Effect of abscisic acid on physiological indices of aged seeds of *Brassica napus* under low temperature stress

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Abscisic acid is considered as one of the most important plant regulator substances in response to abiotic stresses. Seed germination is the critical stage in plant establishment under low temperatures. Effects of hormonal seed priming on physiological indices of two *Brassica napus* cultivars, Zarfam and RGS003, were investigated. Germination tests were conducted at 7 °C, with a factorial arrangement based on a completely randomized design with three replications. Priming treatments were consisted of control, hydro-priming, priming with abscisic acid (ABA) in three concentrations (50, 100, 150 μM) and two priming durations (24 and 40 hours). Controlled deterioration test was conducted either before or after priming. Results showed that seed priming with abscisic acid promoted germination indices of both cultivars under low temperature stress. Deterioration induced greater damage to seeds with no priminig treatments.

Key words: abscisic acid, deterioration, low temperature stress, *Brassica napus*, priming

Introduction

Low temperature stress during germination of *Brassica napus*, an important oily and biofuel crop, causes great defects (Russo et al., 2010). Seed storage especially under adverse condition could reduce germination indices due to deterioration. Abscisic acid is an important plant hormone with several effects especially stress response. It is a suitable material for seed priming, a technique for increasing seed germination and establishment (Bittencourt et al., 2005). In this study we investigated the integration effect of low temperature stress and deterioration on germination indices and surveyed if priming with ABA could improve seed performance.

Materials and Methods

Canola (*Brassica napus* cv. Zarfam and RGS003) seeds were obtained from the 2008 harvest at Plant and Seed Improvement Institute, Karaj, Iran. Seeds were soaked in water (18 h) or abscisic acid (24 or 40 h) in dark at 20±2 ℃. Concentrations of 50, 100 and 150 μM of ABA (± Abscisic acid, SIGMA, Canada) with either 24 or 40 hours duration, hydropriming (18 hours) and control were used. After priming, seeds were blotted and sterilized. They were placed in 10 cm autoclaved glass Petri dishes containing 5 mL sterile water and 2 autoclaved filter papers and put in the dark at 7±0.5℃. Germination was recorded daily for 7 days and radicle protrusion to 1 mm was scored as germination. Controlled deterioration was carried according to Hampton and Tekrony (1995). Seeds with 20% moisture in sealed pockets were placed in bath (45±1 °C) for 48 hours. They were dried for 24 hours at 20±2℃. Part of samples primed and then deteriorated and some were primed after deterioration. They were compared to non deteriorated primed seeds. Germination percentage, germination rate (reverse of mean germination time multiply 100) and vigor index (multiplying of germination rate and seedling length) were calculated. A factorial experiment with a completely randomized design was used with three factors (cultivar, treatment and deterioration status). 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

Low temperature stress (7° C) caused a mean germination percentage of about 50% (fig. 1). Priming (especially low concentrations of abscisic acid) increased (1.4 fold) *Brassica napus* seed germination percentage. Low temperature could either decrease or fix the germination percentage of Brassicas (Russo et al., 2010). Okuda et al. (1991) showed that low temperature in wheat seedlings caused leakage of electrons from electron transport systems and thereupon increase of reactive oxygen species. Spraying *Stylosanthes guianensis* plants with ABA caused lower electrolyte leakage, lower MDA and higher antioxidant enzymes under chilling stress (Zhou and Guo, 2009). Nerson and Govers (1986) showed that muskmelon seed priming increased seed germination under low temperature.

The effect of deterioration upon seed germination was more severe. Deterioration caused a 12.4 fold decrease in *Brassica napus* seed germination under low temperature stress (fig. 1). Also, Hsu et al. (2002) showed that bitter gourd seed ageing decreased emergence percentage due to increased peroxide accumulation and reduced scavenging enzymes activities. Ageing decreased more severely germination percentage of rice seeds due to low performances of deteriorated seeds under low temperature stress (Ali et al., 2006). Priming treatments, either before or after deterioration, could not improve germination percentage (fig. 1). Low germination of primed seeds encountered to deterioration is due to more susceptibility of primed seeds to stressful storage conditions (Hsu et al., 2002). Asparagus seed priming, especially in low vigor, increased germination percentage after stress of deterioration (Bittencourt et al., 2005). Castellinó, et al., (2010) showed that priming could be a recovery treatment if deterioration was not fully advanced. It did such action via resolubilization of proteins.

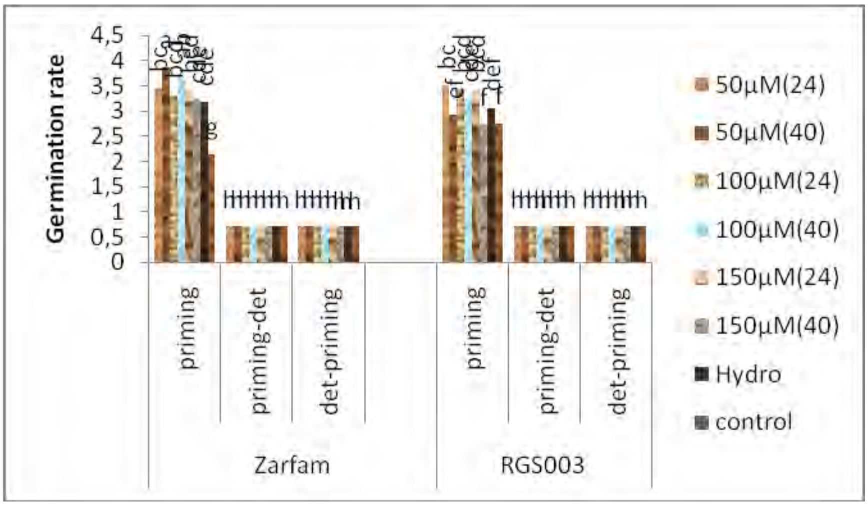

Figure 2. Effect of priming on germination rate of *Brassica napus* cv. Zarfam and RGS003 under chilling stress (7°C) and deterioration. Values are means of three replications. Data were analysed by three-factor (cultivar, deterioration status and treatment) ANOVA and different letters above the bars indicate a

Low temperature stress caused 2.1 and 2.7 germination rates for Zarfam and RGS003 cultivars, respectively (fig. 2). Decraese of germination rate was more severe upon Zarfam cultivar (data not showen). Priming increased germination rate of both cultivars. This effect was more obvious for Zarfam and the best priming treatment was 50 µM concentrations of abscisic acid for 40 hours. The best priming treatment for RGS003 was 50 µM concentrations of abscisic acid for 24 hours. Zhang and Dorna (2005) showed that abscisic acid is a good priming matarial which decreased mean germination time and increased germination index. Pepper seed priming increased germination rate due to increasing respiration rate, decreasing MDA content and increasing antioxidant enzymes activity (Korkmaz and Korkmaz, 2009). Exogenous ABA decreased electrolyte leakage and oxidative stress and so increased growth rate of chickpea (Nayyar et al., 2005).

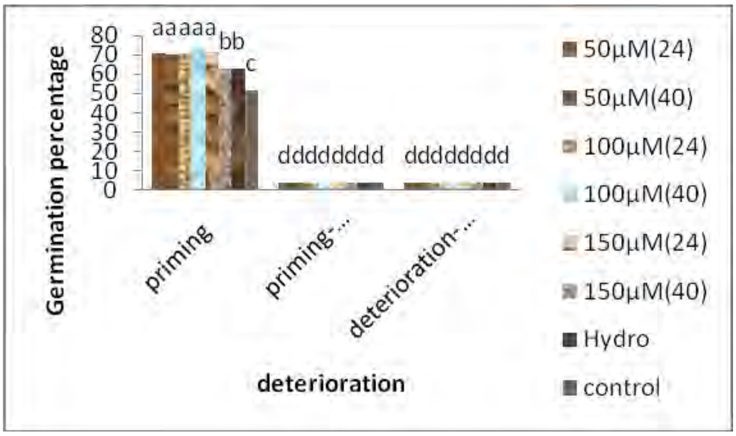

Figure 1. Effect of priming on germination percentage of *Brassica napus* under chilling stress (7°C) and deterioration. Values are means of three replications. Data were analysed by three-factor (cultivar, deterioration status and treatment) ANOVA and different letters above the bars indicate a significant difference at P<0.01 for interaction factors of treatment and deterioration according to Duncan's multiple range test.

Interaction of deterioration and low temperature stress caused a very low germination rate in both cultivars (fig. 2). Ageing decreased germination rate of rice seeds at all temperatures especially at lower one (Ali et al., 2006). Priming, before or after deterioration could not improve germination rate of *Brassica napus*. Ageing increased emergence duration of bitter gourd seeds, especially in primed ones (Hsu et al., 2002). Priming decreased time to 50% emergence and MGT in low vigor seeds of sunflower, but very low vigor seeds after deterioration encountered intense damage that priming could not recover it (Kausar, 2009).

Under low temperature stress, vigor index of Zarfam cultivar was lower than RGS003 (fig. 3). Nykiforuk and Johnson-Flanagan (1999) also showed that low temperature decreased seedling growth. Priming caused increases of 1.9 and 1.2-fold in Zarfam and RGS003, respectively. The best treatments was 50 µM concentrations of abscisic acid for 40 (Zarfam) and 24 hours (RGS003). Prasad et al (1994) showed that ABA increased peroxidase and decreased oxidative stress due to low temperature in maize seedlings.

According to fig. 3, deterioration decreased vigor index of Zarfam (3-fold) and RGS003 (5.5-fold). Accelerated ageing decreased vigor and germinebility of sun flower, either on optimal or sub-optimal temperatures (Kausar et al., 2009). Neither of priminig treatments could conserve nor recover deterioration effects on vigor index of both cultivars. ABA priming improved *Brassica napus* seed viability during storage by decreased dehydration and drying injury (McKee and Finch-Savage, 1989). Priming improved banding pattern and intensity of protein in low vigor seeds (Kausar et al., 2009). Hydroprimig caused lettuce seeds loose viability sooner than control probably due to loss of sucrose and oligosaccharids (Gurusinghe and Bradford, 2001).

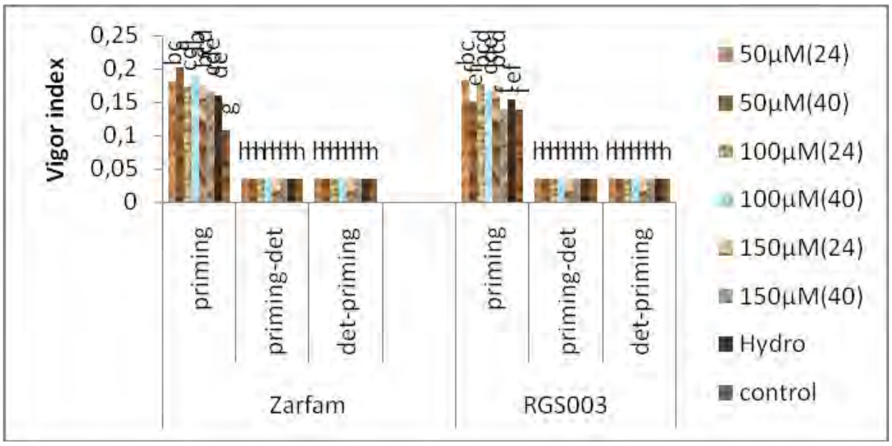

Figure 3. Effect of priming on vigor index of *Brassica napus* cv. Zarfam and RGS003 under chilling stress (7°C) and deterioration. Values are means of three replications. Data were analysed by three-factor (cultivar, deterioration status and treatment) ANOVA and different letters above the bars indicate a significant difference at P<0.01 for triplet interaction factors according to Duncan's multiple

Conclusions

Seed priming with abscisic acid promoted germination indices of both cultivars under low temperature stress. Decreasing oxydative stress by antioxidant enzymes was supposed as the probable reason. Deterioration induced greater damage to seeds which non of priminig treatments could conserve or recover its worst effects on germination percentage, germination rate and vigor index.

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