Polyamines and jasmonic acid induce plasma membrane potential variations in Lima bean

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Abstract

Exogenous polyamines (PAs) [cadaverine (Cad), putrescine (Put), spermidine (Spd) and spermine (Spm)] elicit the production of volatiles in Lima bean (*Phaseolus lunatus*). Among the tested PAs, Spm induces of the production of some volatile terpenoids that are known to be induced by the spider mite *Tetranychus urticae*. Spm treatment elicits the biosynthesis of Jasmonic acid (JA), a phytohormone known to regulate the production of the volatile terpenoids. The treatment with JA together with Spm resulted in the increased volatile emission, and predatory mites *Phytoseiulus persimilis* preferred JA and Spm-treated leaves over those treated with JA alone.[5] JA and Spm treatment has no effects on polyamine oxidase (PAO) and Cu-amine oxidase (CuAO) but has a significant induction of calcium influx, ROS production, enzyme activities for NADPH-oxidase complex, superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase and glutathione peroxidase, and gene expressions except for NADPH-oxidase complex. [5] Here, we report that a plasma membrane potential (V_m) depolarization was observed after polyamine perfusion with an increasing trend: Spm, Cad, Put and Spd. JA perfusion did not alter V_m but the perfusion of JA and the polyamines significantly increased Cad and Put V_m depolarization. When JA was perfused with polyamines, a negative correlation was found between V_m depolarization and the number of amino group of the polyamines tested.

Polyamines are involved in plants' stress responses and growth. By activating biosynthesis of nucleic acids, polyamines concern the plant growth and differentiation.1 Walters DR. Polyamines in plant-microbe interactions. Physiol Mol Plant Pathol 2000; 57:137 - 146 –3 Martin-Tanguy J. Metabolism and function of polyamines in plants: recent developmentn (new approaches). Plant Growth Regul 2001; 34:135 - 148 Furthermore, it has been reported that polyamines are involved in the response against environmental stress and plant disease.1 Walters DR. Polyamines in plant-microbe interactions. Physiol Mol Plant Pathol 2000; 57:137 - 146 –4 Bouchereau A, Aziz A, Larher F, Martin-Tanguy J. Polyamines and environmental challenges: recent development. Plant Sci 1999; 140:103 - 125 We recently reported that exogenously applied polyamines ~diamines [cadaverine (Cad), putrescine (Put)], triamine [spermidine (Spd)] and tetraamine]spermine (Spm)]∽ induce volatile emission in Lima bean leaves.5 Ozawa R, Bertea CM, Foti M, Narayana R, Arimura G-I, Muroi A, et al. Exogenous polyamines elicit herbivore-induced volatiles in Lima bean leaves: involvement of calcium, H_2O_2 and jasmonic acid. Plant Cell Physiol 2009; 50:2183 - 2199 Membrane potentials (V_m) and intracellular calcium variations were also studied in Lima bean leaves after perfusion with the polyamines and with these addition of JA and here we report on these additional results.

The primary candidate for intercellular signaling in higher plants is the stimulus-induced change in V_m.6 Maffei M, Mithöfer A, Boland W. Before gene expression: early events in plant-insect interactions. Trends Plant Sci 2007; 12:310 - 316 The plasma membrane potential (V_m), which lies in the range of −50 to −200 mV in Lima bean leaves,7 Maffei M, Bossi S, Spitfeller D, Miltho'fer A, Boland W. Effects of feeding *Spodoptera littoralis* on Lima beans leaves I. Membrane

potentials, intracellular calcium variations, oral secretions and regurgitate components. Plant Physiol 2004; 134:1752 - 1762 may be shifted either to more negative (hyperpolarization) or to more positive values (depolarization) in response to various biotic or abiotic stresses.

Measurement of V_m were performed and data statistically treated as previously described (ANOVA and Tukey-Kramer's HSD test).7 Maffei M, Bossi S, Spitfeller D, Miltho'fer A, Boland W. Effects of feeding *Spodoptera littoralis* on Lima beans leaves I. Membrane potentials, intracellular calcium variations, oral secretions and regurgitate components. Plant Physiol 2004; 134:1752 - 1762 Perfusion with the polyamines (**Fig. 1** single arrow) shows a specific response of the leaf tissues with a different V_m depolarization, depending on the polyamine. In general, a V_m depolarization was observed after polyamine perfusion with an increasing trend: Spm, Cad, Put and Spd (**Fig. 1**). Spm and Spd V_m depolarization values were significantly different ($p < 0.05$) from all other polyamines, whereas no significant difference was found between Put and Cad V_m depolarization ($p = 0.435$). In all cases, V_m depolarization was reversed by washing polyamine-treated leaves with a fresh buffer solution (**Fig. 1** double arrow); however, a full recovery of the V_m was observed only for Put (**Fig. 1**). The linearization of the data from **Figure 1** allowed to calculate the rate of V_m depolarization after perfusion of the polyamines which was higher for Spd (6.0 mV min^{-1}; $R = 0.96$), equal for Put and Cad (4.8 mV min^{-1}; Put $R = 0.95$; Cad $R = 0.97$) and lower for Spm (3.0 mV min^{-1}; $R = 0.96$).

Perfusion with JA caused a slight and not significant ($p = 0.332$) V_m depolarization (**Fig. 2**) with respect to control. The addition of JA caused a significant increase ($p < 0.01$) in V_m depolarization when perfused with Cad, with respect to the sole perfusion with Cad (**Fig. 1**). The same was observed when JA was perfused with Put, whereas not significant differences were observed when Spm ($p = 0.513$) and Spd ($p = 0.107$) were perfused with JA (**Fig. 2**), with respect to the sole perfusion with Spm and Spd (**Fig. 1**). The linearization of the data from Figure 2 allowed to calculate the rate of V_m depolarization after perfusion of the polyamines + JA, which was higher for Cad (24.40 mV min^{-1}; $R = 0.99$), almost equal for Put and Spd (Put: 14.21 mV min^{-1}, $R = 0.99$; Spd: 13.49 mV min^{-1}, $R = 0.99$) and lower for Spm (1.34 mV min^{-1}; $R = 0.93$). For JA the rate of V_m depolarization was 0.19 mV min^{-1} ($R = 0.96$). With the addition of JA, a negative correlation was found between V_m depolarization and the number of amino group of the polyamines tested.

Since ion fluxes through channels directly influence V_m, it seems reasonable to assume that molecules able to act on channel activity might be considered as important factors inducing electrical signals. Among the various channels, calcium and potassium channels are predominantly involved in cell signaling.8 White PJ. Calcium channels in higher plants. Biochim Biophys Acta 2000; 1465:171 - 218 In the present study, rapid and reversible V_m depolarization observed upon perfusion of Lima bean mesophyll cells with polyamines was found to be significantly increased when JA was added to Cad and Put. The reversibility of the V_m may be linked to the overall physico-chemical amphiphilic properties of polyamines, probably depending on non covalent interaction with plasma membrane molecules, as polyamines occur in plants in free form, bound electrostatically to negatively charged molecules, and conjugated to small molecules and proteins.9 Martin-Tanguy J. Conjugated polyamines and reproductive development: biochemical, molecular and physiological approaches. Physiol Plantarum 1997; 100:675 - 688 Liu et al.10 Liu K, Fu H, Bei Q, Luan S. Inward potassium channel in guard cells as a target for polyamine regulation of stomatal movements. Plant Physiol 2000; 124:1315 - 1326 showed that Spm, Spd, Cad and Put strongly inhibited opening and closing of stomata in *Vicia faba*, suggesting that polyamines target inward potassium channels in guard cells and modulate stomatal movements, so providing a link between abiotic stress, polyamine levels and stomatal regulation. Moreover, the transport of polyamines across the plasma membrane of plant cells is energy-dependent and calcium is involved in the uptake mechanism.1 Walters DR. Polyamines in plant-microbe interactions. Physiol Mol Plant

Pathol 2000; 57:137 - 146 ,11 Antognoni F, Pistocchi R, Casali P, Bagni N. Does calcium regulate polyamine uptake in carrot protoplasts?. Plant Physiol Biochem 1995; 33:701 - 702 Both mechanisms can be correlated to the observed V_m depolarization, and the positive correlation between intracellular Ca^{2+} concentration5 Ozawa R, Bertea CM, Foti M, Narayana R, Arimura G-I, Muroi A, et al. Exogenous polyamines elicit herbivore-induced volatiles in Lima bean leaves: involvement of calcium, H_2O_2 and jasmonic acid. Plant Cell Physiol 2009; 50:2183 - 2199 and V_m depolarizing activity of polyamines confirms the involvement of Ca^{2+} during polyamine uptake.11 Antognoni F, Pistocchi R, Casali P, Bagni N. Does calcium regulate polyamine uptake in carrot protoplasts?. Plant Physiol Biochem 1995; 33:701 - 702

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Figures and Tables

Figure 1 Effect of 1 mM polyamines (arrow) on the V_m of Lima bean palisade cells. Spermine (Spm) caused the lowest V_m depolarization, whereas spermidine (Spd) showed the highest values of V_m depolarization. intermediate values were found when putrescine (Put) and cadaverine (cad) were perfused. after washing the tissues with fresh buffer (double arrow) V_m was always hyperpolarized, however the initial potential was recovered only for Put, while for all other polyamines the V_m never reached the initial values. Metric bars indicate standard deviation.

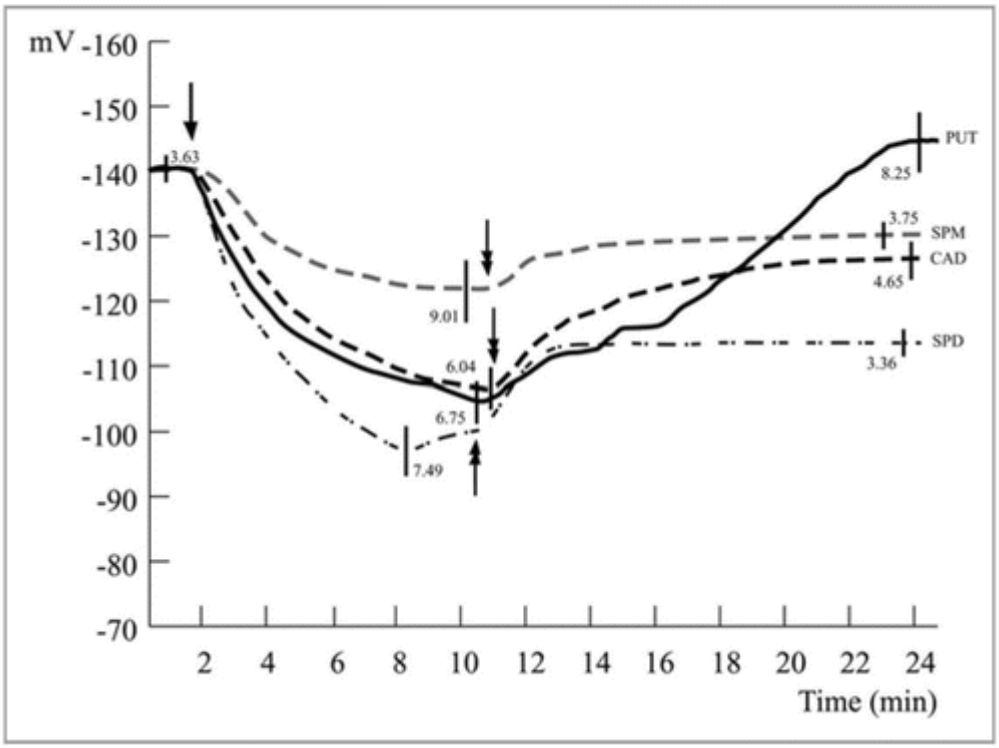
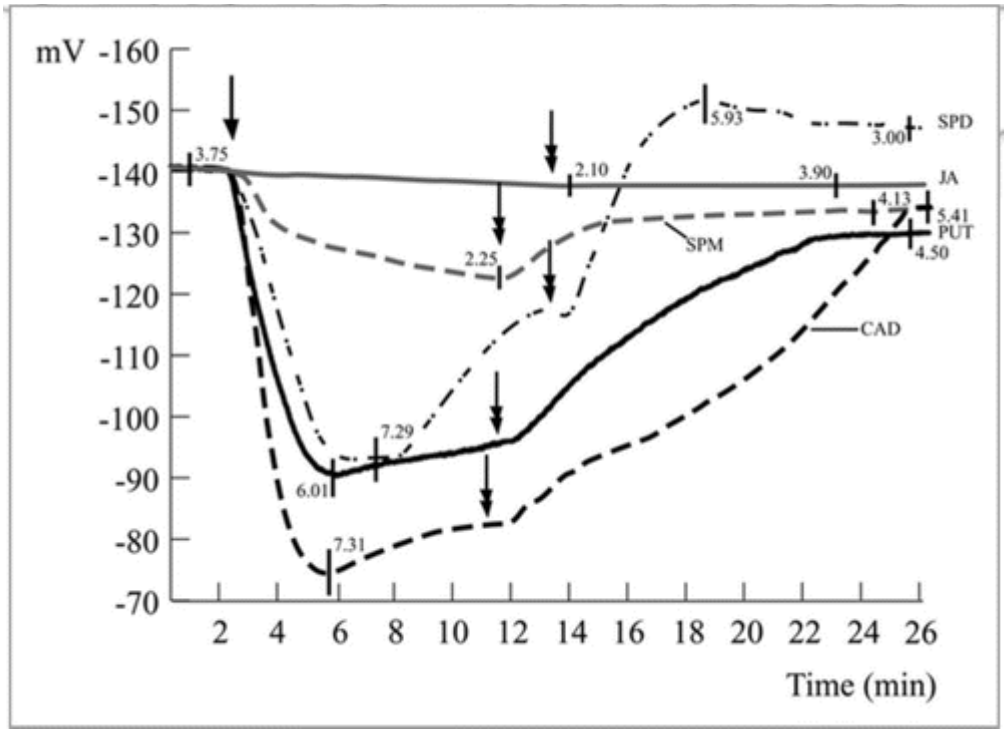


Figure 2 Effect of 1 mM polyamines + 0.1 mMJA (arrow) on the V_m of Lima bean palisade cells. the perfusion with Ja did not cause any variation in the V_m. addition of JA to Spm and Spd caused the same V_m depolarization observed in the absence of JA, whereas when JA was added to Put and Cad a stronger and significantly different V_m depolarization was observed. even in this case washing the tissues with fresh buffer (double arrow) caused a V_m hyperpolarized, however in this

case Spd reached V_m values significantly more negative that the initial V_m. Metric bars indicate standard deviation. For abbreviations see Figure 1.



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Ozawa R, Bertea CM, Foti M, Narayana R, Arimura G-I, Muroi A, et al. Exogenous polyamines elicit herbivore-induced volatiles in Lima bean leaves: involvement of calcium, H_2O_2 and jasmonic acid. Plant Cell Physiol 2009; 50:2183 - 2199

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Maffei M, Bossi S, Spitfeller D, Miltho'fer A, Boland W. Effects of feeding *Spodoptera littoralis* on Lima beans leaves I. Membrane potentials, intracellular calcium variations, oral secretions and regurgitate components. Plant Physiol 2004; 134:1752 - 1762

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