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Alleviation of salt stress-induced inhibition of seed germination in cucumber (*Cucumis sativus* L.) by ethylene and glutamate

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ABSTRACT

Ethylene is an important plant gas hormone, and the amino acid Glu is emerging as a messenger molecule in plants. To evaluate the role of ethylene and Glu in seed germination and radicle growth under salt stress, effects of 1-aminocyclopropane-1-carboxylic acid (ACC), Ethephon and Glu on germination and radicle growth of cucumber (*Cucumis sativus* L.) seeds in the absence and presence of 200 mM NaCl were investigated. Seed germination was markedly inhibited by salt stress, and this effect was alleviated by ACC and Ethephon. In contrast to seed germination, ACC and Ethephon had little effect on radicle growth under salt stress. In addition to ethylene, we found exogenous supply of Glu was effective in alleviating the salt stress-induced inhibition of seed germination and radicle growth. The effect of Glu on the seed germination and radicle growth was specific to L-Glu, whereas D-Glu and Gln had no effect. There was an increase in ethylene production during seed imbibition, and salt stress suppressed ethylene production. Exogenous L-Glu evoked ethylene evolution from the imbibed seeds and attenuated the reduction in ethylene evolution induced by salt stress. The alleviative effect of L-Glu on seed germination was diminished by antagonists of ethylene synthesis, aminoethoxyvinylglycine (AVG) and $CoCl_2$, suggesting that L-Glu is likely to exert its effect on seed germination by modulation of ethylene evolution. These findings demonstrate that ethylene is associated with suppression of seed germination under salt stress and that L-Glu interacts with ethylene in regulation of seed germination under salt stress.

Introduction

Seed germination is a complicated process and is sensitive to many hormonal and environmental cues (Finch-Savage and Leubner-Metzger, 2006). Among the phytohormones, ABA inhibits seed germination, while ethylene and gibberellin acid (GA) antagonize the ABA-induced inhibitory effect on seed germination (Finch-Savage and Leubner-Metzger, 2006; Matilla and Matilla-Vazquez, 2008; Linkies et al., 2009). It has been widely reported that ethylene production is stimulated during seed imbibition and that peak ethylene production is correlated with radicle protrusion (Fu and Yang, 1983). However, it remains to be established whether the ethylene production acts as a trigger to elicit seed germination or the ethylene production is a consequence of seed germination and radicle protrusion. Ethylene is not only involved in seed germination under optimal conditions (Matilla and Matilla-Vazquez, 2008), but may also play an important role in alleviating the inhibitory effect of the stressed environment on seed germination in many species (Kepczynski and Kepczynska, 1997). There have been numerous reports demonstrating that seed germination of both glycophytes (Zhu, 2003) and halophytes (Khan and Huang, 1988; Gul and Weber, 1998; Li et al., 2005) is inhibited by salinity. The inhibitory effect of salt stress on seed germination is alleviated by phytohormones, including cytokinin (Khan and Huang, 1988), ethylene (Khan and Huang, 1988; Kepczynski and Kepczynska, 1997; Gul and Weber, 1998), GA (Khan and Ungar, 1998; Khan et al., 2004) and messenger molecules such as nitric oxide (NO) (Zhao et al., 2007).

In addition to phytohormones, seed germination is also sensitive to external nitrogen (N) in general and nitrate/nitrite in particular (Bethke et al., 2006) and glucose (Zhao et al., 2009). Recent studies have also revealed that organic N, particularly L-Glu, plays a regulatory role in modulation of root growth and development (Sivaguru et al., 2003; Walch-Liu et al., 2006; Walch-Liu and Forde, 2008). Glu has also been shown to interact with signaling cascades of Ca^{2+} (Dennison and Spalding, 2000) and ABA in plants (Kang et

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinylglycine; Glu, glutamate; Gln, glutamine; NO, nitric oxide.

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al., 2004). There is evidence suggesting the involvement of glutamate receptors (GLRs) in plant development and stress responses (Ford and Lea, 2007; Roy et al., 2008). Given that seed germination is sensitive to ABA (Finch-Savage and Leubner-Metzger, 2006) and Glu is associated with ABA signaling cascades (Kang et al., 2004), it is conceivable that Glu may also be involved in seed germination. However, to our knowledge, there have been no studies to evaluate the role of Glu in seed germination and the interactions between ethylene and L-Glu in physiological processes. In the present study, we investigated the effect of Glu and ethylene on seed germination and radicle growth of cucumber under salt stress. Our results indicate that both Glu and ethylene alleviate salt stress-induced inhibition of seed germination. The possible interactive mechanisms underlying the alleviative effect of ethylene and Glu on seed germination exposed to salt stress are discussed.

Materials and methods

Determination of seed germination and radicle length

Seeds of cucumber (*Cucumis sativus* L., cv Zhongnong 8) were surface-sterilized for 15 min in 10% (v/v) sodium hypochlorite solution, and rinsed thoroughly with deionized water. Seeds were then used to investigate the effect of NaCl on seed germination. Cucumber seeds were placed randomly in Petri dishes (9.0 cm diameter) containing filter paper soaked with 0.5 mM $CaCl_2$ solution containing either 0 (control) or different concentrations of NaCl (100 and 200 mM) at 28 °C for 48 h in a growth chamber in the dark. There were 40 seeds in each Petri dish and the seeds were soaked with 5 mL treatment solution. Seeds were considered to be germinated at the emergence of the radicle and scored. The effects of Glu and ethylene on seed germination in the absence and presence of NaCl were investigated by treating the seeds with the solutions containing Glu (both L-Glu and D-Glu), ethylene synthesis precursor (1-aminocyclopropane-1-carboxylic acid, ACC), ethylene releaser (Ethephon) and antagonists of ethylene synthesis (aminoethoxyvinylglycine, AVG and $CoCl_2$). All the compounds were freshly prepared as stock solutions in sterilized deionized water for each experiment, and diluted to the appropriate concentrations just before use.

Measurements of ethylene evolution

Ten cucumber seeds that were exposed to solutions with and without 10 mM L-glutamate in the absence and presence of 200 mM NaCl for varying time periods were put in to 5 mL gas-tight vials. One milliliter of the headspace was taken from the vials, and then injected into a gas chromatograph equipped with an alumina column (GDX502) and a flame ionization detector (GC-7AG; Shimadzu Japan) for measuring the ethylene concentration as described previously (Sun et al., 2007).

Statistical analysis

Analysis of variance (ANOVA) was conducted between different treatments. Significant differences between treatments were evaluated by ANOVA, and the dates are expressed as the mean values ± SD of at least 4 replicates for seed germination percentage.

Results

Effect of NaCl on seed germination and radicle growth

The cucumber seeds used in the present study had an average germination rate of 96.9% after 48 h imbibition under control conditions. The germination rate was reduced to 90.0% and 55.7% when 100 and 200 mM NaCl were added to the incubation solution, respectively (data not shown). The mean radicle length was 38.7.0 ± 0.75 mm (n = 40) after 48 h imbibition in the control medium. The radicle length was reduced to 23.1 ± .0.43 and 7.72 ± 0.30 mm (n = 40), respectively after 48 h imbibition in the presence of 100 and 200 mM NaCl in the medium, suggesting that radicle growth is more sensitive to NaCl than seed germination. Given that 200 mM NaCl significantly inhibited seed germination and radicle growth, this concentration of NaCl was chosen for further study, and results are reported throughout the remainder of the paper.

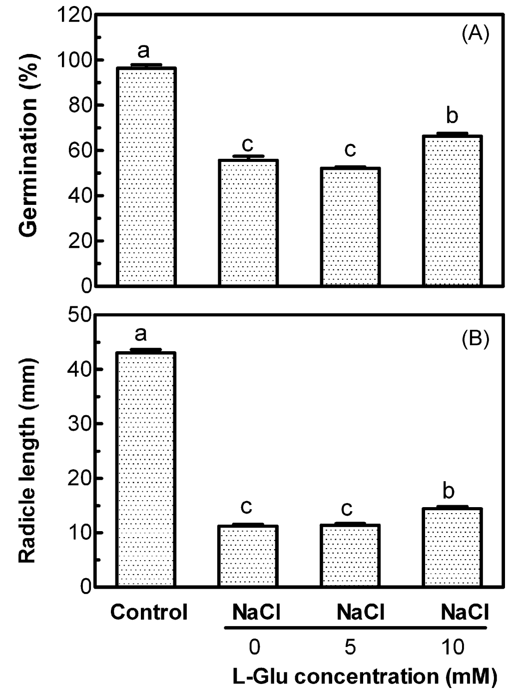

Fig. 1. Effects of L-Glu on seed germination (A) and radicle length (B) in the presence of NaCl. Seed germination rates and radicle length were codetermined after imbibition for 48 h under various treatments: control; treatment with 200 mM NaCl plus 0, 5 and 10 mM L-Glu. Data are mean ± SE for 4 replicates for calculation of seed germination, and values for radicle length were obtained from measurements of 40 radicles. Different letters shown in the error bars mean significant differences among control and treatments.

Glu alleviated the inhibitory effect of NaCl on seed germination

Glu, particularly L-Glu, plays an important regulatory role in both animal and plant cells (Ford and Lea, 2007). To examine whether Glu is involved in NaCl-induced inhibition of seed germination and radicle growth, the effect of Glu on seed germination and radicle growth in the absence and presence of NaCl was investigated. Neither seed germination and radicle length were affected by L-Glu up to 20 mM in the absence of NaCl (Data not shown). In contrast, L-Glu at 10 mM enhanced seed germination in the presence of NaCl, while 5 mM L-Glu did not have any effect on germination of cucumber seeds exposed to NaCl (Fig. 1A). Like seed germination, 10 mM L-Glu, but not 5 mM L-Glu, increased the radicle length in the presence of NaCl (Fig. 1B). To test whether the observed effect of L-Glu on NaCl-induced inhibition of seed germination and radicle growth is specific, we also investigated the effect of D-Glu and Gln on seed germination and radicle growth in the presence of NaCl. As shown in Table 1, 10 mM D-Glu and Gln did not alleviate NaCl-induced reduction in seed germination and radicle length. Rather, both D-Glu and Gln further inhibited seed germination and radicle