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Seed priming stimulate germination and early seedling growth of Chinese cabbage under drought stress

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A B S T R A C T

Drought stress influence seed germination and seedling growth of many plants. Seed priming could be used to alleviate the depressive effects of drought stress. The improving effects are influenced by many factors including priming methods, plant species and drought intensity. The mechanisms of drought tolerance induced by seed priming have not been clearly elucidated. The study was carried out to assess whether drought tolerance could be enhanced by seed priming at the germination stage and characterize the potential physiological and biochemical alternation of drought tolerance in Chinese cabbage. The seeds were soaked at 20 °C for 8 h in distilled water, 200 mmol/l potassium nitrate (KNO_3), 200 mmol/l urea, respectively. Both primed and unprimed seeds germinated under six levels of drought stress (0, −1.0, −2.0, −3.0, −4.0 and −5.0 MPa osmotic potential) induced by PEG 6000. Results indicated that germination traits (germination percentage, potential and seedling vigor index) of Chinese cabbage all decreased gradually with increasing drought intensity. Three seed priming types including water, KNO_3 and urea all increased germination traits at all levels of drought stress as compared to the unprimed seed. The enhanced drought tolerance conferred by seed priming treatments is associated with the modulating peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) activities and levels of soluble sugar and proline. The results suggested that priming could serve as an appropriate treatment to increase the germination and early seedling growth of Chinese cabbage under drought stress conditions.

1. Introduction

As a major constraint limiting crop production worldwide, water deficit during germination stage results in a decline or even complete inhibition of seedling emergence and stand establishment (Kaya et al., 2006). Under drought stress, seed germination and seedling establishment were inhibited due to the drop of water potential, which results in the decline in water uptake (Farooq et al., 2009). Oxidative damage caused by the overproduction of reactive oxygen species (ROS) is another major problem when plants are exposed to drought stress (Gill and Tuteja, 2010). It is necessary to alleviate the adverse effects of drought stress for achieving good crop yields (Ashraf and Rauf, 2001).

Among various strategies adopted to improve plant drought tolerance, seed priming is thought to be an easily applied, low-cost and effective approach (Ashraf and Foolad, 2005). Different types of priming treatments were recorded to enhance drought tolerance in many plants. Seed priming with ascorbic acid, potassium salts improved the drought resistance of wheat (Farooq et al., 2013). Hydropriming improved seed germination and seedling emergence of lentil (Saglam et al., 2010). KNO_3 and hydropriming increased sunflower germination and seedling growth under salt and drought stresses (Kaya et al., 2006). Seed priming with PEG increased the germination and early seedling growth of barley under drought stress condition (Rouhollah, 2013; Tabatabaei, 2013). Seed priming with PEG and water increased drought stress tolerance in seeds of rice cultivars at the germination stage (Sun et al., 2010).

These positive effects of priming are associated with a wide range of metabolic and physiological improvement (Shehab et al., 2010). Among them, activating protective enzymes, such as SOD, POD, CAT and accumulating osmoprotectants, such as proline, soluble sugar and soluble protein are the typical stress-avoidance responses (Farhad et al., 2011). Activation of enzymatic antioxidants can reduce ROS-induced oxidative damages (Posmyk et al., 2009). Osmoprotectants helps to improve water uptake by improving the water status (Farooq et al., 2009).

Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) is a very popular vegetable around the world. The major Chinese cabbage growing areas in Asia are often threatened by drought stress. Although the effects of seed priming on improving drought tolerance have been extensively investigated in many crops and vegetables, experimental data of seed germination and seedling growth under drought stress subsequent to seed-priming are lacking in Chinese cabbage.

Abbreviations: GP, germination potential; SVI, seedling vigor index.

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The purpose of the current study is to evaluate the effects of three priming treatments on enhancing seed germination and early growth of Chinese cabbage under drought stress, and characterize the physiological and biochemical responses to drought stress in Chinese cabbage under laboratory conditions.

2. Materials and methods

2.1. Plant material and priming

Seeds of Chinese cabbage (*B. rapa* subsp. *pekinensis*) cv. Lainong 50 were soaked in distilled water, 200 mmol/l urea and 200 mmol/l KNO_3 at 20 °C for 8 h, respectively. The treated seeds were rinsed with distilled water and air-dried at 20 °C for 48 h back to their initial moisture content.

2.2. Germination tests

Primed and unprimed seeds were germinated in plastic boxes (12 × 12 × 6 cm) with two layer filter papers moistened with 10 ml appropriate solutions, 6 levels of osmotic potentials including 0 (distilled water), −1.0, −2.0, −3.0, −4.0 and −5.0 MPa induced by PEG 6000. Four replicates with 100 seeds per replicate were used for each treatment. The boxes were placed in a germinator at 20 ± 1°Cunder 8/16 h day/night light for 7 days. The papers belong to each box were replaced every two days to maintain water potential. Seeds were considered germinated when the radicle visibly protruded through the seed coat and recorded daily. At the end of experiment, the final germination percentage was calculated. The germination potential was calculated as $GP = \sum(Gt/Tt)$, Gt is the number of germinated seeds on day t, and Tt is the number of days from the start of the test. Seedling vigor index (SVI) was calculated as SVI = $GP \times$ *seedling dry weight*. The seedlings were dried at 80 °C for about 48 h and seedling dry weight was determined (Yan et al., 2010).

2.3. Antioxidant enzyme assays in Chinese cabbage seedlings

Two gram of Chinese cabbage seedlings were ground in liquid nitrogen and the powders were extracted with 10 ml 50 mmol/l phosphate buffer (pH 7.0), then centrifuged at 14,000 *g* for 15 min at 4 °C. The supernatant was collected to assess the antioxidant enzyme activities and soluble protein content.

CAT activity was measured following the method of Aebi (1974). SOD activity was assayed using the nitroblue tetrazolium method described by Dhindsa et al. (1980). POD activity was measured using the guaiacol oxidation method (Chance and Maehly, 1955).

2.4. Assaying for compatible solutes in Chinese cabbage seedlings

Soluble sugar content was measured by the Anthrone method (Irigoyen et al., 1992) and expressed as $mg.g^{-1}$ FW of seedlings. Proline content was assessed as described by Bates et al. (1973). Soluble protein content was determined according to the method of Bradford (1976).

2.5. Statistical analyses

A completely randomized design was used in the experiments. Analysis of variance (ANOVA) was used to compare priming treatments effect, and significant differences of means were separated using Duncan's test ($p < 0.05$). To correlate the results obtained with different methods, a regression analysis was performed and correlation coefficients were calculated.

3. Results

3.1. Effects of seed priming on seed germination of Chinese cabbage under drought stress

The germination traits (germination percentage, potential and vigor index) both of primed and unprimed seeds decreased gradually with increasing drought stress levels (Table 1). Three priming treatments studied showed depressive effects of drought stress on germination traits compared with the unprimed control. The germination traits of the primed seeds were higher than those of unprimed ones under normal and drought stress conditions (Table 1).

3.2. The antioxidant enzyme activities in primed and unprimed Chinese cabbage seedlings under drought stress

Results showed that POD activity in unprimed plants increased at the drought stress (−1.0, −2.0, −3.0 and −4.0 MPa), then decreased at −5.0 MPa (Table 1). The activity of POD in primed plants was significantly higher than that in non-primed plants under normal and water stress conditions (0, −1.0, −2.0 and −3.0 MPa) (Table 1). These results indicated that seed priming enhances POD activity in Chinese cabbage seedlings under drought stress. Therefore, POD activity is significantly positively correlated with the germination percentage ($r = 0.597$, $p < 0.05$), potential ($r = 0.480$, $p < 0.01$) and vigor index ($r = 0.590$, $p < 0.05$) (Table 2).

The activities of SOD in unprimed and primed plants were markedly increased with the increasing drought stress levels. SOD activities in primed seedlings were higher than that in non-primed one both normal and stress conditions (Table 1). The results indicated that SOD activity was significantly negatively correlated with the germination percentage ($r = -0.736$, $p < 0.05$), potential ($r = -0.632$, $p < 0.01$) and vigor index ($r = -0.642$, $p < 0.05$) (Table 2).

The activity of CAT in primed and non-primed Chinese cabbage seedlings increased with elevating drought stress levels and then decreased at higher levels of drought stress (Table 1). CAT activity in unprimed, hydroprimed, KNO_3 and urea primed seedlings peaked at −2.0, −3.0 and −1.0 MPa, respectively (Table 1). Results indicated that CAT activity was positively correlated with the germination percentage ($r = 0.468$, $p < 0.01$) (Table 2).

3.3. The osmolyte concentration in primed and unprimed Chinese cabbage seedlings under drought stress conditions

Soluble sugars in unprimed and primed seedlings increased with increasing drought stress levels (Table 1). Results indicated that sugar content was significantly negatively correlated with the germination percentage ($r = -0.645$, $p < 0.05$), potential ($r = -0.547$, $p < 0.01$), vigor index ($r = -0.550$, $p < 0.01$), and positively correlated SOD ($r = 0.646$, $p < 0.05$) (Table 2). Soluble protein content in unprimed and primed seedling decreased dramatically at −1.0 MPa, and recovered at higher drought stress levels. In the present study, no significant correlation was observed for soluble protein with any of the parameters studied (Table 2).

Proline content in primed seedlings markedly increased with the increasing drought stress. Proline contents in three priming seedlings were significantly higher than those in unprimed ones. Results indicated that proline content was significantly negatively correlated with the germination percentage ($r = -0.801$, $p < 0.05$), potential ($r = -0.726$, $p < 0.05$), vigor index ($r = -0.655$, $p < 0.05$), and positively correlated SOD ($r = 0.721$, $p < 0.05$) and sugar ($r = 0.590$, $p < 0.05$) (Table 2).

4. Discussion

Drought stress is a major abiotic agent that seriously decreases crop productivity in arid and semi-arid regions of the world (Lipiec et al.,