old wild-type tomato plants had 20 times more aboveground adventitious roots than NR plants. When combined with the observations of normal belowground root growth in NR, these data suggest a fundamentally different regulation of control for normal root formation versus adventitious root formation.

Under less than optimal soil medium conditions (i.e. sand versus commercial potting media), 7-d-old NR seedlings performed significantly different than wild-type tomato seedlings and with less consistency. Normal growth was observed on 100% of wild-type and 96% of NR seedlings germinated and grown on commercial potting medium. When germinated and grown on sand, 96% of wild-type seedlings grew normally, while 47% of NR seedlings grew elongated roots and shorter hypocotyls, and never penetrated the medium. The commercial potting medium used in this experiment had a bulk density of approximately 100 lb/yd³, whereas the sand had a bulk density of approximately 1,600 lb/yd³, meaning that there was much greater physical resistance to downward growth.

A similar phenomenon has been reported previously with tomato seedlings grown on agar plates in the presence of inhibitors of ethylene action. Zacarias and Reid (1992) showed that seedlings germinated on 2% agar plates containing silver thiosulfate or in the presence of 2,5-norbornadiene failed to insert their radicles into the medium, whereas seedlings germinated on 0.5% agar with similar treatments penetrated the medium. Seedlings that did not grow into the medium had a corkscrew morphology, with increased root length and decreased hypocotyl elongation, and these characters were all observed in our experiments as well. We do not know why 53% of NR seedlings grown on sand in the present study displayed a normal phenotype. Since sand is a less homogenous medium than agar, it is likely that some roots were able to grow downward between the sand particles. This idea would support the results of Zacarias and Reid (1992), who hypothesized that inhibition of ethylene action may reduce developmental movement of the root cap, thus decreasing the ability of the root to penetrate through compacted media.

When we germinated and grew wild-type and NR tomato seedlings in the dark on 2% agar, we observed a reduction in root hair length on NR roots compared with wild-type roots (data not shown). This observation supports the results of Pitts et al. (1999), who reported that ethylene is a positive regulator of root hair elongation in *Arabidopsis*. It is possible that decreased root hair formation reduces the ability of NR seedlings to anchor in soil during emergence. In real terms, these results suggest that ethylene-insensitive plants may be hindered in normal root growth under adverse growing conditions. Further experiments need to be conducted to determine the full extent of this response, because it could be a major limitation in determining the utility of genetically manipulated ethylene insensitivity in horticultural crops.

Over a range of applied IBA concentrations that induced maximal numbers of adventitious roots in wild-type tomato cuttings, NR cuttings did not show corresponding increases in rooting. These results were further supported in studies with 44568 petunia plants treated with auxin, which showed no increase in adventitious root formation at IBA concentrations that doubled the number of roots in control wild-type cuttings. The fact that cuttings of ethylene-insensitive plants never produced comparable numbers of adventitious roots compared with controls under any auxin treatment is clear evidence of a central role for ethylene in adventitious root formation of tomato and petunia cuttings.

We also observed that increased auxin concentration decreased root length of wild-type tomato adventitious roots, but not of NR roots. It is possible that this observation was due to a higher demand for carbon allocation because of more adventitious roots to support in wild-type cuttings treated with higher IBA concentrations. In experiments with vegetative tomato cuttings, we observed that rooting was not totally inhibited in NR cuttings, and application of ACC to both wild-type and NR cuttings increased the number of adventitious roots formed. This observation was likely due to the fact that the NR tomato mutation is semidominant or "leaky" (Yen et al., 1995), and has a small amount of endogenous ethylene sensitivity. When constitutive ethylene insensitivity was engineered into petunia by use of the CaMV 35S-*etr1-1* construct (Wilkinson et al., 1997), rooting was almost completely inhibited. It is possible that CaMV35S-driven ethylene insensitivity is more effective than an endogenous mutation, or observed differences may reflect the inherent differences in adventitious root formation between petunia and tomato. These observations led us to conclude that the commercial utility of plants that have been genetically manipulated for ethylene insensitivity is chiefly dependent on the ability to drive tissue-specific expression in plants if they need to be vegetatively propagated.

It is apparent from these experiments that ethylene may have a different role(s) in normal root formation than in adventitious root formation. Ethylene sensitivity appears to be temporally regulated during normal root formation of plants grown from seed. Morphological differences were observed between NR and wild-type seedlings for root hair growth and for their response to mechanical impedance,

suggesting a critical role for ethylene during early seedling root development. We predict that with further investigation of NR seedlings, many of the characteristic root hair responses seen with *etr1-1* (Masucci and Schiefelbein, 1996) will be observed. Although indistinguishable by visual appearance, greenhouse-grown 35-d-old NR plants actually had more total root mass than wild-type tomato plants. This result suggests that ethylene does not play a major role in root formation of plants established in soil and growing under optimal growth conditions. Future experiments to determine how ethylene-insensitive plants respond to adverse soil conditions leading to abiotic stress should help further characterize the role of ethylene in normal root formation and growth at the whole-plant level.

The role of ethylene during adventitious root formation appears to be more centralized. On tomato stems, adventitious root formation was greatly reduced by ethylene insensitivity. A similar reduction in adventitious rooting was observed with vegetative cuttings, and could not be overcome with application of auxin at levels that increased root formation in wild-type plants. Results from these experiments led to the hypothesis that ethylene has a significant role in adventitious root formation of tomato and petunia. It is likely that similar experiments with auxin-insensitive mutants will be needed to help clarify the interaction between ethylene and auxin during adventitious root formation.

Received January 11, 1999; accepted May 19, 1999.

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