Elevated Levels of Ethylene During Germination Reduces the Time to Emergence in Impatiens

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Abstract
Impatiens seeds treated with the ethylene biosynthetic precursor 1-aminocyclopropane-1-carboxylic acid (ACC) had reduced times to radicle protrusion and seedling emergence. Sixty hours after imbibition, 68% more seeds showed radicle protrusion on media containing 0.1 or 1.0 mM ACC compared to seeds germinated on water alone. Subsequently, ACC was effectively “loaded” into impatiens seeds using an aqueous soak prior to being dried back to their original fresh weight. Seeds treated with 15 mM ACC for 24 hours showed a 10-fold increase in ethylene production following imbibition as well as a reduced time for initiation of germination. Similarly, seedling emergence in plug flats began sooner in the 24 hour, 15 mM ACC-treated seeds and required 24 hours less time to emerge compared to untreated seeds.

INTRODUCTION
The speed and uniformity at which seedlings emerge is critical for stand establishment and a quality seedling plug tray. The ability for seedlings to emerge under a variety of environmental conditions can be assessed as seed vigor. The Association of Official Seed Analysts defines seed vigor as comprising “those seed properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions” (AOSA, 1983). Compared with seed viability, seed vigor is a better predictor of seedling emergence (TeKrony and Egli, 1991). It is not uncommon for seeds to have high (> 90%) standard germination tests and yet have a low vigor rating. This indicates that characteristics that maintain high vigor are lost prior to any noticeable loss in viability.

Ethylene production is correlated with seed vigor in a number of species. These include corn, tomato, leek and lettuce (Khan, 1994); snap bean (Samimy and Taylor, 1983); radish (Holubowicz et al., 1993); cocklebur and pea (Gorecki et al., 1991); and sunflower (Chojnowski et al., 1997). In all cases, lower vigor seed lots commence producing ethylene later after imbibition and produce less ethylene overall than higher vigor seed lots.

In our previous studies, we have observed that sweet corn, tomato and Arabidopsis seeds treated with ACC showed increased ethylene production and reduced time to radicle emergence compared to non-treated seeds (Siriwitayawan, 2002). Impatiens is the most important bedding plant crop in the USA. The influence of ACC or ethylene on impatiens germination is not known. Therefore, the objectives of this study were to:
1. Evaluate the impact of ACC treatment on time to radicle protrusion.
2. Develop a method to load ACC into seeds prior to germination.
3. Evaluate radicle and seedling emergence in ACC-treated seeds.

MATERIAL AND METHODS
Experiments used impatiens (Impatiens wallerana “Infinity Salmon”). Seeds were stored in closed containers at 5°C until used.

To determine the impact of ACC-treatment on radicle and seedling emergence,
seeds were either germinated in the presence of ACC in the medium or infiltrated with ACC and dried back prior to germination. Seeds were germinated in Petri dishes on germination paper (Anchor Inc., Hudson, Wisconsin, U.S.A.) wetted with water or ACC (0.1 or 1 mM). Seeds were germinated in an incubator with constant temperature (25°C) with an 18-h photoperiod. Time to radicle emergence was recorded every 4 h. Each Petri dish contained 25 seeds and treatments were replicated 4 times.

Seeds were also infiltrated at 25°C with ACC (0, 10, 15 mM) in water for 12, 24 and 48 hours or solvent carriers (ethanol or acetone) for 15, 30, and 60 minutes. Two hundred seeds were placed in 100 ml Erlenmeyer flasks with enough liquid to cover twice the depth of the seeds. The solution was bubbled using an air pump. At the end of the infiltration time, the seeds were removed and dried to their initial moisture content. The dried seeds were stored at 4°C until used. Petri dish germination was as previously described.

Seedling emergence was determined for seeds infiltrated with ACC or water. Seeds were sown into Metromix plug mix in modified 288 plug flats cut to contain 25 cells. Time to seedling emergence was evaluated daily. Three plug flats were used per treatment.

For ethylene measurements, seeds were placed in 25 ml Erlenmeyer flasks (25 seeds per flask, 3 flasks per treatment) containing two pieces of germination paper wetted with 2 ml of deionised water. Flasks were sealed with Parafilm® and kept in germination conditions previously described. Flasks were capped with serum stoppers and incubated for three hours prior to ethylene determination. A 1 ml gas sample was evaluated for ethylene using a Buck Scientific gas chromatograph with flame ionization detector (155°C) and porapak Q column (125°C) with a nitrogen flow rate of 1 ml per minute.

**RESULT AND DISCUSSION**

During radicle protrusion, there is a concomitant rise in ethylene production following imbibition that peaks just after radicle emergence (Matilla, 2000). Increased ethylene production during the early stages of germination has been documented for numerous species (Khan, 1994; Lalonde and Saini, 1992; Petruzzelli et al., 1993). We have shown that seeds from several species (tomato, sweet corn, and Arabidopsis) have reduced time to radicle emergence when germinated in the presence of ACC or ethylene (Siriwitayawan, 2002). This was also the case with impatiens seeds (Fig. 1). Sixty hours after imbibition, 68% more seeds showed radicle protrusion on media containing 0.1 or 1.0 mM ACC compared to seeds germinated on water alone.

In order for this seed treatment to be effective for seedling plug production, a system needed to be developed to load ACC into seeds prior to sowing. In preliminary studies, impatiens seeds were exposed to ACC in either water or solvent carriers for various times. Seeds exposed to the solvents showed reduced germination capacity (data not shown). However, seeds exposed to ACC in water showed reduced time to both radicle and seedling emergence (Fig. 2 and 3). Time to radicle emergence was reduced to a similar extent in the 24 and 48 hour ACC-treatment, but the 12-hour treatment was similar to water control (data not shown). Using 15 mM ACC for 24 hours, seeds required only 65 hours to reach 50% radicle protrusion, while the water and untreated controls required approximately 80 hours (Fig. 2). Similarly, seedling emergence began sooner in the 24 hour, 15 mM ACC-treated seeds and required 24 hours less time to emerge compared to untreated seeds. Ethylene production during germination was higher in seed exposed to ACC for 24 or 48 hours (Fig. 4). For example, seeds exposed to 15 mM ACC for 24 hours produced 10 times more ethylene 3 days after imbibition compared to untreated seeds.

There have been several suggestions concerning the mode of action for ethylene during germination. The most compelling involves a role for ethylene in promoting cell enlargement or elongation leading to an increase in “germination force” (also termed growth potential) resulting in radicle emergence. Abeles (1986) working with lettuce seeds, concluded that ethylene increased the enlargement of cells in the radicle leading to
germination. Similarly, osmoregulation was thought to be the mechanism of action for ethylene-stimulated germination in cocklebur (Esashi et al., 1990). In this study, ethylene increased the osmotic potential in radicle and cotyledon tissue presumably increasing the driving force for radicle emergence leading to germination. One mechanism to explain an increase in osmotic potential would be the production of osmotically active solutes cleaved from storage macromolecules such as starch or proteins. Direct evidence for ethylene’s role in initiating storage protein metabolism was found in chickpea (Cervantes et al., 1994). Using PCR amplification, they were able to clone a cDNA from chickpea that was undetected in dry seeds, but increased following imbibition. The identity of this cDNA corresponded to a class of cysteine proteinases involved in storage protein degradation. They were further able to demonstrate that expression of this gene was dependent on ethylene.

Previous studies have demonstrated that the ability to produce ethylene during germination is an indicator of seed vigor (Khan, 1994) and that for many seeds exogenous application of ethylene or ACC can reduce the time to radicle emergence (Siriwitayawan, 2002). It remains to be seen whether this treatment is a cost effective alternative to other seed treatments such as priming that are used to accelerate germination in bedding plant crops.

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Literature Cited


Figures

Fig. 1. Radicle protrusion in impatiens seeds germinated in a medium containing ACC. Means with the same letter for each hour were not significantly different by Tukey’s HSD (P ≤ 0.05).

Fig. 2. Radicle protrusion in impatiens seeds following an aqueous soak in ACC for 24 hours. Means with the same letter for each hour were not significantly different by Tukey’s HSD (P ≤ 0.05).
Fig. 3. Seedling emergence in impatiens following an aqueous soak in ACC for 24 hours. Means with the same letter for each hour were not significantly different by Tukey’s HSD (P ≤ 0.05).

Fig. 4. Ethylene evolution from impatiens seeds soaked for 24 or 48 hours in ACC. Means with the same letter for each hour were not significantly different by Tukey’s HSD (P ≤ 0.05).