Tomato Root Penetration in Soil Requires a Coaction between Ethylene and Auxin Signaling1[C][W][OA]

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During seed germination, emerging roots display positive gravitropism and penetrate into the soil for nutrition and anchorage. Tomato (Solanum lycopersicum) seeds germinated in the presence of 1-methylcyclopropene (1-MCP), an inhibitor of ethylene action, failed to insert roots into Soilrite and grew in the air, forming loops. Time-lapse video imaging showed that 1-MCP-grown root tips retained positive gravitropism and made contact with the surface of Soilrite but failed to penetrate into the Soilrite. Time-course studies revealed that the effect of 1-MCP was most prominent when seed imbibition and germination were carried out in the continual presence of 1-MCP. Conversely, 1-MCP was ineffective when applied postgermination after penetration of roots in the Soilrite. Furthermore, treatment with 1-MCP caused a reduction in DR5:β-glucuronidase auxin-reporter activity and modified the expression of SIIAA3 and SIIAA9 transcripts, indicating interference with auxin signaling. The reduced ethylene perception mutant, Never-ripe, displayed decreased ability for root penetration, and the enhanced polar auxin transport mutant, poly-1cotyledon, showed a nearly normal root penetration in the presence of 1-MCP, which could be reversed by application of auxin transport inhibitors. Our results indicate that during tomato seed germination, a coaction between ethylene and auxin is required for root penetration into the soil.

Seed germination is a crucial phase in the life cycle of plants, when the emerging seedlings first encounter the external environment and regulate growth to optimize survival. Generally, the primary root is the first organ to emerge from the seed, and its subsequent growth and development are strongly influenced by the local environment. The roots of plants perform multifaceted roles, of which two functions are foremost: to firmly anchor the plant into the soil, and to supply water and nutrients to the aerial parts of the plant (Hodge et al., 2009). To efficiently perform these functions, roots are endowed with a variety of sensory mechanisms for detecting gravity, water, nutrients, and the mechanical resistance of the soil (Arnaud et al., 2010). A large body of physiological experiments in conjunction with surgical dissection have pointed out that the root tip is the site for the perception and integration of many of these signals (Iijima et al., 2008; Arnaud et al., 2010). The root tip harbors the root apical meristem, the quiescent center, and the initial cells that give origin to all cell types of the root. The root tip is surrounded by the root cap covering the apical meristem, which consists of two tissues: columella and lateral root cap cells (Arnaud et al., 2010). The root cap offers physical protection to the root apical meristem against mechanical resistance during penetration of roots into the soil. In addition, the root cap also functions as the site for gravity sensing, and surgical removal and laser or genetic ablation of root cap cells result in a loss of gravitropic response (Blancaflor et al., 1998; Barlow, 2003; Iijima et al., 2003; Morita, 2010).

Consistent with the classic Cholodny-Went hypothesis, a large number of reports have indicated that the differential growth of roots after gravistimulation is initiated by asymmetric redistribution of the plant hormone auxin (Rashotte et al., 2001; Ottenschläger et al., 2003; Muday and Rahman, 2008; Vanneste and Friml, 2009). The reverse-fountain model of auxin transport in the root tip proposes that shoot-derived auxin is acropetally transported to the root cap through the root vascular tissue, and after radial distribution in the root cap, in the epidermal tissues it is basipetally transported back to the elongation zone of the roots, where it regulates growth (Muday

1 This work was supported by the International Atomic Energy Agency, by the Department of Biotechnology, New Delhi, India (grants to R.S. and Y.S.), by the University Grants Commission, New Delhi, by the Department of Biotechnology-Center for Education and Research in Biology and Biotechnology (fellowship to P.S.), by a University of Hyderabad fellowship to N.S., and by the U.S. Department of Agriculture National Research Initiative Competitive Grants Program (grant no. 2006–03434 to M.G.I.).

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www.plantphysiol.org/cgi/doi/10.1104/pp.111.177014
and De Long, 2001; Mudad and Rahman, 2008; Vanneste and Friml, 2009). Studies of the expression of auxin-response reporters such as DRS::GUS have shown that the maximal DRS activity in the root is in the root tip region, indicating higher levels of auxin (Sabatini et al., 1999), which is asymmetrically distributed during gravitropic curvature of root (Rashotte et al., 2001; Ottenschläger et al., 2003). Root elongation growth is also modulated by the accumulation of auxin at the root tip (Persson et al., 2009), and the accumulation of auxin above a threshold level inhibits root elongation. In accordance with this, seedlings of the enhanced polar auxin transport mutant of tomato (Solanum lycopersicum, polycoyledon pct1-2), have shorter roots, and inhibition of auxin transport in the mutant stimulates root elongation (Al-Hammadi et al., 2003; Madishetty et al., 2006).

Although auxin is the most important hormone regulating root growth, its action is modulated by several other hormones, particularly by the gaseous hormone ethylene (Benková and Hejátko, 2009). The synergistic interaction between auxin and ethylene has been studied in several root developmental responses in plants, such as regulation of root gravitropism (Buer et al., 2006), root growth (Rahman et al., 2001), lateral root development (Ivanchenko et al., 2008, 2010; Negi et al., 2008), and differentiation and elongation of root hairs (Pitts et al., 1998). One mode of interaction is at the level of hormone synthesis, where auxin stimulates the synthesis of ethylene by up-regulation of 1-aminocyclopropane-1-carboxylate (ACC) synthase, a key enzyme in ethylene production (Abel et al., 1995). Conversely, analysis of root-specific ethylene-insensitive mutants led to the discovery that the expression of genes such as ASA1, ASB1, TAA1, and TAA2, which are involved in Trp-mediated auxin biosynthesis, is up-regulated by ACC or ethylene in the root tip (Stepanova et al., 2005, 2008). Likewise, it has also been reported that ethylene-mediated up-regulation of auxin efflux transporters, PIN1 and PIN2, and the auxin influx transporter, AUX1, leads to the stimulation of basipetal transport of auxin toward the elongation zone of the root in Arabidopsis (Arabidopsis thaliana) and tomato (Růžička et al., 2007; Stepanova et al., 2007, 2008; Negi et al., 2008, 2010). Ethylene also inhibits the gravireponse of roots by regulating the synthesis of flavonoids that are considered as likely in vivo regulators of auxin transport (Buer et al., 2006). In the Arabidopsis root tip, ethylene stimulates auxin biosynthesis, leading to the inhibition of root elongation (Růžička et al., 2007; Swarup et al., 2007). The roots of mutants such as tir1, which has a defect in auxin perception, and pin2 and aux1, with defects in auxin efflux and auxin influx transporters, respectively, are resistant to growth inhibition in the presence of ethylene (Stepanova et al., 2007). Similar ethylene-auxin interaction in regulating auxin transport and synthesis has also been reported for opening of the hypocotyl hook in Arabidopsis (Vandenbussche et al., 2010; Zádniková et al., 2010). Thus, although the molecular details regarding the modes of interaction between the auxin and ethylene pathways are still unclear, numerous studies point out that there is extensive cross talk between the hormones in the regulation of root growth.

Despite the important roles of auxin and ethylene in regulating root development and growth, little information is available about the relative functions of these hormones in regulating the penetration of roots into the soil (Clark et al., 2003; Benková and Hejátko, 2009; Hodge et al., 2009). Examination of roots exposed to mechanical impedance to penetrate the medium has shown that increases in ethylene synthesis and/or signaling mediate the reduction of root growth, which is accompanied by an increase in root diameter and a decrease in root cell elongation (Sarquis et al., 1991; Okamoto et al., 2008). These morphological changes probably increase the ability of roots to overcome the physical resistance during soil penetration. As demonstrated with maize (Zea mays) roots, the root cap is important in this process, as it reduces friction, assisting in the root penetration into the soil (Iijima et al., 2003). Localized synthesis of auxin and ethylene has been shown to occur in the root tip. For example, transcripts of ACC synthase and ACC oxidase, the key enzymes regulating ethylene biosynthesis, have been detected in maize root tips (Gallie et al., 2009). Similarly, auxin is synthesized within the root tip of Arabidopsis (Růžička et al., 2007; Swarup et al., 2007). Inhibition of ethylene action enhances root growth in maize, indicating that endogenously produced ethylene causes a reduction of root growth (Whalen and Feldman, 1988). Exogenously applied ethylene rapidly inhibits root growth by inhibiting cell elongation in the region proximal to the root tip in maize (Whalen and Feldman, 1988) and Arabidopsis (Le et al., 2001). Roots of the maize mutant Zmaces6, defective in ACC synthase activity, show reduced growth in soil, signifying the importance of ethylene in overcoming physical resistance (Gallie et al., 2009). The roots of tomato seedlings treated with inhibitors of ethylene action are also unable to penetrate into 2% agar but can penetrate 0.5% agar (Zacarias and Reid, 1992). Similarly, the ethylene-insensitive mutant of tomato, Never-ripe (Nr), shows a decreased ability for soil penetration in the presence of higher mechanical impedance (Clark et al., 1999). Genetic approaches have been applied for the identification of quantitative trait loci (QTLs) responsible for root penetration in soil, particularly in crop species such as rice (Oryza sativa; Price et al., 2000). Courtos et al. (2009) combined QTL detection studies conducted by different groups in rice on different root traits using meta-QTL analysis involving the whole genome and identified 35 QTLs for root penetration index in rice. It is expected that in the near future, the genes regulating root penetration in crop plants will be identified.

To better understand the relative contributions of ethylene and auxin in the process of root penetration, we examined this response in wild-type tomato and in
the enhanced polar auxin transport mutant, pct1-2, in conditions of decreased ethylene signaling. We also analyzed the interaction between these two hormones by using an auxin-responsive reporter gene and by examining the expression of ethylene and auxin signaling genes using quantitative reverse transcription (qRT)-PCR. The results obtained in this study demonstrate that a coaction between auxin and ethylene is required for the penetration of tomato roots into the soil.

RESULTS

Inhibition of Ethylene Signaling Impairs Root Penetration in Soil

To study the role of ethylene in root growth in tomato, we germinated tomato seeds on Soilrite in the presence of 1-methylcyclopropene (1-MCP), an inhibitor of ethylene receptors (Sisler et al., 1996; Sisler, 2006). Amazingly, in contrast to control seedlings, which showed normal penetration, the roots of the 1-MCP-treated seedlings failed to penetrate into the Soilrite and grew in the air, forming loops (Fig. 1A). To ascertain that the observed lack of root penetration in the presence of 1-MCP was specifically related to the inhibition of ethylene receptors, we examined root penetration in the Nr tomato mutant, which carries a truncated version in one of the tomato ethylene receptors, ETR3 (Wilkinson et al., 1995). The majority of untreated Nr seedlings (67%) showed a lack of root penetration on Soilrite, whereas 1-MCP-treated Nr seedlings displayed a complete loss of root penetration (Fig. 1B; Supplemental Fig. S1A). The reduced root penetration in Nr on Soilrite agrees with an earlier study by Clark et al. (1999), who reported a 47% loss of root penetration in Nr on sand medium. Altogether, these observations show that 1-MCP inhibits root penetration due to the inhibition of ethylene-regulated pathways.

Given the natural propensity of emerging roots to grow into the soil, it was interesting to examine whether 1-MCP would cause aerial growth of roots in species other than tomato. Roots of monocot seedlings, wheat (Triticum aestivum; Supplemental Fig. S1B) and rice (data not shown), and dicot seedlings, tobacco (Nicotiana tabacum) and lettuce (Lactuca sativa; data not shown), grown in the presence of 1-MCP also displayed similar aerial growth of roots with loop formation. In wheat and rice, three to six primary root axes emerged on germination, and in the presence of 1-MCP, all formed aerial loops. In the absence of 1-MCP, the roots of respective control seedlings penetrated normally and grew inside the Soilrite.

Examination of ethylene evolution from seedlings showed that 1-MCP did not affect this process prior to the radicle emergence stage, which is about 48 h from sowing. However, after emergence of the radicle at 3 d from sowing, the ethylene evolution from 1-MCP-treated seedlings was reduced in comparison with control seedlings (Fig. 1C). We also examined whether exogenous application of ethylene could reverse the aerial growth of roots caused by 1-MCP and allow for root penetration into the Soilrite. Figure 1D shows that seedlings germinated in the presence of both 1-MCP and ethylene displayed increases in root penetration with increasing ethylene concentration, indicating that ethylene could counteract the 1-MCP-induced inhibition of root penetration. This agrees with previous reports that exogenously applied ethylene can partially restore the 1-MCP-induced inhibition of ethylene receptors in some plant species (Sisler et al., 1996; Binder and Bleecker, 2003; Feng et al., 2004; Sisler, 2006). Taken together, these observations strongly support a role of ethylene in regulating root penetration.

1-MCP Elicits Specific Growth Responses Related to Ethylene Signaling

It is known that exposure to ethylene leads to the promotion of hypocotyl elongation in light-grown
seedlings (Smalle et al., 1997; Vandenbussche et al., 2003a, 2003b), reduction of gravitropic curvature (Buer et al., 2006), and inhibition of root elongation in several species (Smalle and van der Straeten, 1997; Buer et al., 2006). To further confirm that 1-MCP acts in tomato by inhibiting ethylene action, we examined the effect of different concentrations of 1-MCP on the elongation of hypocotyls and roots of Soilrite-grown tomato seedlings. Figure 2A shows that 1-MCP caused a progressive dose-dependent reduction of hypocotyl length while increasing the elongation of roots. Simultaneous exposure to ethylene partially negated the above growth responses of 1-MCP (Supplemental Fig. S1C). The effect of 1-MCP on growth response was also observed in seedlings grown on vertical agar plates (Supplemental Fig. S1D), demonstrating that these effects did not arise secondarily due to a failure of root penetration. The roots of 1-MCP-treated as well as control seedlings grown on vertical agar plates were more elongated than those of seedlings grown on Soilrite, probably due to the absence of any physical impedance to root growth as well as to differences in the growth conditions. The effect of 1-MCP on seedling growth was most striking when observed as an alteration in the ratio of root versus hypocotyl length. On average, root versus hypocotyl length ratios of 2.84 and 4.4 were observed for untreated seedlings grown on Soilrite or vertical agar plates, respectively, and much higher ratios of 9.87 and 10.6 were observed upon addition of 1-MCP ($P < 0.005$; $n = 15$). Thus, 1-MCP acted in tomato in an opposite fashion in hypocotyls and roots, as known for the effects of ethylene in different plant species.

To further test whether the 1-MCP-elicited developmental effects were specifically related to ethylene signaling, we examined the growth and 1-MCP responses of the Nr mutant. On control medium, Nr roots were longer than those of the wild type, and exposure to 1-MCP did not increase but rather slightly decreased the Nr root length (Fig. 2B). Interestingly, Nr hypocotyls had similar length when compared with the wild type and displayed normal elongation inhibition in the presence of 1-MCP. This may be related to different relative participation of ETR receptors in the regulation of root and hypocotyl elongation. While 1-MCP is expected to affect multiple ethylene receptors, in Nr only the ETR3 receptor is mutated, although the non-functional receptor might inhibit other ethylene receptors by associating with them (Hackett et al., 2000).

The effect of 1-MCP on tomato seedlings was not restricted to the modification of root and hypocotyl growth; the treatment also stimulated organogenesis. The 1-MCP-treated seedlings initiated more lateral roots (Fig. 2, C and D), displaying a phenotype similar to that reported for Nr (Negi et al., 2010), and showed faster appearance of primary leaves on the shoot apical meristem (Fig. 2C). However, 1-MCP did not affect the initiation of adventitious roots, which was similar to that of the control seedlings (data not shown). Similarly, 1-MCP did not affect the timing of seed germination, which was similar in treated and untreated samples (Fig. 2E). These observations demonstrate that 1-MCP affects specific aspects of the growth and development of tomato seedlings and acts in tomato by inhibiting ethylene responses.

Figure 2. 1-MCP has specific effects on the growth and development of tomato seedlings. A, Effect of different concentrations of 1-MCP on root and hypocotyl (Hypo) lengths of 7-d-old tomato seedlings. B, Comparison of the 1-MCP (2 μL L$^{-1}$) effect on root and hypocotyl lengths of 7-d-old wild-type (WT) and Nr tomato seedlings. C, Images of 9-d-old tomato seedlings grown on vertical agar plates showing increased root development and advanced organogenesis of primary leaves (arrow). D, Quantification of the 1-MCP effect on lateral root formation in seedlings grown on vertical agar plates. E, Absence of the 1-MCP effect on tomato seed germination. Seeds were sown on agar in the presence or absence of 1-MCP, and germination was monitored by quantifying the emergence of the radicle from the seed coat at the time points indicated on the abscissa. F, The effect of 1-MCP on root penetration into Soilrite depends on the timing of application. At the time points indicated on the abscissa, 1-MCP was added and the boxes were sealed. The percentage of root penetration was determined at 5 d from sowing. Asterisks and hash marks indicate statistically significant differences between treated and control plants and between wild-type and mutant plants, respectively ($P < 0.005$; $n = 15–30$ per group). [See online article for color version of this figure.]
The 1-MCP Effect Is Strongest When Applied prior to the Beginning of Root Penetration

After sowing of tomato seeds, it normally takes at least 24 h or longer for the emergence of the radicle from the seeds. Prior to emergence, the radicle must overcome the mechanical barrier of four layers of cells in the endosperm cap and the testa layer (Toorop et al., 2000). To precisely determine the developmental stage at which 1-MCP triggered maximal inhibition of root penetration into the Soilrite, tomato seeds were transferred to 1-MCP either right from sowing or at progressive time intervals after sowing. Figure 2F shows that highest inhibition of root penetration was observed when 1-MCP was applied right from sowing or before penetration of roots into the Soilrite. The inhibitory effect of 1-MCP was drastically reduced when it was applied after root penetration. In such cases, the roots continued to grow in the Soilrite and did not emerge out of the Soilrite (data not shown). Apparently, 1-MCP was most effective in blocking root penetration when present prior to the initial stage of root penetration into the Soilrite.

1-MCP-Treated Roots Retain a Normal Gravitropic Response

Roots are positively gravitropic in nature, and this response is required for root growth toward the soil. Arabidopsis mutants such as aux1 that are defective in root gravitropism display roots that grow randomly on the surface of the medium (Bennett et al., 1996). Therefore, we considered the possibility that roots of seedlings germinated in the presence of 1-MCP grew in the air, forming loops, because they have lost their gravitropic response. Using time-lapse imaging, we compared the time course of root gravitropic curvature in untreated and 1-MCP-treated tomato seedlings grown on vertical agar plates after orienting the seedlings horizontally. We detected no significant difference in the root gravitropic curvature between treated and untreated seedlings (Fig. 3A), indicating that 1-MCP did not have any effect on the root gravitropic response at the concentrations tested.

To be able to monitor the root gravitropic response simultaneously with root penetration, we performed time-lapse imaging of tomato seedlings grown on Soilrite in the presence or absence of 1-MCP (Supplemental Movies S1 and S2). It was evident that although the roots of 1-MCP-treated seedlings could not penetrate in the Soilrite and formed aerial loops, the root tips remained in close contact with the Soilrite at all times, clearly showing positive gravitropism (Fig. 3B, Supplemental Movie S2). As the roots elongated, they displayed much larger loops, but their tips still remained in close contact with the Soilrite surface. This root behavior is similar to that observed in Arabidopsis, when on encounter of an obstacle the root forms a bend, allowing the root cap to remain in contact with the object while the main root elongates parallel to the surface of the obstacle (Massa and Gilroy, 2003). Thus, in seedlings grown on Soilrite, 1-MCP inhibited root penetration but had no inhibitory effect on the root gravitropic response at the concentrations tested.

By using a unidirectional light source during the time-lapse imaging, we were able to also analyze the effect of 1-MCP on the hypocotyl phototropic response during the process of root penetration. We did not find any significant difference between 1-MCP-treated and untreated seedlings (Fig. 3B, Supplemental Movies S1 and S2). Both treated and untreated seedlings showed hypocotyl orientation toward the direction of the light, displaying normal phototropic responses. Although moisture condensation in the sealed boxes blurs the image by 4 d, the phenotypes are clearly visible. Taken together, these observations show that 1-MCP can inhibit root penetration through a process that is independent from effects on gravitropic or phototropic responses.

1-MCP Treatment Causes Changes in the Expression of Ethylene-, Auxin-, and Mechanoperception-Related Genes

We asked what signaling components participated in the ability of 1-MCP to inhibit root penetration. The ability of ethylene to promote hypocotyl elongation in light-grown plants depends on auxin signaling (Vandenbussche et al., 2003a, 2003b). Similarly, the ethylene inhibition of root elongation in etiolated seedlings correlates with the expression of both ethylene- and auxin-related genes (Stepanova and Alonso, 2005; Stepanova et al., 2007). The signal transduction pathway regulating the inhibition of root growth by ethylene has been hypothesized to involve a linear sequence of events employing first ethylene and then auxin signaling components (Roman et al., 1995). Therefore, we first examined whether 1-MCP was affecting the tomato ethylene receptors at the transcriptional level in addition to its known effect on receptor activity. Transcripts of all six ETR receptors were detectable in tomato roots by qRT-PCR, and four receptors, ETR2, -4, -5, and -6, showed prominently increased expression in roots upon 1-MCP treatment, whereas the expression of ETR1 and NR (ETR3) was reduced. In comparison, the expression of NR, ETR4, -5, and -6 was reduced, and that of ETR1 and ETR2 was up-regulated, in 1-MCP-treated hypocotyls (Fig. 4, A and B). Apparently, exposure to 1-MCP affected ETR expression in both hypocotyls and roots, although the 1-MCP effect varied in different tissues, somewhat similar to its differential effect on root and hypocotyl elongation discussed above (Fig. 2B).

In tomato, information about the interaction between auxin and ethylene signaling pathways is limited. However, expression analysis has shown that two of the auxin signaling genes, SlIAA3 and SlIAA9, play prominent roles in ethylene-mediated regulation of fruit development and hypocotyl hook opening, respectively (Wang et al., 2005; Chaabouni et al., 2009). By examining the transcript levels of these two genes,
we found that their expression was increased in the root tips of 1-MCP-treated seedlings. In contrast, in hypocotyls, SlIAA3 was up-regulated and the SlIAA9 transcript level was reduced by 1-MCP treatment, displaying differential responses, as observed with the ETR transcripts.

We also asked if 1-MCP could be inhibiting root penetration by inhibiting the expression of genes involved in mechanoperception. Although no specific plant mechanosensor has been identified to date, there is evidence that touch-activated ion channels in the plasma membrane participate in the plant touch response (Monshausen et al., 2008). In Arabidopsis, penetration of root tips in the medium has been closely associated with expression of the MID1-COMPLEMENTING ACTIVITY1 (MCA1) gene, a Ca\textsuperscript{2+}-permeable mechanosensitive channel (Nakagawa et al., 2007). Using the Arabidopsis MCA1 transcript for sequence alignment, we identified the putative tomato MCA1 transcript (EST SGN-E 1245095 in Unigene SGN-U565830) with high homology to AtMCA1. qRT-PCR showed that the expression of the MCA1-like tomato gene was elevated in 1-MCP-treated root tips (Fig. 4A), whereas in hypocotyls, its expression was reduced by the 1-MCP treatment (Fig. 4B). Because the MCA1-like tomato transcript is up-regulated in 1-MCP-treated roots, it is unlikely that the inability of roots to penetrate in the medium was caused by an inhibition of mechanoperception. Altogether, these findings show that 1-MCP modulates the expression of ethylene-, auxin-, and mechanoperception-related genes simultaneously with inhibition of root penetration.

**1-MCP Treatment Likely Alters Auxin Accumulation in the Root Tip**

Given the observed changes in the expression of auxin-response gene transcripts upon 1-MCP treatment, it was necessary to investigate in greater detail the correlations between the inhibition of ethylene signaling by 1-MCP, root penetration, and changes in auxin response in the root tip. Several studies have indicated that activity of the synthetic consensus auxin reporter, DR5::GUS, closely reflects the auxin-induced...
gene expression in tissues (Ulmasov et al., 1997; Sabatini et al., 1999). By analyzing roots grown in the presence or absence of 1-MCP, we observed that the DR5 reporter clearly demonstrated a difference between treated and control seedlings. In roots of control seedlings, maximal DR5 activity was seen in the root tips, and the activity progressively decreased to undetectable levels toward the elongation zone. In 1-MCP-treated seedlings, DR5 activity was less intense and extended into a much smaller region. Similar differences were observed in both 3- and 5-d-old seedlings (Fig. 4, C and D). These findings demonstrated that DR5 activity could be used to investigate changes in the auxin response during 1-MCP-induced inhibition of root penetration into Soilrite.

We next examined in a time course whether the removal of 1-MCP would restore the level of DR5 expression in tomato root tips, along with the resumption of root penetration into the Soilrite. For this, we first grew seedlings for 3 d in the presence of 1-MCP, followed by growth in open boxes under normal atmosphere conditions. At 12 h after the removal of 1-MCP, the roots began to penetrate into the Soilrite, and almost all roots penetrated within 48 h (Fig. 5A). Simultaneously, there was an increase in DR5 activity in the root tips, with a maximum at 24 h (Fig. 5B), a time point when nearly 60% of the roots entered the Soilrite (Fig. 5A). Thereafter, although DR5 activity declined in a similar manner in both treated and untreated seedlings, roots continued to penetrate into the Soilrite, and the process was nearly completed by 48 h. Taken together, these observations suggest a likely interaction between ethylene and auxin signaling in regulating root penetration in tomato seedlings.

To further explore this model, we examined whether exogenously applied auxin would be able to counteract the loss of root penetration in the presence of 1-MCP. Germination of seeds in the presence of 1-MCP together with different auxins, naphthylacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and IAA, showed that the addition of auxin significantly stimulated the penetration of roots in the Soilrite in a concentration-dependent fashion (Fig. 5C). Among the three auxins tested, NAA was the most effective in restoring root penetration, leading to more than 50% penetration at 0.5 μM concentration. Application of 2,4-D restored the root penetration at a threshold concentration, with maximal effect in the range of 2.5 to 5 μM. In contrast, IAA showed a gradual restoration of root penetration with increasing concentration.

Since 1-MCP application stimulates root elongation and auxin application inhibits root elongation, we examined whether auxin counteracted the effect of 1-MCP on root growth. For all auxins, NAA, IAA (data not shown), and 2,4-D (Supplemental Fig. S2, A and B), the application of auxin reduced the root elongation in 1-MCP-treated seedlings and at the same time promoted root penetration in a dose-dependent fashion (Fig. 5C). The application of auxin also restored root penetration in the Nr mutant (Supplemental Fig. S2C), similar to the reversal of the 1-MCP effect by auxins. The differential effect of different auxins on the restoration of root penetration may be related to their respective permeability to cells. While NAA permeates into cells via diffusion through the plasma membrane, both IAA and 2,4-D are believed to require
active transport for entering the cell (Kramer and Bennett, 2006). The observation that exogenously supplied auxin was able to counteract the 1-MCP-induced inhibition of root penetration suggests that 1-MCP acted by reducing the auxin level in the root tip.

An Auxin Transport Mutant Displays Normal Root Penetration in the Presence of 1-MCP

We hypothesized that if the inhibition of root penetration by 1-MCP was related to a reduction of auxin supply, then an enhanced polar auxin transport mutant of tomato should be able to resist the 1-MCP-induced inhibition of root penetration by virtue of higher auxin levels in the root tip. The pct1-2 mutant of tomato displays nearly 3-fold faster polar auxin transport that is associated with increased expression of the PIN1 auxin transporter in roots (Al-Hammadi et al., 2003; Kharshiing et al., 2010). Consistent with our hypothesis, time-lapse imaging of root penetration in the wild type and pct1-2 roots had nearly normal penetration in the Soilrite in the presence of 1-MCP, with only occasional seedlings showing an aerial root (Fig. 6A; Supplemental Movie S3). To further confirm that the pct1-2 mutation counteracted the 1-MCP action due to increased polar auxin transport, we tested whether the auxin transport inhibitor 2,3,5-triiodobenzoic acid (TIBA; Dhonukshe et al., 2008) is able to reduce the 1-MCP resistance of pct1-2 in root penetration. We observed that pct1-2 seedlings were less resistant to 1-MCP when it was applied simultaneously with TIBA, showing nearly 50% inhibition of root penetration (Fig. 6, A and B; Supplemental Movie S4). The auxin influx inhibitor 3-chloro-4-hydroxyphenylacetic acid (CHPAA; Parry et al., 2001) was also effective in reducing the resistance of pct1-2 to 1-MCP with respect to root penetration (Fig. 6C). The reversal of the 1-MCP resistance in pct1-2 by the application of auxin transport inhibitors shows that the resistance of this mutant to 1-MCP results, at least in part, from increased polar auxin transport.

To further explore the hypothesis that 1-MCP inhibits root penetration by reducing auxin transport, we performed direct measurements of auxin transport in wild-type and pct1-2 roots after treatment of seedlings with 1-MCP. Consistent with our earlier results, wherein we observed enhanced polar auxin transport in pct1-2 hypocotyls (Al-Hammadi et al., 2003), we found higher basipetal as well as acropetal auxin transport in pct1-2 roots compared with the wild type. Both in the wild type and pct1-2, 1-MCP application caused a reduction in polar auxin transport; however, 1-MCP-treated pct1-2 roots still demonstrated increased auxin transport in comparison with untreated wild-type roots (Fig. 6D). These observations suggest that enhanced polar auxin transport in the pct1-2 mutant increases
the level of auxin in the root tip, which counteracts the action of 1-MCP.

Further analyses demonstrated that DR5 activity was increased in pct1-2 root tips compared with the wild type (Fig. 6E). Although exposure to 1-MCP reduced the DR5 activity in both wild-type and pct1-2 root tips, the DR5 expression still remained much higher in pct1-2 root tips compared with the wild type. Application of TIBA along with 1-MCP caused a drastic reduction in DR5 activity both in wild-type and pct1-2 root tips (Fig. 6E). This was associated with an inhibition of root penetration in pct1-2 (Fig. 6, A and B). Taken together, the above results are in conformity with the hypothesis that the enhanced auxin transport in the pct1-2 mutant counteracts the ability of 1-MCP to inhibit root penetration, and they support the interaction between auxin and ethylene in regulating this physiological response.
DISCUSSION

Inhibition of Ethylene Action Hampers Root Penetration and Induces Aerial Growth of Roots

To fulfill the important task of providing anchorage and nutrients to plants, roots must grow continuously and penetrate into the soil. One of the major agricultural problems is mechanical impedance of root growth in compact soils. Although crop scientists have investigated the phenomenon of root penetration through compact soils under various environmental conditions, very limited information is available about the molecular processes underlying this phenomenon (Clark et al., 1999). Investigations have shown that certain morphological alterations, such as increases in the root diameter behind the root tip on encountering higher mechanical impedance, can be related to the ability of roots to penetrate in hard soils (Wilson et al., 1977). Physiological evidence has indicated that on encountering mechanical impedance, the localized increase in ethylene synthesis causes increases in the diameter of the root (Le et al., 2001). Consistent with this view, the roots of a maize mutant defective in ACC synthase activity, a key enzyme regulating ethylene synthesis, have shown reduced growth in soil, signifying the importance of ethylene in overcoming physical resistance (Gallie et al., 2009). The aim of our study was to analyze the role of ethylene in regulating root penetration and the possible role of auxin in aiding this response in tomato seedlings.

The inability of the primary root of tomato to penetrate in the medium under conditions of inhibition of ethylene receptors, either by the use of specific inhibitors (Zacarias and Reid, 1992) or by mutations such as in the Nr gene (Clark et al., 1999), demonstrates the important role of ethylene in regulating root penetration. In conformity with previous studies, roots of tomato seedlings grown in the presence of 1-MCP, an inhibitor of ethylene receptors (Sisler et al., 1996; Binder and Bleecker, 2003; Feng et al., 2004; Sisler, 2006), failed to penetrate in the Soilrite. Likewise, roots of the ethylene-resistant mutant Nr also failed to enter the Soilrite, a response that was exacerbated by the application of 1-MCP. The effect of 1-MCP was not limited to the inhibition of root penetration; it also affected the growth of both hypocotyls and roots, with stimulation of root growth and inhibition of hypocotyl elongation. Since ethylene promotes hypocotyl elongation in light-grown seedlings (Smalle et al., 1997; Vandenbussche et al., 2003a, 2003b) and inhibits root elongation (Smalle and van der Straeten, 1997; Buer et al., 2006), the observed inhibition of hypocotyl length and increase in root length are consistent with inhibition of the ethylene action in 1-MCP-treated seedlings. The increase in the number of lateral roots in seedlings treated with 1-MCP also resembled that reported for Nr seedlings (Negi et al., 2010), further supporting that 1-MCP acts by affecting the ethylene response. Furthermore, the remarkable phenotype of aerial growth of the roots that was associated with the absence of root penetration in the presence of 1-MCP was observed in monocot plants (rice and wheat) and dicots (tobacco and lettuce), indicating that the essential role of ethylene in assisting root penetration into the growth medium is evolutionarily conserved.

Experiments with the transfer of tomato seedlings to a 1-MCP atmosphere at different time intervals from sowing indicated that the signaling pathway enabling root penetration is initiated during a very early phase of seed germination, perhaps before the emergence of the radicle from the seed coat. Once the radicle emerges from the seed coat and the root penetrates into the Soilrite, the effect of 1-MCP on root penetration is reduced. However, the ability of 1-MCP to inhibit root penetration is not related to the inhibition of radicle emergence from the seed or to a decrease in ethylene evolution at the early stage of radicle emergence.

Since the root tip plays an important role in sensing the direction of gravity as well as the mechanical interaction between the root and the soil (Massa and Gilroy, 2003; Bengough et al., 2006) and ethylene modulates root gravitropism in Arabidopsis (Buer et al., 2006), 1-MCP could be hypothesized to affect root penetration by inhibiting either the graviresponse or mechanosensing of roots. However, the aerial growth of roots does not appear to result from loss of the gravitropic response, as the root tips of 1-MCP-treated seedlings remained positively gravitropic. Time-lapse imaging consistently revealed that in these seedlings, the root tips remained in close contact with the Soilrite surface, and the failure of penetration forced the roots to grow in the air, forming loops. Similarly, the idea that 1-MCP could inhibit root penetration by reducing mechanosensing was not supported by the observation that the potential mechanosensation-related transcript, SlMCA1, was up-regulated in 1-MCP-treated roots. The fact that the inhibitory effect of 1-MCP on root penetration was reversed by ethylene shows that 1-MCP did not permanently reduce the ability of the root tip to penetrate the soil. A resumption of root penetration into the Soilrite on withdrawal of 1-MCP was observed by 12 h, and by 48 h, all roots penetrated into the Soilrite. Because 1-MCP is believed to form a stable bond with the ethylene receptors (Sisler et al., 1996; Binder and Bleecker, 2003; Feng et al., 2004), the 12- to 48-h time period could reflect the time required for receptor recovery and/or synthesis of new ethylene receptors to reactivate the ethylene response and the process of root penetration. In essence, these observations indicate a pivotal role for ethylene in regulating root penetration.

Root Penetration Is Likely Regulated via Ethylene-Auxin Cross Talk

In Arabidopsis, auxin and ethylene have synergistic effects in several root growth and developmental
responses, including root hair initiation and growth (Pitts et al., 1998), gravitropism (Buer et al., 2006), and root elongation (Pickett et al., 1990; Rahman et al., 2001; Swarup et al., 2002; Rüczika et al., 2007). Therefore, it was not surprising that the 1-MCP inhibition of root penetration was accompanied by changes in the expression of both ethylene- and auxin-response genes. This suggests that the two hormones closely interact to regulate root penetration. Interestingly, 1-MCP had a differential effect on the expression of ETR genes in roots and hypocotyls. While the expression of ETR2, -4, -5, and -6 receptor transcripts increased in roots, the expression of ETR3, -4, -5, and -6 declined in hypocotyls. 1-MCP has been reported before to reduce the expression of ETR4/5/6 but to have a marginal effect on ETR1/2 and Nr (ETR3) expression in tomato fruit at the breaker stage, in correlation with a delay in fruit ripening (Tassoni et al., 2006). Therefore, the differential response of ETR receptors to 1-MCP treatment could be related to their differential participation in different developmental responses. However, the increase in the expression levels of some ETR receptors in 1-MCP-treated roots could also be related to a compensatory response resulting from the receptor inhibition at the protein level to help maintain the ethylene homeostasis and action. A compensatory interaction between different receptors has been observed in tomato, where a decrease in the expression of the ETR1/3 receptors was compensated by an increase in the expression of ETR4 (Tieman et al., 2000).

In Arabidopsis, genetic evidence suggests that ethylene inhibits the growth of roots in part by up-regulating auxin biosynthesis (Stepanova et al., 2005). Therefore, it is possible that the inhibition of ethylene signaling by 1-MCP in turn leads to a decline in the auxin level in roots. Consistent with this view, our observations demonstrated that the 1-MCP treatment was distinctly associated with a decline in the activity of the auxin-response reporter DR5 in the root tips, suggesting that the inability of roots to penetrate into the soil was linked to a decline of the auxin response in the root tip. Moreover, we observed that DR5 activity was reversibly restored after withdrawal of 1-MCP, in parallel with the resumption of root penetration. This strongly supports a causal association between auxin signaling and root penetration, which is modulated by ethylene. In agreement with ethylene-auxin cross talk being involved in the ability of roots to penetrate the soil, we also observed that SIIAA3 and SIIAA9 transcript levels were modulated during the 1-MCP-induced inhibition of root penetration. The decline in auxin-responsive gene expression in 1-MCP-treated roots, as monitored by the reduction in DR5 activity, could result in an increased expression of components inhibiting auxin action. The auxin signaling in higher plants is modulated by Aux/IAA proteins, which act as transcriptional repressors of auxin-regulated genes, including their own, and auxin regulates their expression both at the transcriptional and protein stability levels (Woodward and Bartel, 2005). The functions of SIIAA3 and SIIAA9 have been demonstrated in fruit and vegetative shoot development (Wang et al., 2005; Chaabouni et al., 2009). For example, down-regulation of the SIIAA3 transcript in tomato using RNA interference results in an apical hook with exaggerated curvature in dark-grown plants and a reduced petiole epinasty in light-grown plants (Chaabouni et al., 2009).

Ethylene Enhancement of Polar Auxin Transport Is Involved in Root Penetration

How can ethylene affect the auxin level and/or response during root penetration? Previous reports have shown that ethylene can stimulate both auxin transport and biosynthesis in roots (Stepanova et al., 2005, 2007; Rüczika et al., 2007; Swarup et al., 2007). Roots of the tomato Nr mutant, which shows reduced perception to ethylene similar to 1-MCP-treated seedlings, show reduced polar transport of auxin, whereas the ethylene-overproducing Epi mutant shows increased auxin transport (Negi et al., 2010). In Arabidopsis, ethylene regulates the transcription of several auxin transporters, including PIN1, PIN2, and AUX1 (Rüczika et al., 2007), thus enhancing the auxin transport in roots. Therefore, the observed reduction of DR5 activity in 1-MCP-treated roots could result from a reduction in auxin biosynthesis, polar auxin transport, or a combination of both of these processes. Our findings that the application of 1-MCP reduced both the acropetal and basipetal auxin transport in wild-type tomato roots support that ethylene acts in root penetration at least in part by increasing polar auxin transport. This view was further supported by the observation that the pct1-2 mutant, which has nearly 3-fold higher auxin transport than the wild type (Al-
Hammadi et al., 2003), displayed nearly normal root penetration in the Solirite in the presence of 1-MCP. The higher level of auxin in pct1-2 roots may be counteracting the inhibitory effect of 1-MCP on root penetration. Although 1-MCP treatment reduced auxin transport in pct1-2 roots, the polar auxin transport was still considerably higher in 1-MCP-treated pct1-2 roots than in untreated wild-type roots. The fact that the DR5 activity in pct1-2 roots was significantly higher than in wild-type roots, even in the presence of 1-MCP, supports the notion that the increased auxin level aided the root penetration of pct1-2 seedlings. In essence, these results suggest that an increase in the auxin level in pct1-2 roots overcomes the inhibitory action of 1-MCP on root penetration.

If the ability of pct1-2 roots to normally penetrate the Solirite in the presence of 1-MCP is due to increased auxin transport, then upon inhibition of polar auxin transport, the pct1-2 mutant should show inhibited root penetration similar to that observed in the wild type. Several inhibitors have been reported to inhibit polar auxin transport, such as TIBA, which interferes with vesicle trafficking of auxin transporters (Dhonukshe et al., 2008), and CHPAA, an inhibitor of auxin influx (Parry et al., 2001). Consistent with pct1-2 being able to resist the inhibitory effect of 1-MCP on root penetration due to higher auxin transport, simultaneous application of TIBA or CHPAA along with 1-MCP partially inhibited the root penetration in pct1-2, leading to the formation of distinct aerial loops. Simultaneously, DR5 activity also declined in pct1-2 root tips, potentially revealing an overall decrease in auxin-responsive gene expression.

In summary, our results indicate that a coaction between ethylene and auxin likely regulates the penetration of tomato roots in the soil. Although the molecular mechanisms regulating root penetration are still to be deciphered, in tomato there seems to be an obligatory requirement for ethylene action for executing the penetration of roots. Ethylene signaling thereafter modulates auxin synthesis and/or transport to trigger the pathway responsible for the penetration of roots in the soil. While our study shows that auxin transport plays a pivotal role in regulating root penetration, it is possible that the cross talk between ethylene and auxin can occur at many different levels, including the modulation of auxin sensitivity and accumulation. In the future, more detailed analysis of the interaction between these two hormones and also other regulatory molecules may uncover the precise mechanism governing the process of root penetration.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Wild-type tomato (Solanum lycopersicum), Nr mutant, and pct1-2 mutant (Madishetty et al., 2006) seeds, all in the Ailsa Craig background, were used in this study. The pct1-2 mutant was crossed with a DR5:GUS transgenic line (Dubrovsky et al., 2008), and homozygous pct1-2 seedlings with GUS activity in the F4 generation were used. Seeds were surface sterilized with 20% (v/v) sodium hypochlorite for 10 min, washed, and directly sown (unless indicated otherwise) on the surface of moist Solirite (a mixture of horticulture-grade expanded perlite, Irish peat moss, and exfoliated vermiculite in equal ratios; Karnataka Explosive) in transparent plastic germination boxes (9.5 cm long × 9.5 cm broad × 5 cm high). Plants were grown under continuous white light (100 µmol m⁻² s⁻¹) for 7 d, unless noted otherwise. Commercially available seeds of rice (Oryza sativa), wheat (Triticum aestivum), tobacco (Nicotiana tabacum), and lettuce (Lactuca sativa) were sterilized and grown on Solirite following the same procedure. For experiments with vertical growth of seedlings, after sterilization, seeds were germinated on 1.2% (w/v) agar on petri plates. Unless otherwise stated, all experiments were repeated at least three times.

Treatment with 1-MCP

Unless stated otherwise, 30 mg of 1-MCP-releasing powder (0.14% active ingredient by weight; SmartFresh [Rohm and Haas]) was dissolved in 5 mL of water in a plastic vial to provide a final gas concentration of 2 µL L⁻¹ 1-MCP (Tassoni et al., 2006). The vial was taped to the wall of the germination box, and the boxes were immediately sealed tightly with a sealing tape. In the control treatments, 1-MCP was taped to the side of the germination boxes, and the boxes were tightly sealed. For simultaneous exposure to ethylene and 1-MCP, the 1-MCP vial was included in the germination boxes as described above. In addition, different amounts of ethylene gas were injected in the tightly sealed box through a rubber septum. The frequency of root penetration was quantified after 7 d from sowing.

To examine the effect of 1-MCP on lateral root formation, seedlings were grown on petri plates (14 cm deep) on 1.2% (w/v) agar on 9.5 cm broad, 5 cm high). Plants were grown under continuous white light (100 µmol m⁻² s⁻¹) for 7 d, unless noted otherwise. Commercially available seeds of rice (Oryza sativa), tobacco (Nicotiana tabacum), lettuce (Lactuca sativa), and Nr mutant seedlings were grown for 5 d without 1-MCP treatment. The vial containing 1-MCP was taped to the side of the germination boxes, and the boxes were tightly sealed. For simultaneous exposure to ethylene and 1-MCP, the 1-MCP vial was included in the germination boxes as described above. In addition, different amounts of ethylene gas were injected in the tightly sealed box through a rubber septum. The frequency of root penetration was quantified after 7 d from sowing.

Time-Lapse Video Imaging

Time-lapse images were captured using a Quickcam Pro 4000 (Logitech) as described at http://plantsmotion.bio.indiana.edu/plantsmotion/stuthere.html. The seeds were sown on Solirite in germination boxes and were placed in a closed chamber with a unidirectional white light source (30 µmol m⁻² s⁻¹). Images were captured at every 160 s immediately from sowing for 4 d. The frames were combined to produce a continuous movie of 4 d using video editing (MGI Video Wave4). The .avi movie files were compressed using Windows Movie Maker version 2.6 (http://www.softh2.com/get/download/Microsoft_Windows_Movie_Maker) and converted to mov using Oxelon Media Converter version 1.1 (http://www.oxelon.com/media_converter.html). The still picture frames at required time points were extracted from the movies using FxFrameCapture software (http://www.jhepple.com/framcap/framcap.htm).

Determination of Ethylene Evolution

For measuring ethylene evolution from seedlings, plants were grown in tightly sealed germination boxes. At 24-h intervals from sowing, a 1-mL volume of the headspace was removed from the vials through a rubber septum using a syringe. The headspace gas was then injected into a gas chromatograph equipped with Porapak T column and a flame ionization detector (GC-17A; Shimadzu) to determine ethylene levels. The total ethylene evolution was
normalized to the number of seedlings and the time of incubation. At every time point, ethylene in the headspace gas was measured three times.

GUS Staining

The root tips were incubated in 1 mL of GUS staining solution containing 10 mM sodium phosphate buffer (pH 7.2), 10 mM EDTA, 1 mM 5-bromo-4-chloro-3-indolyl-b-D-glucuronide (X-gluc) and 1% (v/v) Triton X-100. To aid infiltration, the tips were subjected to vacuum for 5 min and left under vacuum for 30 min. Thereafter, the tips were incubated at 37°C in the dark for 16 h. The root tips were cleared overnight in formaldehyde:acetic acid:ethanol (2:1:1) solution and observed with an Olympus BX41 compound microscope fixed with a digital camera (Olympus C-7070).

Gene Expression Analysis by qRT-PCR

The root tips (1 cm) and hypocotyls (1.5 cm) below the cotyledons were excised from 5-d-old seedlings grown on 0.8% agar in germination boxes in the presence or absence of 1-MCP to facilitate root tip harvest. Tissues were frozen in liquid N2 and homogenized to a fine powder using a mortar and pestle. About 100 mg of homogenized powder was used for total RNA extraction with the RNeasy kit (Qiagen) according to the manufacturer’s instructions. Concentration of RNA was determined by UV absorbance at A 260 and A 280 in a NanoDrop spectrophotometer. The integrity of RNA was verified by agarose gel electrophoresis. RT was performed with 2 μg of total RNA in a total reaction volume of 20 μL using a cDNA synthesis kit (Bioline). qRT-PCR was performed with cDNAs corresponding to 5 ng of total RNA in 20-μL reaction volumes using the SYBR Green PCR Master Mix (Takara) on a 7300 Fast Real Time PCR system (Applied Biosystems). The MCA1-like gene sequence (At4g35920) against the SGN Unigene database (http:// unigene). Gene-specific primers used for qRT-PCR were designed using PRIMER3 software. qRT-PCR analyses were performed as described previously (Pirrello et al., 2006). The optimal primer concentration was 280 nM for the gene-specific primers. To determine relative fold differences for each sample in each experiment, the cycle threshold (Ct) values for SIER2 and SLIN2 genes were normalized to the Ct value for SlAct51 and were calculated relative to a calibrator using the equation 2 ΔΔCt (Livak and Schmittgen, 2001).

Plasmid sequences were as follows: for 18SrRNA, forward (FP), 5′-CGCA-GGACATATTCAATTC-3′, and reverse (RP), 5′-TCCGGAATCGAACC- TAATTC-3′; for ETR1, FP, 5′-GTTGCCGTCATGACGACTTG-3′, and RP, 5′-GCACGACTCGCAAAAGAC-3′; for ETR2, FP, 5′-TCGGCTGACCTTCG- GAGGATC-3′, and RP, 5′-GAAATGCGACACCCGATG-3′; for ETR3, FP, 5′-GCACGACTCGCAGCATTCC-3′, and RP, 5′-TGGGCGTCTCATTG-3′; for ETR4, FP, 5′-AGCCAAGCCGACCATGG-3′, and RP, 5′-CCCAAGAACGACAGCCATGC-3′; for ETR5, FP, 5′-ATCCGAGCCAACGACGATG-3′, and RP, 5′-GTTGCCTGCTGACGACTTGC-3′; for ETR6, FP, 5′-CACTGACAGTTCCGCTG-3′, and RP, 5′-CACTGCGAGTTCCGCTG-3′; for IAA9, FP, 5′-GATTGTGTCGTGGACCGACG-3′, and RP, 5′-AAGGGTGCAAGGGGGAACG-3′; for IAA3, FP, 5′-CGATGCGATGAC- CACCTTTATTGG-3′, and RP, 5′-TGTTGACTCCTACAAAGAAGATCC-3′; and for MCA1-like, FP, 5′-GGCCCTCTCTGTGATCC-3′, and RP, 5′-CCCAT-TAAGCTCCTACCGGATC-3′.

Auxin Transport Assays

Polar auxin transport was measured using the method of Negi et al. (2010) and Lewis and Muday (2009) with minor modifications. For acropetal transport assay, seeds were germinated on blotting paper in the presence or absence of 1-MCP, and after radicle emergence, they were transferred to agar plates. After 48 h, a 100 μM [3H]IAA agar line was layered just below the aligned root-shoot junctions, and the seedlings were incubated in light in inverted position for 18 h. At the end of treatment, the apical 5 mm of each root tip was excised and used to measure radioactivity. Ten root tips per treatment were combined in 2.5 mL of scintillation fluid, and radioactivity was measured for 2 min in a liquid scintillation counter. For measuring basipetal auxin transport, seedlings grown as described above were treated by applying a 100 μM [3H]IAA agar line adjacent to the root tip, and the seedlings were incubated in light for 5 h. After treatment, the apical 2 mm of the roots was excised and discarded, and the 5-mm segments above the apex were taken for quantification of radioactive IAA.

Root Gravitropism Analysis

Root gravitropism analysis of control and 1-MCP-treated seedlings was performed as described by Al-Hammadi et al. (2003).

Statistical Analysis

Statistically significant differences between genotypes and treatments were determined by one-way ANOVA using the Student-Newman-Keuls method with P < 0.005.

The following genotypes (with accession nos.) were examined in this study: ETR1, AF0430841; ETR2, AY600436; ETR3, AY600437; ETR4, AF118843; ETR5, AY600439; ETR6, AY600440; IA9, AJ937282; IA3, AF022014; and MCA1-like, SGN-E1245095.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. 1-MCP-induced loss of root penetration is specific to ethylene.

Supplemental Figure S2. Reversal of the 1-MCP effect by exogenous application of 2,4-D.

Supplemental Movie S1. Time-lapse imaging of tomato seedlings.

Supplemental Movie S2. Time-lapse imaging of tomato seedlings grown in the presence of 1-MCP.

Supplemental Movie S3. Time-lapse imaging of pct1-2 mutant seedlings grown in the presence of 1-MCP.

Supplemental Movie S4. Time-lapse imaging of pct1-2 mutant seedlings grown in the presence of 1-MCP and TIBA.

ACKNOWLEDGMENTS

We thank Dr. Sangeta Negi for helpful discussions and Rohm and Haas for the kind gift of SmartFresh.

Received March 25, 2011; accepted May 10, 2011; published May 12, 2011.

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