Ethylene activates a plasma membrane 
Ca\textsuperscript{2+}-permeable channel in tobacco suspension cells

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Summary

- Here, the effects of the ethylene-releasing compound, ethephon, and the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), on ionic currents across plasma membranes and on the cytosolic Ca\textsuperscript{2+} activity ([Ca\textsuperscript{2+}]\textsubscript{c}) of tobacco (Nicotiana tabacum) suspension cells were characterized using a patch-clamp technique and confocal laser scanning microscopy.
- Exposure of tobacco protoplasts to ethephon and ACC led to activation of a plasma membrane cation channel that was permeable to Ba\textsuperscript{2+}, Mg\textsuperscript{2+} and Ca\textsuperscript{2+}, and inhibited by La\textsuperscript{3+}, Gd\textsuperscript{3+} and Al\textsuperscript{3+}.
- The ethephon- and ACC-induced Ca\textsuperscript{2+}-permeable channel was abolished by the antagonist of ethylene perception (1-metycyclopropene) and by the inhibitor of ACC synthase (aminovinylglycin), indicating that activation of the Ca\textsuperscript{2+}-permeable channels results from ethylene. Ethephon elicited an increase in the [Ca\textsuperscript{2+}]\textsubscript{c} of tobacco suspension cells, as visualized by the Ca\textsuperscript{2+}-sensitive probe Fluo-3 and confocal microscopy. The ethephon-induced elevation of [Ca\textsuperscript{2+}]\textsubscript{c} was markedly inhibited by Gd\textsuperscript{3+} and BAPTA, suggesting that an influx of Ca\textsuperscript{2+} underlies the elevation of [Ca\textsuperscript{2+}]\textsubscript{c}.
- These results indicate that an elevation of [Ca\textsuperscript{2+}]\textsubscript{c}, resulting from activation of the plasma membrane Ca\textsuperscript{2+}-permeable channels by ethylene, is an essential component in ethylene signaling in plants.


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Introduction

Ethylene is a gaseous plant hormone that is involved in a wide spectrum of important physiological processes in plants, ranging from seed germination to nodulation initiation (Bleecker & Kende, 2000; Kwak et al., 2006). Ethylene also plays an important role in perception and transduction signals associated with both biotic (van Loon et al., 2006) and abiotic (Munné-Bosch et al., 2004) stresses. There is substantial evidence that ethylene is closely related to other plant hormones, including auxin, cytokinin, abscisic acid (ABA), salicylic acid and jasmonate, in terms of its signaling...
pathways and responses to biotic and abiotic stress in plants (Guo & Ecker, 2004; Etheridge et al., 2006; Kwak et al., 2006). Ethylene is synthesized from methionine through S-adenosyl-L-Met and 1-aminocyclopropane-1-carboxylic acid (ACC) (Kende, 1993). The rate-limiting step of ethylene biosynthesis is believed to lie in the production of ACC by ACC synthase, which is followed by the conversion of ACC to ethylene by ACC oxidase (Kende, 1993; Bleecker & Kende, 2000).

Recent studies have revealed that there is a functional link between ethylene and reactive oxygen species (ROS) in general, and hydrogen peroxide in particular, in mediating stomatal closure (Desikan et al., 2005, 2006) and programmed cell death (de Jong et al., 2002). For instance, ethylene-induced stomatal closure in Arabidopsis is dependent on H2O2 production in guard cells, and the ethylene receptor mutants etr1-1 and etr1-3 are insensitive to ethylene in terms of stomatal closure and H2O2 production (Desikan et al., 2006). In this context, the function of ethylene is analogous to ABA, etr1-1 mutants (Klusener et al., 2002; Wang et al., 2003), are dependent on Ca2+/c, as a result of activation of the plasma membrane Ca2+-permeable channels, is involved in the ethylene-induced stomatal closure. In addition to stomatal movement, there is evidence indicating that the ethylene-mediated pathogenesis (Raz & New Phytologist, 2006) in tobacco and root-hair formation, and growth cating that the ethylene-mediated pathogenesis (Raz & New Phytologist, 2006). Ethylene is synthesized from methionine through ACC oxidase (Kende, 1993; Bleecker & Kende, 2000).

In this context, the function of ethylene is analogous to ABA, which elicits H2O2 production and stomatal closure via elevating the cytosolic Ca2+ activity ([Ca2+]c) through activating Ca2+-permeable channels in the plasma membranes of guard cells (Grabov & Blatt, 1998; Hamilton et al., 2000; Pei et al., 2000; Murata et al., 2001). However, it remains to be characterized whether an elevation of [Ca2+]c, as a result of activation of the plasma membrane Ca2+-permeable channels, is involved in the ethylene-induced stomatal closure. In addition to stomatal movement, there is evidence indicating that the ethylene-mediated pathogenesis (Raz & Fluhr, 1992) in tobacco and root-hair formation, and growth in pea (Petruzelli et al., 2003), are dependent on Ca2+. However, there has been no direct experimental evidence to show whether ethylene affects [Ca2+]c and modulates the plasma membrane Ca2+-permeable channels. Elevations of [Ca2+]c of plant cells by ABA (Gehring et al., 1990; McAinsh et al., 1990), auxin (Gehring et al., 1990), nitric oxide (Garcia-Mata et al., 2003), jasmonic acid (Sun et al., 2006), H2O2 (Pei et al., 2000; Foreman et al., 2003), free oxygen radicals (Demidchik et al., 2003) and calmodulin (Shang et al., 2005) have been reported.

Two types of voltage-gated Ca2+-permeable channels in the plasma membranes of plants have been identified: depolarisation-activated (Thuleau et al., 1994; Thion et al., 1998); and hyperpolarisation-activated (Kiegle et al., 2000; Hamilton et al., 2000; Pei et al., 2000; Very & Davies, 2000; Murata et al., 2001; Klusener et al., 2002; Lecourieux et al., 2002; Perfus-Barbeoch et al., 2002; Wang et al., 2004; Shang et al., 2005). Depolarisation-activated channels are likely to play a role in signal transduction (Miedema et al., 2001), whereas hyperpolarisation-activated Ca2+ channels (Miedema et al., 2001), together with voltage-independent Ca2+-permeable nonselective cation channels (Demidchik et al., 2002; White & Davenport, 2002), may function in the acquisition of Ca2+. The depolarisation-activated Ca2+-permeable channels are regulated by microtubules (Thion et al., 1996, 1998). Activity of the hyperpolarisation-activated plasma membrane Ca2+ channels is modulated by ROS (Pei et al., 2000; Murata et al., 2001; Klusener et al., 2002; Lecourieux et al., 2002; Demidchik et al., 2003; Foremann et al., 2003), fungal elicitors (Gelli et al., 1997), cAMP (Lemtriri-Chlieh & Berkowitz, 2004), calmodulin (Shang et al., 2005) and cytochalasin D (Wang et al., 2004). In the present study, we demonstrated, using the whole-cell patch-clamp technique, that ethylene activates a Ca2+-permeable channel in the plasma membranes of tobacco suspension cells, and found, using the Ca2+-sensitive fluorescent probe, Fluo-3, and confocal laser scanning microscopy, that ethylene elicits an increase in the cytosolic Ca2+ activities.

Materials and Methods

Tobacco suspension cells (Nicotiana tabacum L. cv. Samsun), initiated from pith parenchyma cells, were grown in Gamborg’s B5 liquid medium, supplemented with 1 µM 2,4 di-chlorophenoxyacetic acid, under dim lighting with photosynthetic flux density of approx. 20 µmol m−2 s−1 at 25°C, on a rotary shaker at a speed of 100 r.p.m. and were subcultured every 14 d. Protoplasts of tobacco cells were isolated by incubating the suspension cells in an enzyme solution of 0.4% (w/v) cellulase (Onozuka RS; Yakult Ltd., Honsha, Japan) and 0.04% (w/v) pectolyase (Sigma-Aldrich) polyvinylpyrrolidone (PVP) containing 0.5% (w/v) bovine serum albumin, 1 mM CaCl2, 500 mM sorbitol, 2 mM ascorbic acid and 10 mMMES/Tris (pH 6.0), for 1 h at 25°C. A sucrose density gradient, as described previously (Zhang et al., 1997), was used to collect clean protoplasts. The protoplasts were kept on ice until patch clamped.

Electrophysiology and data analysis

Patch pipettes, pulled from borosilicate glass blanks (Clark Electromedical, Reading, UK), were coated with Sylgard® (Dow Corning, Midland, MI, USA). The voltage across the patch was controlled and the current measured using an Axopatch 200 A (Axon Instruments, Foster City, CA, USA). Series resistance and capacitance were compensated. Voltage pulses of approx. 4 s in duration were used to study the voltage-dependent current. Current–voltage curves from whole-cell recordings were constructed from a series of voltage steps. Current–voltage curves were also obtained by applying repetitive voltage ramps of 4 s in duration, ranging from −200 mV to 40 mV. Data were sampled at 2 kHz and filtered at 0.5 kHz by a low-pass 4 pole Bessel filter. Sufficient time between voltage pulses was given to allow the current to settle to a steady-state level at each holding potential before a new pulse protocol was initiated. Records were stored and analysed using pClamp 8.0 (Axon Instruments, Foster City, CA, USA). All experiments were carried out at room temperature (20–22°C). Junction potentials were calculated, and corrected for, by using the program JPCALC (P. H. Barry, University of New South Wales, Sydney, Australia).
**Experimental solutions**

The pipette solution was composed of 10 mM BaCl₂, 0.1 mM dithiothreitol, 4 mM EGTA, 10 mM HEPES, pH 7.2, and had an osmolality of 720 mOsm kg⁻¹, adjusted with Tris and sorbitol, respectively. All bath solutions contained 5 mM Mes, pH 6.0, and had an osmolality of 700 mOsm kg⁻¹, adjusted with Tris and sorbitol, respectively. Details of bath solutions are given in the appropriate figure legends.

**Measurement of cytosolic Ca²⁺ activity**

Tobacco suspension cells were incubated in a solution containing 0.5 mM CaCl₂, 20 µM Ca²⁺-sensitive fluorescent dye, Fluo-3/AM (Molecular Probe, Eugene, OR, USA), at 4°C for 1 h, followed by a 3-h incubation at 25°C in the dark, as described by Zhang et al. (1998). After washing with the incubation solution (0.5 mM CaCl₂), the suspension cells were mounted in a specifically designed chamber flooded with the standard incubation solution for imaging using a confocal microscope. The Fluo-3 fluorescence of the suspension cells was measured with a laser confocal scanning microscope (LSM 510; Zeiss, Oberkochen, Germany). The wavelength of excitation light was 488 nm, and the emission signals of > 515 nm were used to collect the images. Three-dimensional scanning was performed every 5 min with a 2-µm Z-series project step, and three-dimensional reconstructed images of individual suspension cells were used to calculate the relative fluorescence. The fluorescence intensity of the entire individual suspension cells was determined using the ZEISS LSM 510 software and was expressed in pixel numbers on a scale ranging from 0 to 255.

**Results**

**Ethyphon-activated whole-cell current**

A small inward and outward current was elicited by membrane hyperpolarisation and depolarisation in the bath solution containing 10 mM BaCl₂ in the absence of the ethylene-releasing compound, ethephon (Fig. 1a). Exposure of tobacco protoplasts to ethephon led to a marked increase in the whole-cell current, and the ethephone-induced whole-cell current was strongly dependent upon the voltages (i.e. the current became more activated with membrane hyperpolarizing in the presence of ethephone) (Fig. 1b,c). The ethephon-elicited current was observed within 1–2 min of exposure to ethephon, and the current increased with the exposure time and reached a maximum approx. 30 min after the addition of ethephon to the bath solution (Fig. 1b, inset). The ethephon-activated current was found in 74% of the protoplasts examined (n = 32).

When protoplasts were pretreated with 1-metylcyclopropene (1-MCP), which inhibits ethylene binding to the receptor, whole-cell currents across plasma membranes of the tobacco protoplasts remained relatively unchanged upon exposure to ethephon (Fig. 1d), suggesting that the observed activation of inward current by ethephon is attributed to the effect of ethylene.

**Ethyphon-activated current is carried by an influx of divalent cations**

To test whether the ethephone-dependent inward current is caused by cation (Ba²⁺) influx or anion (Cl⁻) efflux, the response of the inward current to changes in concentration of the external BaCl₂ was investigated. When external BaCl₂...
was reduced from 100 to 10 mM in the bath solution, the magnitude of the ethephon-dependent inward currents was reduced and the reversal potential of the currents shifted following the equilibrium potential for Ba$^{2+}$ ($E_{Ba}$), but it shifted opposite the equilibrium potential for Cl$^-$ ($E_{Cl}$) (Fig. 2a). This indicates that the ethephon-activated channel is highly permeable for Ba$^{2+}$ over Cl$^-$, with the permeability ratio between Ba$^{2+}$ and Cl$^-$ ($P_{Ba}/P_{Cl}$), of 23.4 ± 4.5 ($n = 3$), calculated using the Goldman equation (Bertil, 1992). The relative permeability of the ethephon-activated channels to other cations was also evaluated by substitution of Ba$^{2+}$ in the bath solution with other equimolar divalent cation ions (Ca$^{2+}$, Mg$^{2+}$, Mn$^{2+}$) and monovalent cation ions (200 mM Na$^+$ and TEA$^+$) while maintaining a constant Cl$^-$ concentration. The channel responsible for the ethephon-activated inward current was almost equally permeable to Ba$^{2+}$, Mg$^{2+}$ and Ca$^{2+}$, but less permeable to Mn$^{2+}$ and displayed little permeability to the monovalent cations of Na$^+$ and TEA$^+$ (Fig. 2b,c).

The ethephon-activated current was sensitive to the broad-spectrum calcium-channel blockers, Gd$^{3+}$ and La$^{3+}$, and to Al$^{3+}$ (Fig. 2d). For example, 100 µM Gd$^{3+}$, La$^{3+}$ and Al$^{3+}$ (pH 4.0) inhibited the ethephon-activated current at ~182 mV by 68.3 ± 4.6%, 70.5 ± 5.7% and 58.7 ± 6.4% ($n = 3$), respectively. Taken together, these findings reveal that ethephon activates a cation channel permeable to Ba$^{2+}$, Mg$^{2+}$ and Ca$^{2+}$, and that the ethephon-dependent channel is inhibited by Gd$^{3+}$, La$^{3+}$ and Al$^{3+}$.

Ethylene precursor, ACC, also activated the divalent cation channels

To test, in more detail, whether the ethephon-activated Ca$^{2+}$-permeable channels results from ethylene, the effect of the ethylene precursor (ACC) on the whole-cell current of tobacco protoplasts was also investigated. As shown in Fig. 3, the exposure of tobacco protoplasts to 500 µM ACC also elicited an inward current, and the ACC-induced inward currents were inhibited by La$^{3+}$ and Gd$^{3+}$ (Fig. 3d).

To test whether the ACC-induced inward current results from the conversion of ACC to ethylene by ACC synthase, the effect of the ACC synthase inhibitor, aminovinylglycine (AVG), on the ACC-dependent current was studied. When protoplasts preincubated in 100 µM AVG for 1 h were challenged with ACC, no ACC-induced current was found ($n = 5$) (Fig. 4a–c), suggesting that the ethylene derived from ACC accounts for the ACC-activated inward current.

The ACC-induced inward current was smaller than the ethylene-induced current at the same concentrations. Therefore, ethylene is more potent in activating the divalent cation channels in tobacco protoplasts than ACC. For example, ethylene, at concentrations as low as 10 µM, resulted in a significantly enhanced current (Table 1). By contrast, ACC at 100 µM only marginally stimulated the current, and the current magnitude activated by 500 µM ACC was approx. 50% of that activated by 100 µM ethephon (Table 1). Seal resistance between the patch-pipette and the protoplasts often deteriorated when higher concentrations of ethephon were used (data not shown), making it impossible to examine their effect on the whole-cell current.

Effect of ethephon on cytosolic Ca$^{2+}$ activity in tobacco cells

Activation of the Ca$^{2+}$-permeable channel in the plasma membranes of tobacco cells by ethephon will mediate an influx of Ca$^{2+}$ into the protoplasts, leading to an elevation of
To test whether ethephon induces an increase in \([Ca^{2+}]_c\), the effect of ethephon on the \([Ca^{2+}]_c\) of the tobacco cells was investigated using a \(Ca^{2+}\)-sensitive fluorescent probe, Fluo-3, and confocal laser scanning microscopy. When the tobacco suspension cells were imaged under confocal microscopy, relatively weak fluorescence from the suspension cells was detected, and a marginal increase in the fluorescence over time was observed for cells incubated in the control solution (Fig. 5a,e). By contrast, a marked increase in the Fluo-3-dependent fluorescence was observed upon addition of ethephon to the incubation solution (Fig. 5b,e). The increase in Fluo-3-dependent fluorescence indicates that ethephon elicits an increase in \([Ca^{2+}]_c\). The increase in the \([Ca^{2+}]_c\) could result from either an influx of \(Ca^{2+}\) from the apoplasm and/or \(Ca^{2+}\) release from intracellular stores. To identify the source of \(Ca^{2+}\) underlying the ethephon-elicited increase in \([Ca^{2+}]_c\), the effects of the \(Ca^{2+}\)-channel blocker, Gd\(^{3+}\), and of the \(Ca^{2+}\) chelator, BAPTA, on the ethephon-dependent increase in \([Ca^{2+}]_c\) were studied. The ethephon-elicited increase in \([Ca^{2+}]_c\) was abolished when the cells were treated with Gd\(^{3+}\) and BAPTA (Fig. 5c–e). As shown in Fig. 5(e), the Flu-3 fluorescence intensity (expressed as pixel numbers) from suspension cells that were not challenged by ethylene remained relatively constant, whereas the fluorescence intensity exhibited a rapid increase in suspension cells upon exposure to ethephon. The ethylene-elicited increase in fluorescence intensity was abolished when the suspension cells were pretreated with Gd\(^{3+}\) and BAPTA (Fig. 5e). Therefore, these findings suggest that the ethylene-induced increase in \([Ca^{2+}]_c\) is likely to result from \(Ca^{2+}\) influx through \(Ca^{2+}\)-permeable channels in the plasma membranes of tobacco cells.

**Discussion**

In the present study, we found that, using the whole-cell patch-clamp technique, the treatment of tobacco suspension cells with the ethylene-releasing compound, ethephon, and the ethylene precursor, ACC, activated \(Ca^{2+}\)-permeable cation channels in plasma membranes of tobacco suspension cells (Fig. 1). Inhibitors of ethylene binding to receptors (1-MCP) and ethylene biosynthesis (AVG) diminished the ethephon- and ACC-induced plasma membrane \(Ca^{2+}\)-permeable channels (Figs 1c and 3), indicating that the activation of \(Ca^{2+}\)-permeable channels by ethephon and ACC can be attributed to ethylene. Ethephon elicited a rapid elevation of \([Ca^{2+}]_c\) of the same tobacco suspension cells, and the elevation of \([Ca^{2+}]_c\) induced by ethephon was diminished by the \(Ca^{2+}\)-channel blocker, Gd\(^{3+}\), and the \(Ca^{2+}\) chelator, BAPTA (Fig. 5), suggesting that the influx of \(Ca^{2+}\) through ethephon-activated \(Ca^{2+}\)-permeable channels underpins the elevation of \([Ca^{2+}]_c\). Free cytosolic \(Ca^{2+}\) ions play a pivotal role in cellular processes, such as cell growth and development, in response to ethylene.

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**Table 1** Effect of ethephon and 1-aminocyclopropane-1-carboxylic acid (ACC) on the whole-cell current density \(I_m\) of tobacco (Nicotiana tabacum) suspension cells measured at \(-182\) mV

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(I_m) (mA m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-15.7 ± 5.9 (17)</td>
</tr>
<tr>
<td>+0.01 mM ethephon</td>
<td>-33.2 ± 7.2 (4)</td>
</tr>
<tr>
<td>+0.1 mM ethephon</td>
<td>-58.7 ± 8.6 (17)</td>
</tr>
<tr>
<td>+0.1 mM ACC</td>
<td>-21.8 ± 5.5 (6)</td>
</tr>
<tr>
<td>+0.5 mM ACC</td>
<td>-29.5 ± 7.8 (7)</td>
</tr>
</tbody>
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Data represent the mean ± standard error (SE), with the number of protoplasts given in parenthesis.
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role in the transduction of various hormonal and environmental signals to the responsive element of cellular metabolism in plants (Sanders et al., 2002). It is conceivable that the ethylene-induced increase in \([\text{Ca}^{2+}]_c\) acts as a signal to activate \(\text{Ca}^{2+}\)-dependent proteins and protein kinases, thus modulating ethylene-dependent physiological processes. To the best knowledge of the authors, the results presented in the present study show that, for the first time, ethylene modulates \([\text{Ca}^{2+}]_c\) of higher plant cells through activating \(\text{Ca}^{2+}\)-permeable channels in the plasma membranes. Therefore, these findings highlight that interactions between ethylene and \([\text{Ca}^{2+}]_c\) could be of critical importance in the perception and signaling of ethylene in plants.

Hyperpolarisation-activated \(\text{Ca}^{2+}\)-permeable channels in the plasma membranes have been characterized in many plant cells, including guard cells (Grabov & Blatt, 1998; Hamilton et al., 2000; Pei et al., 2000), root elongating cells (Kiegle et al., 2000), root hairs (Very & Davies, 2000), pollen and pollen tubes (Wang et al., 2004; Shang et al., 2005), and tomato suspension cells (Gelli et al., 1997). The ethylene-induced, hyperpolarisation-activated \(\text{Ca}^{2+}\)-permeable channels identified in the present study were comparable to the hyperpolarisation-activated \(\text{Ca}^{2+}\)-permeable channels in the literature in terms of permeability, pharmacological profiles and activation kinetics (cf. Kiegle et al., 2000; Pei et al., 2000; Very & Davies, 2000; Shang et al., 2005). Therefore, it is likely that the ethylene-dependent cation channels belong to the same family of plant divalent cation channels.

The observation that plasma membrane \(\text{Ca}^{2+}\)-permeable channels in tobacco suspension cells were activated by ethylene suggests that modulation of \(\text{Ca}^{2+}\) influx through the ethylene-dependent \(\text{Ca}^{2+}\) channels may be a key event in the ethylene signaling cascade. In this context, the hyperpolarisation-activated \(\text{Ca}^{2+}\)-permeable channels have been shown to be modulated by ROS (Pei et al., 2000; Demidchik et al., 2003; Foremann et al., 2003), ABA (Grabov & Blatt, 1998; Hamilton et al., 2000), fungal elicitor (Gelli et al., 1997), actin cytoskeleton (Wang et al., 2004), cAMP (Lemtiri-Chlieh & Berkowitz, 2004) and calmodulin (Shang et al., 2005). Interestingly, ethylene is closely associated with ABA in modulating seed germination (Beaudoin et al., 2000) and stomatal closure (Desikan et al., 2005), with ROS in programmed cell death (de Jong et al., 2002; Yakimova et al., 2006), with calmodulin in heat stress (Larkindale & Knight, 2002), and in leaf senescence and death (Yang & Poovaiah, 2000) and fungal and bacterial pathogen attack (van Loon et al., 2006).

The activation of the plasma membrane \(\text{Ca}^{2+}\)-permeable channels by ethylene, and the subsequent elevation of \([\text{Ca}^{2+}]_c\), may provide an explanation for the findings that ethylene elicits stomatal closure in Arabidopsis (Desikan et al., 2005), as an increase in \([\text{Ca}^{2+}]_c\) often precedes stomatal closure induced by ABA and ROS (McAinsh et al., 1990; Pei et al., 2000). In addition to guard cells, there have been reports indicating the potential involvement of plasma membrane \(\text{Ca}^{2+}\)-permeable channels in ethylene signaling and response. For instance, ethylene markedly stimulates cadmium-induced cell death (Yakimova et al., 2006). This can be accounted for by the fact that ethylene stimulates cadmium influx through \(\text{Ca}^{2+}\)-permeable channels, thus stimulating cadmium-dependent

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Fig. 4 Whole-cell currents (\(I_m\)) in tobacco (Nicotiana tabacum) protoplasts pretreated with 500 µM aminovinlyglycin (AVG), an inhibitor of ethylene synthase, before (a) and after challenge with 500 µM 1-amino-cyclopropane-1-carboxylic acid (ACC) (b). Current-voltage curves for the currents were determined in the absence and presence of ACC in protoplasts pretreated with AVG. Data represent the mean ± standard error of five protoplasts.
cell death. There is evidence that cadmium influx into plant cells occurs through Ca\(^{2+}\)-permeable channels (Perfus-Barbeoch et al., 2002). Moreover, our findings also provide some insights into the ethylene-dependent enhancement of root hair elongation. Elongation of root hair is enhanced by ethylene (Tanimoto et al., 1995; Petruzzelli et al., 2003) and is positively dependent on \([\text{Ca}^{2+}]_c\) at the tip of root hairs (Wymer et al., 1997). Therefore, the activation of Ca\(^{2+}\)-permeable plasma membrane channels by ethylene will lead to a greater \([\text{Ca}^{2+}]_c\) in the tips of root hairs, which in turn promotes the elongation of root hairs. Furthermore, our findings also provide direct experimental evidence to account for the observations that ethylene-dependent induction of the pathogenesis-related gene is inhibited and stimulated by reducing the Ca\(^{2+}\) influx with EGTA and artificially elevating cytosolic Ca\(^{2+}\) with Ca\(^{2+}\) ionophore (Raz & Fluhr, 1992).

In summary, we demonstrated that an ethylene-releasing compound (ethephon) and an ethylene precursor (ACC) rapidly activated a Ca\(^{2+}\)-permeable channel in the plasma membrane, eliciting an elevation of the \([\text{Ca}^{2+}]_c\) of tobacco suspension cells. These findings unequivocally demonstrate that activation of the plasma membrane Ca\(^{2+}\)-permeable channel underlies the ethylene-elicited elevation of \([\text{Ca}^{2+}]_c\). Given that a number of phytohormones (e.g. ABA, auxin and jasmonic acid) and messenger molecules (e.g. nitric oxide, cAMP) act on \([\text{Ca}^{2+}]_c\), and that there is a close functional link between ethylene to these phytohormones and messenger molecules, the findings that ethylene elicited an elevation of \([\text{Ca}^{2+}]_c\) of plant cells by activating the Ca\(^{2+}\)-permeable channels highlight the fact that \([\text{Ca}^{2+}]_c\) may serve as a key node for cross-talk between ethylene and other phytohormones and messenger molecules in plants.
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References


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