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Research Paper

Comparative assessment for the effects of reactive species on seed germination, growth and metabolisms of vegetables

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ABSTRACT

Reactive oxygen and nitrogen species (RONS) play an important role as signaling molecules in various biological pathways in plants. The aim of this study is to assess the effects of H_2O_2 (ROS) and nitric oxide (RNS) on seed germination, seedling growth and metabolism of two vegetables, coriander and carrot. Seed germination and seedling length of vegetables were significantly increased after treatment with H_2O_2 for 12 and 24 h. An optimal concentration of H_2O_2 giving maximum effect was 25–50 mM for both vegetables. The seed germination and seedling growth of coriander were significantly increased after treatment with 12.5 and 25 μM sodium nitroprusside (SNP; generating about 10–25 μM NO) for 12 and 24 h. On the other hand, SNP treated carrot seeds showed no significant difference in seed germination, compared to control. Amounts of total soluble proteins extracted from both vegetables at 72 h post-germination were significantly greater in treatment with 25 mM H_2O_2. Amounts of total soluble proteins were significantly increased in coriander seeds treated with SNP during germination but not in carrot. The α-amylase activity measured as the amount of reducing sugar was significantly increased in coriander and carrot after H_2O_2 treatment. Our results suggest that the effect of RONS on seed germination, growth and development, and seed metabolisms can be various depending on dose and plant species.

1. Introduction

Vegetables are an important source for healthy foods because of their richness in nutrients and vitamins. Coriander (*Coriandum sativum* L.), a member belonging to the *Apiaceae* family, is an annual herbaceous vegetable and usually used as a flavoring worldwide ingredient in several foods (Laribi et al., 2015). Coriander has been also used in traditional medicinal treatment such as aromatherapy and drugs (Msaada et al., 2017) because they contain a lot of essential oils and phenolic compounds, especially in fruit part (Laribi et al., 2015; Msaada et al., 2017). Carrot (*Daucus carota* L.), a well-known root vegetable and also a member of *Apiaceae* family, contains many antioxidant compounds such as numerous α and β carotenes and provitamin A (Silva Dias, 2014). In spite of being useful sources for nutrition and medicine, coriander and carrot often exhibit poor efficiency in production yield because of the low and inconsistent rate of seed germination (Rithichai et al., 2009; Pereira et al., 2008). Low quality and vigor of coriander seeds may be caused by the staggered flowering behavior of plant (Rithichai et al., 2009). Quality of carrot seeds is highly variable depending on umbel order of inflorescence and this may cause inconsistent germination efficiency (Pereira et al., 2008). Moreover, coriander and carrot seeds require highly optimized environmental conditions for efficient germination such as moisture, temperature, light, pH and oxygen (Koger et al., 2004; Pereira et al., 2008).

Seed germination as an initial step of growth and development in plants is the most important stage. During seed germination process, stored nutrients inside an endosperm (monocot plants) or cotyledon (dicot plants) are catabolized by degradation enzymes to produce energy (Gallardo et al., 2001). On the other hand, the anabolic pathway such as protein synthesis is progressed inside the seeds after imbibition (Miransari and Smith, 2014). These metabolic pathways are triggered by water uptake of seeds as well as oxygen gas diffused into the seed (Gallardo et al., 2001). Some plant seeds go through a long term dormancy after ripening and are very hard to germinate because of thicker and stronger seed coat, germination inhibitors on seed surface, hormone level and unsuitable environmental conditions (Arc et al., 2013).

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The structure of seed coat is important for water absorption and oxygen gas penetration during germination stage (Gutterman, 1994). Indeed, phytohormones, especially gibberellins (GA) and abscisic acid (ABA), are key players for controlling seed germination (gibberellins) and dormancy (abscisic acid) (Bewley, 1997). Several chemical methods have been tried for breaking seed dormancy to enhance seed germination rate, for example, soaking seeds in saline solutions (Panuccio et al., 2014), and treating seeds with plasma generated reactive species (Ji et al., 2015) and chemically produced reactive species (Barba-Espin et al., 2012).

Reactive species in plants can play dual roles, beneficial or harmful, depending on the amount. They are essential for stimulating physiological and developmental processes and resisting stresses (Liu et al., 2007), whereas high level of reactive species triggers senescence, cell death, cell cycle arrest and other harmful reactions (Graves, 2012). Chemically generated and exogenously applied reactive species can play critical roles during plant development such as seed germination and growth as demonstrated in various plants; rice, barley, legume, and maize (Liu et al., 2007; Gondim et al., 2010; Cavusoglu and Kabar, 2010; Barba-Espin et al., 2012). These studies suggest that exogenous reactive species enable to induce and stimulate seed germination and subsequent seedling growth. However, there are still limited number of studies on comparative analysis among individual reactive species in determining effectiveness as an activator and interaction with plant species.

In this study, we compared the effectiveness of chemically generated RONS in enhancing seed germination, seedling growth and seed metabolisms of two vegetable plants in the *Apiaceae* family, coriander and carrot. The results from this study will provide the useful information in understanding the effectiveness of individual reactive species on plant development and the interaction between reactive species and plant species.

2. Materials and methods

2.1. Plant materials and growth condition

Coriander and carrot seeds were used for testing the effect of chemically generated reactive species on seed germination, growth and metabolisms. Variety 3A (AAA) and 333 seeds were used for coriander and carrot, respectively. Germinated seeds were planted in sterilized soil and kept in the natural greenhouse of Agroforestry program, Maejo University Phrae Campus, Thailand.

2.2. Treatment with chemically generated reactive species

All chemicals used in the experiments were analytical grade. H_2O_2 solution and sodium nitroprusside (SNP) were used as a donor for H_2O_2 and NO, respectively. For H_2O_2 treatment on coriander and carrot seeds, seeds (150 seeds per treatment) were placed in a beaker containing 30 ml of H_2O_2 solution of 25, 50, 100, and 200 mM and incubated at room temperature for 12 and 24 h. For NO treatment, we dissolved sodium nitroprusside (SNP) in distilled water to make the concentration of 12.5, 25, 50 and 100 μM. After placing coriander and carrot seeds in the beakers containing SNP solutions, the beakers were incubated for 12 and 24 h. After incubation, seeds were moved onto two layers of wet-cultivate paper in petri-dish (∅ 9 centimeters) and then germination was monitored every day.

2.3. Seed germination and seedling growth analysis

For analysis of coriander and carrot seed germination, we counted number of germinated seeds every 24 h. Germinated seed was determined by the emergence of the radicle from seed coat. Seed germination rate was calculated as follows;

$$\text{The percentage of seed germination} = \frac{\text{Number of seeds germinated}}{\text{Total number of seed}} \text{x } 100$$

In addition, germination index (GI) was also calculated for assessing seed vigor as described in below;

$$\text{Germination index} = \frac{\text{No. of seed germination}}{\text{Day of first count}} + \ldots + \frac{\text{No. of seed germination}}{\text{Day of final count}}$$

In the case of seedling growth, we randomly selected 10 coriander (6 days old) and 10 carrot (5 days old) seedling plants in each treatment, and then measured the seedling length.

2.4. Analysis for the amount of total soluble proteins and reducing sugars

Coriander and carrot seeds were treated with H_2O_2 and SNP solutions as described in earlier section. Geminated seeds were harvested 0 and 72 h after H_2O_2 and SNP treatment. Then, seeds were dried by incubating at 60 °C for 24 h and then grinded in liquid nitrogen. To extract total soluble proteins, 6 ml of Tris-buffer (0.05 M Tris hydroxylmethionine, 0.02 M $CaCl_2$, adjust pH 7.4 by using 6 M HCl) solution was added to grinded powder and the mixture was shaken on platform mixer for 1 h. Homogenates were centrifuged at 2879 × *g* for 5 min. Supernatant was transferred to a new tube and kept on ice. Concentration of proteins was measured at 280 nm using spectrophotometer. Reducing sugar amounts were measured by dinitro-salicylic (DNS) method. Extracted solutions (0.25 ml) were mixed with Tris-buffer (0.25 ml), and then 0.5 ml starch solution (1% w/v) (starch prepared in 0.02 M phosphate buffer pH 7.0) was added. Mixture was incubated at 37 °C for 1 h. Then, 0.5 ml of DNS (1% (w/v) 3,5-dinitrosalic acid, 0.02 M NaOH, 30% (w/v) sodium potassium tartrate solution was added to the mixture for detection of reducing sugars and incubated at 100 °C for 5 min. After 5 ml of distilled water was added to samples, absorbance was measured at 540 nm using spectrophotometer. The amounts of reducing sugar were calculated from a standard curve made by using maltose as a reference sugar.

2.5. Statistical analysis

The data of seed germination rate, seedling length, and concentration of total soluble proteins and reducing sugars were expressed as mean and standard error (SE) of the mean for indicated number of replicates (≥3). Statistical analysis was performed by using student's *t*-test to establish significance between data points, and significant differences were based on the $p < 0.05$ or $p < 0.01$ ($^*p < 0.05$ and $^{**}p < 0.01$).

3. Results

3.1. Exogenous H_2O_2 enhanced seed germination and seedling growth of both vegetables

Coriander and carrot seeds were treated with several concentrations of H_2O_2 solution under room temperature. The pH of all H_2O_2 solutions before and after soaking seeds was between 5.43 and 5.59. Fig. 1A shows the percentage of coriander seed germination after treatment with H_2O_2 for 12 and 24 h. The percentage of seed germination was significantly increased after treatment with all concentrations of H_2O_2 solutions. Particularly, 25 mM H_2O_2 solution showed the greatest effect; 82 and 87% germination after 12 and 24 h, respectively. In the treatment with 200 mM H_2O_2 solution, the percentage of seed germination was initially reduced but became higher than that of control (no H_2O_2) after 120 h. Fig. 1B shows the morphology of germinated coriander seeds. Radicle of embryo was emerged through seed coat earlier in seeds treated for 12 h than 24 h, Germination index was also