Canola seed priming by abscisic acid improved antioxidant enzymes activity under low temperature stress

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Abstract
Low temperature stress is one of the most important stresses during canola germination in spring. A study was conducted to study the effect of priming with abscisic acid (50 µM concentration) on *Brassica napus* cv. Zarafm seed germination under chilling stress (3℃). Primed seeds showed more and faster germination. Also, the effects of ABA priming on antiodiant enzymes during first three days of germination in different seed parts (embryo axis, seed coat and cotyledon) were investigated. ABA-priming promoted superoxide dismutase and peroxidase activity for detoxification of superoxide and hydrogen peroxide radical, especially in seed coat.

Keywords: abscisic acid (ABA), priming, *Brassica napus* (canola), germination, chilling stress, polyphenol oxidase (PPO), peroxidase (POX) and superoxide dismutase (SOD)

Introduction

Canola (*Brassica napus)* is an important worldwide oily plant and biofuel that chilling injury could limit its cultivation. Chilling stress caused delay or defect at the phase of germination that might affect yield due to increasing reactive oxygen species (ROS) reduce antioxidant activity and decreasing respiration. Priming is an approach for improving germination characteristics which means a partial imbibition of seeds for pre-germinative metabolisms without radicle protrusion. ABA is a plant hormone by several roles in germination, development and encountering different stresses. Antioxidant system consists of different members like superoxide dismutase (SOD), peroxidase (POD), and polyphenol oxidase (PPO), which would detoxify ROS. Polyphenol oxidase (PPO, EC 1.14.18.1) is a copper enzyme which could hydroxylate monophenols to *o*-diphenols and also oxidize *o*-diphenols to *o*-quinones. Superoxide dismutase (SOD; EC 1.15.1.1) is a group of metalloenzymes that catalyze the dismutation of superoxide radicals and convert them to oxygen and hydrogen peroxide. Peroxidase (POX, EC 1.11.1.7) is a group of glycoproteins with different roles which decompose hydrogen peroxide. In this investigation, we studied the effect of ABA priming on antioxidant enzymes activities in different parts of seed (embryo axis, seed coat and cotyledon) during the first 3 days of germination.

Materials and methods

Canola (*Brassica napus* cv. Zarfam) seeds were obtained from the 2008 harvest at Plant and Seed Improvement Institute, Karaj, Iran. Seeds were soaked for 40 hours in 50 µM abscisic acid (± Abscisic acid, SIGMA, Canada) solution in the dark at 20±2℃. After priming they were blotted and sterilized. They were placed in 10 cm autoclaved glass petri dishes containing 5ml sterile water and 2 autoclaved filter papers and put in the dark at 3±0.5℃. Germination was recorded daily for 7 days and radicle protrusion to 1 mm was scored as germination.
For enzyme assays, seeds were collected after 24, 48 and 72 hours of imbibitions and separated into seed coats, cotyledons and embryo axis. 100 mg of each sample was homogenized in liquid nitrogen, suspended in 1mL of extraction buffer [0.1 M Tris-HCl pH 7.8 and 10 % (v/v) glycerol], vortexed, filtered and centrifuged for 20 min at 13000 *rpm* at 4 ℃. Supernatant was served as the enzyme preparation by using a UV – 160 A – SHIMADZO spectrophotometer. Protein concentration was determined using BSA as a standard. Polyphenol oxidase assayed according to modified method of Kar and Mishra, (1976). Increase in absorbance g^{-1} min^{-1} (ΔA g^{-1} min^{-1}) of sample at 420 nm during 180 sec was used. SOD assay was a modified method of Lee and Lee (2000). One unit of SOD was defined as the amount of enzyme which caused a 50% decrease of the SOD-inhibitable NBT

reduction at 560 nm. Peroxidase assayed according to a modified method of Lee and Lee (2000). Increase in absorbance g^{-1} min^{-1} (ΔA g^{-1} min^{-1}) of sample at 470 nm during 180 sec was used.
A factorial experiment with a completely randomized design was used with two factors (treatment and time) for cumulative germination and three factors (treatment, time and seed part) for enzymes. Data were transformed and then subjected to analyses of variance, which were carried out on all data collected using MSTAT-C. When a significant F ratio occurred, Duncan's test was used for separating mean values.

Results and discussion

Effect of ABA-priming on cumulative germination percentage of *Brassica napus* seed under chilling stress

At first hours of chilling stress, there wasn't significant difference between ABA-primed and control seeds. In control seeds, cumulative germination percentage increased slowly under chilling stress and 96 hours later was the start point for phase III of germination (Fig. 1). As a thermal dependent event, it is possible that respiration rate decreased due to low temperature and caused lower germination rate. Priming with 50 µM ABA, caused the seeds to germinate faster (after 48 hours since start of imbibition). Faster germination with seed priming could be related to higher antioxidant activity Seed priming with ABA caused a three-fold increase in cumulative germination percentage (about 61%).

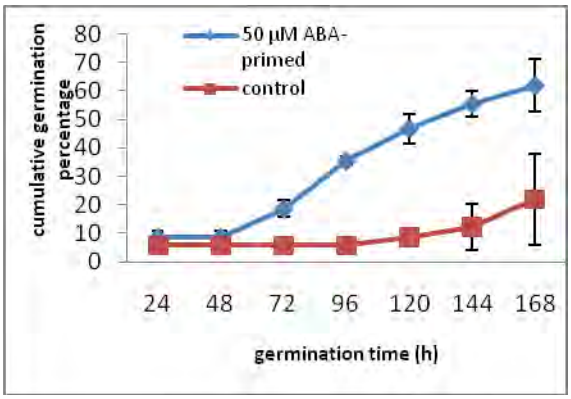

Figure 1. Cumulative germination percentage for 50 µM ABA-primed and control seeds of *Brassica napus* cv. Zarfam under chilling stress (3°C) during 7 days germination. Data were analyzed by two-factor (time and treatment) completely randomized design and were significant at P<0.01 according to Duncan's Multiple Range Test. Data points are average ± SD for 3 replications.

Effect of ABA-priming on biochemical indexes of *Brassica napus* seed under chilling stress

a. ***Polyphenol oxidase activity:***
Priming did not influence PPO activity. In contrast to our observation, some papers showed an increase of PPO activity in primed seeds (Moosavi et al., 2009**).** Totally, PPO had the maximum activity (0.077 ΔA mg^{-1} min^{-1}) under chilling stress, in *Brassica napus* seed coat (Fig. 2A). Under chilling stress, plant growth decreased due to increase of phenolics content. This meant lower activity of polyphenol oxidase. For producing pigments, PPO used O_2 and produced H_2O.

b. ***Superoxide dismutase activity:***
ABA-primed whole seed showed a 1.5-fold SOD activity more than control, under chilling stress (Fig. 2B).The maximum activity of SOD was for seed coat (23.5 U mg^{-1}) (Fig. 4C). This increase might be related to oxidative stress and production of superoxide radicals since radicle protrusion. Significant difference was not observed between SOD activity of cotyledon and embryo (Fig. 2C).

c. Peroxidase activity
Fig. 3 showed that seed priming with ABA increased peroxidase activity during seed imbibition under chilling stress. 10-fold and 4.6-fold increases were observed in primed seeds during 48 and 72 hours, respectively. Low temperature decreased respiration and caused leakage of electrons from electron transport systems. Their reaction with O_2 increased H_2O_2 suddenly. POX would detoxify this enzyme; otherwise it might cause further damages. ABA treatment under chilling stress increased POX which may cause more respiration and ATP production by protecting mitochondria from oxidative stress (Prasad et al., 1994). Maximum peroxidase activity measured for seed coat. Peroxidase activity was rather low at both cotyledons and embryo axis, and

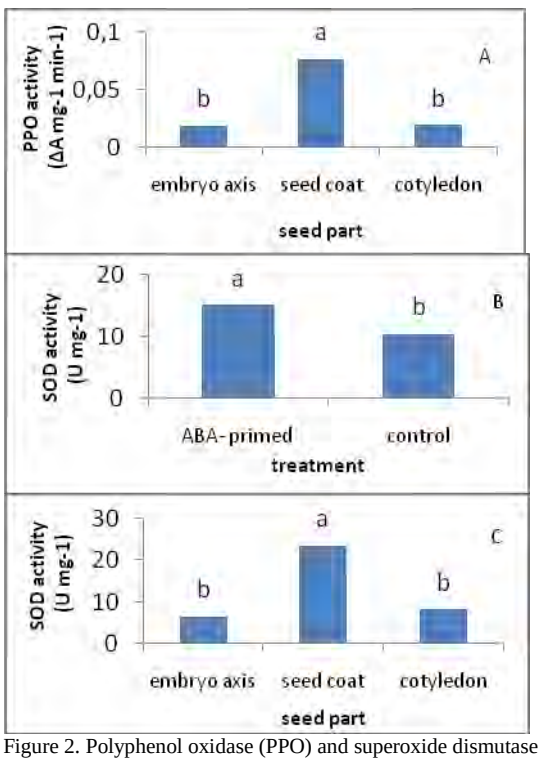

Figure 2. Polyphenol oxidase (PPO) and superoxide dismutase (SOD) activities in *Brassica napus* cv. Zarfam during first three days of germination under chilling stress (3° C). Seeds either primed with 50 µM concentration of abscisic acid or control were separated into embryo axis, cotyledons and seed coat. Data were analyzed by three factor (time, treatment and seed part) completely randomized design. PPO activity showed significant difference at P<0.01 for the main factor of seed part (A). SOD activity showed significant difference at P<0.05 for the main factors of treatment (B) and seed part (C). Different letters above the bars indicate significant differences according to Duncan's Multiple Range Test

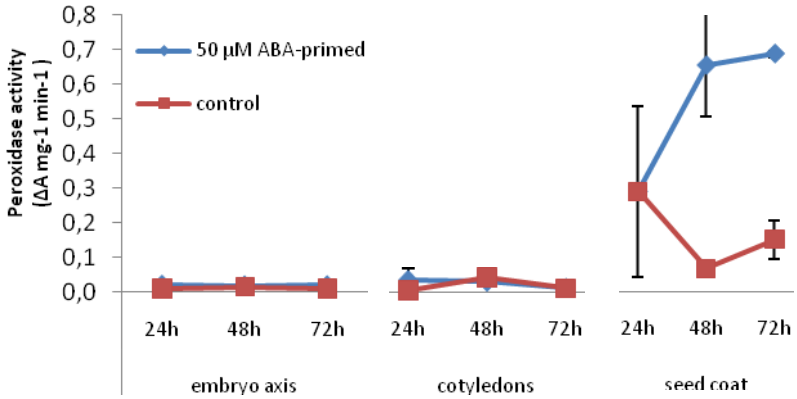

Figure 5. Peroxidase activities of *Brassica napus* cv. Zarfam during first three days of germination under chilling stress (3 ° C). Seeds either primed with 50 µM concentration of abscisic acid or non-primed were separated into embryo axis, cotyledons and seed coat. Data were analyzed by three factor (time, treatment and seed part) completely randomized design and

ABA did not have any significant effect on it (Fig. 3). POX activity increased sharply in seed coat of *Brassica napus* during seed germination, but in cotyledon and embryo axis increased slowly.

Conclusion

Chilling stress decreased germination percentage of *Brassica napus* cv. Zarfam seed and lowered its rate which would deeply influence its yield. Probable decrease in seed respiration would cause electron leakage, O_2 accumulation and their combination would produce ROS. Abscisic acid is a hormone with several characteristics like stress counter. Increasing O_2 during first 3 days of germination under chilling stress could not influence PPO. It seems that electron leakage would convert O_2 to superoxide radicals. ABA-Priming promoted SOD activity of canola seed coats. This enzyme detoxified superoxide radicals to hydrogen peroxide. On the other hand, priming improved POX activity which would detoxify H_2O_2. These phenomena occurred after 48 hours of germination start and would be coincided with completion of germination and seed coat protrusion of primed *Brassica napus* under chilling stress.

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