Adventitious Root Formation in Tomato Hormone Mutants©

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INTRODUCTION

The role of plant hormones during adventitious rooting has been studied for many years, yet their specific interaction(s) during rooting is still difficult to determine. It is accepted that auxin is the key hormone responsible for initiating adventitious roots. The other major hormones—gibberellin (GA), abscisic acid (ABA), and ethylene—have been shown to promote, have no effect, or inhibit rooting depending on the species or rooting environment (Hartmann et al., 2002).

Part of the reason for this confusion is that traditional model systems used to study rooting (i.e., pea, mung bean, sunflower) were selected based on their ease of rooting and experimental manipulation rather than their genetic characteristics as a rooting system. Ernst (1994) described the characteristics of an ideal model system for conducting meaningful rooting studies. These included important genetic and developmental characteristics of the model species. He felt that *Arabidopsis* and tomato best approximated the characteristics of a model system for studying rooting. Importantly, *Arabidopsis* and tomato have numerous, characterized genetic mutants for plant development and hormone function (Arabidopsis Biological Resource Center, Columbus Ohio; Tomato Genetics Resource Center, Davis, California; SOL genomics network, Cornell, New York). Also, the genome sequence is available for *Arabidopsis* and should be available for tomato in the near future.

The objective of this research was to study hormone interactions during adventitious rooting in tomato leaf discs taken from stock plants with mutations for hormone synthesis or perception. Leaf discs were chosen because they fail to root without exogenous auxin application (Coleman and Greyson, 1977) and exogenous hormones were easily applied in the in vitro rooting medium.

METHODS AND MATERIALS

Hormone mutants of tomato (*Lycopersicon esculentum* Mill.) deficient in gibberellin (*gib-1*) and abscisic acid (*not*) production or ethylene perception (Nr) were obtained from Tomato Genetics Resource Center (University of California, Davis). Tomato stock plants were grown under greenhouse conditions with a day/night temperature of 24/20 °C and supplemental lighting in commercial potting substrate (Metro Mix 280, SunGro, Belleve, Washington) in Com-pack 606 deep cells (T.O. Plastics, Bloomington, Minnesota). Plants were fertilized at each watering with 200 ppm N from Peat-lite Special (Peter's 20N–10P–20K Fertilizer Products, Fogelsville, Pennsylvania).

To approximate normal phenotypes in *gib-1* and *not*, stock plants were sprayed with 10 μM GA_3 once per week or 50 μM ABA every 3 days, respectively. A gibberellin deficient phenotype was attained by germinating seeds in Petri dishes containing 34 μM paclobutrazol (gibberellin biosynthesis inhibitor) prior to moving seedlings to pots in the greenhouse.

Stock plants were grown to the seven-leaf stage (approximately 3 weeks) and the third leaf was harvested for rooting experiments. Six-mm diameter leaf discs

were cut over a mid-vein using a cork borer, surface sterilized for 15 min with 10% Clorox and rinsed three times with sterile water. Five leaf discs were placed in 9-cm Petri dishes with 25 ml sterile MS media (Murashige and Skoog, 1962) supplemented with 30 $g \cdot L^{-1}$ sucrose, 7 $g \cdot L^{-1}$ agar. Treatments were 25 µM K-IBA alone or in combination with 50 µM GA_3, ABA, or ACC (1-aminocyclopropane-1-carboxylic acid—immediate precursor to ethylene). Leaf discs were cultured under a 16/8 h photoperiod provided by cool white fluorescent lamps (PAR 45 $\mu mol \cdot sec^{-1} \cdot m^{-2}$) at ~22 °C. There were four dishes per treatment and roots were counted after 12 days.

RESULTS

Untreated leaf discs from wild type and hormone mutants or leaf discs treated with GA_3, ABA, or ACC failed to root unless treated with auxin (data not shown). There was no difference in rooting between wild type and *gib-1* leaf discs treated with IBA, while rooting was reduced for leaf discs from *not* and *Nr* (Table 1).

Wild type discs showed reduced rooting when placed on media containing GA_3, ABA, or ACC, but there was no difference in rooting when leaf discs were taken from wild type stock plants treated with GA_3 or ABA (Table 1).

GA_3 applied to *gib-1* stock plants induced growth that resembled wild type stock plants. Leaf discs taken from these plants responded to IBA in a similar manner to discs taken from wild type and *gib-1* plants with only a slight reduction in root number (Table 1). However, rooting was reduced in *gib-1* leaf discs placed on GA_3 medium.

Stock plants from paclobutrazol-treated seeds showed a phenocopy to *gib-1* stock plants, and leaf discs taken from these plants showed no difference in rooting compared to wild type or *gib-1* plants (Table 1).

Leaf discs from wild type and mutant stock plants showed reduced rooting on ABA media (Table 1). However, rooting in leaf discs from ABA-treated wild type stock plants was not different from wild type alone and ABA treatment partially recovered rooting in *not* stock plants to wild type levels (Table 1).

Leaf discs from *not* stock plants showed reduced rooting on ACC media and discs from *Nr* on ABA media were severely impaired for rooting (Table 1).

DISCUSSION

As previously described, auxin was required for rooting in isolated leaf discs from tomato (Coleman and Greyson, 1977). Therefore, the effects observed in the current study were for interactions with auxin.

Gibberellin is generally thought to be inhibitory to rooting (Hansen, 1988). This is based on studies where exogenous application of gibberellin (mainly GA_3) reduced rooting, while gibberellin biosynthesis inhibitors promoted rooting (Davis and Sankhla, 1988). In the few studies where endogenous gibberellin levels have been measured, they were negatively correlated with rooting. For tomato leaf discs, exogenous GA_3 inhibited auxin-induced rooting (Table 1; Coleman and Greyson, 1977). However, since there were no effects on rooting in the gibberellin biosynthesis mutant (*gib-1*) or wild type stock plants dwarfed by reducing gibberellin biosynthesis with paclobutrazol, it does not appear that endogenous gibberellin plays a significant role in mediating auxin-induced rooting in tomato.

There have been two postulated roles for ABA in rooting (Hartmann, et al., 2003). It possibly acts to antagonize the inhibition of rooting by gibberellin and to attenuate

Table 1. Root initiation in tomato leaf discs taken from mutants for gibberellin (*gib-1*), abscisic acid (*not*), and ethylene (*Nr*) treated with a combination of indolebutyric acid (IBA) and various growth regulators.

	Genotype							
	Wild type		*gib-1*		*not*		*Nr*	
Growth regulator	Percentage	Root number	Percentage	Root number	Percentage	Root number	Percentage	Root number
IBA (25 µM) alone	95a[z]	14.8a	95a	15.8a	70b	9.7c	85b	60d
IBA (25 µM) plus								
GA_3 (50 µM)	65c	1.7e	100a	3.9d				
GA_3 treated stock plant			100a	12.5b				
Paclobutrazol treated stock plant	90a	15.3a						
ABA (50 µM)	60c	4.1d	35d	2.2e	40d	1.6e	30d	0.6e
ABA treated stock plant	95a	16.6a			90a	13.4b		
ACC (50 µM)	70b	10.5b			80b	6.1d		

[z]Means followed by the same letter were not significantly different at the 5% level by Tukey's HSD test.

water stress in cuttings prior to rooting. However, exogenous application of ABA has both promoted and inhibited rooting depending on the species (Davis and Sankhla, 1988). In general, endogenous ABA levels have positively correlated with rooting, particularly in seasonal variation observed in woody plants (Blakesley et al., 1991). In addition, ABA has been suggested as one of the cofactors postulated to positively interact with auxin during rooting (Basu et al., 1968). Previous work with tomato showed that exogenous ABA had no effect on auxin-stimulated rooting and ABA could not reverse GA_3 rooting inhibition (Coleman and Greyson, 1977). In the current study, exogenous ABA inhibited rooting in leaf discs in wild type as well as all the mutant backgrounds (Table 1). However, in the ABA deficient *not* mutant, auxin-induced rooting was reduced and this reduction could be complemented with exogenous application of ABA to *not* stock plants. The mutant data suggests that ABA could have a direct physiological role in rooting, but the impact of stock plant water stress in the ABA mutant could also account for the observed differences in rooting.

The effects of ethylene on rooting have also been mixed depending on the system used to evaluate rooting (Hartmann, et al., 2002). However, ethylene has been previously shown to inhibit rooting in tomato leaf discs (Coleman et al., 1980) and the authors concluded that ethylene was an endogenous inhibitor of the rooting process. The current study confirms the inhibitory effect of ethylene (via ACC application) on rooting in tomato leaf discs (Table 1). However, its endogenous role as a rooting inhibitor is doubtful given the reduced rooting in the ethylene perception *Nr* mutant (Table 1) as has been previously shown tomato stem cuttings by Clark et al. (1999).

Alternatively, there is also the possibility that the reduced rooting seen in *Nr* was caused by increased tissue sensitivity to ABA. Ethylene and ABA share downstream elements in the signal transduction pathway and ethylene mutants can be more sensitive to ABA compared to wild type (Gazzarrini and McCourt, 2003). However, the reduction in rooting with ABA application affected all genetic backgrounds in a similar manner (Table 1), although it was most severe in the ethylene perception mutant where rooting percentage and number were reduced 64 and 90%, respectively.

In conclusion, the current study demonstrates that tomato is a useful model system for studying adventitious root formation. Results with the hormone mutants often contradicted conclusions drawn by exogenous application of hormones alone. The combination of a genetic approach complimented with exogenous application of hormones to stock plants and rooting media provided a more powerful tool for interpreting the endogenous physiological roles for these hormones in rooting.

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