Cercetări Agronomice în Moldova
Vol. XLVI , No. 4 (156) / 2013

HEAT SHOCK TREATMENT CAN IMPROVE SOME SEED GERMINATION INDEXES AND ENZYME ACTIVITY IN PRIMED SEEDS WITH GIBBERELLIN OF MOUNTAIN RYE (*SECALE MONTANUM*) UNDER ACCELERATED AGING CONDITIONS

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Received February 8, 2013

ABSTRACT. Seed priming with gibberellin (GA) enhances seed germination performance; but the quality of primed seeds in aging condition often reduces more than non-primed seeds. An experiment was conducted to evaluate the effect(s) of heat shock treatments on germination characteristics and enzyme activity of primed mountain rye (*Secale montanum*) seeds with gibberellin under accelerated aging. Heat shock treatments, can substantially decrease the speed of quality reduction of mountain rye (*Secale montanum*) primed seeds. In primed seeds with gibberellin, which has non-aged, the highest germination percentage (GP) and normal seedling percentage (NSP) was attained from heat shock treatment at 35°C for 3 h, also after 3 days aging, it was attained from heat shock treatment at 35°C for 3 h. After 3 days of aging the highest germination index (GI) was attained from unprimed seeds, but no significant difference with heat shock treatment at 35°C for 3 h. The minimum means time germination (MTG) was in heat shock treatment at 30°C for 3 h in non-aged seeds. After 3 days of aging, heat shock treatment reduce MTG as compared to the primed seeds. Heat shock treatment at 35°C for 3 h increased seed vigor index (SVI) as compared to the unprimed and primed seed in non-aged seeds and after 3 days aging. Seedling length (SL) increases with heat shock treatment at 30°C for 4 h in non-aged seeds as compared to the primed and unprimed seeds, but after 3 days of aging heat shock treatment except at 35°C for 3 h and 40°C for 4 h reduced SL as compared to the primed and unprimed seeds. Also, heat shock treatments increase some antioxidant

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enzymes [Catalase (CAT), Ascorbat peroxidase (APX)].

Key words: Germination characteristics; Heat shock treatment; Priming; Catalase; Ascorbic peroxidase; Accelerated aging.

INTRODUCTION

The mountain rye (*Secale montanum*) is a native wild species in southern Europe, Morocco, Iran and Iraq (De Bustos and Jouve, 2002). The value of *S. montanum* as a pasture crop has been tested successfully in the United States (Buman *et al.,* 1988), Australia and New Zealand (Oram, 1996). Montanum rye (*Secale montane*) is an important plant in world that has more feed uses.

Successful stand establishment requires highquality seeds, i.e. seeds that: 1) germinate completely; 2) germinate quickly and simultaneously; 3) produce normal and vigorous seedlings; 4) have germination which shows little sensitivity to external factors, enabling them to germinate in a wide range of agroclimatic conditions (Corbineau and Côme, 2006).

Methods of evaluation of seed quality, providing accurate prediction of seed performance under field conditions, must be developed. Such methods are also required for assessing the effectiveness of technologies used to enhance seed performance, such as priming or seed health treatments (Corbineau, 2012). McDonald (2000) and Ansari *et al.* (2012) reported that; seed priming is a process in which seeds are imbibed in water or osmotic solutions followed by drying before radicle emergence. Bruggink *et al.* (1999) reported that an important practical limitation of seed priming is the strong reduction in longevity that is associated with the desired increase in speed of germination. Various treatments imposed after priming but before dehydration have been reported to improve seed longevity in storage (Bruggink *et al.,* 1999). Longevity can be restored by the combination of reducing seed moisture content (MC) slightly and then incubating at elevated temperature immediately following priming, or used of heat shock treatments can substantially restore potential longevity of the primed seeds (Bruggink *et al.,* 1999; Gurusinghe and Bradford, 2001; Schipper *et al.,* 2001). Post priming treatments including a reduction in seed water content followed by incubation at 37 or 40°C for 2 to 4 h can substantially restore potential longevity in tomato (*Lycopersicon esculentum* Mill.) seeds (Gurusinghe *et al.,* 2002). The seeds that deteriorate rapidly under accelerated aging conditions generally show a marked depression in their vigor (McDonald, 1999, Siadat *et al.,* 2012).

Today, application of accelerated aging treatment is used to assess seed vigor and quality (TeKrony *et al.,* 1989; Moradi and Younesi, 2009). Many hypotheses have been proposed regarding causes of seed ageing such as lipid peroxidation mediated by free radicals, inactivation of enzymes or decrease in proteins, disintegration of cell membranes and genetic damage (Murthy *et al.,* 2003; Smith and Berjak, 1995; Walters, 1998). There are some reports that showed degradation and inactivation of enzymes due to changes in their macromolecular structures is one of the most important hypotheses proposed regarding causes of ageing in seeds (Bailly, 2004; Goel *et al.,* 2002; McDonald, 2004). Most of these studies suggest that decreases occur in the activity of enzymes such as superoxide dismutase, catalase, peroxidase and glutathione reductase in aged seeds. The general decrease in enzyme activity in the seed lowers the respiratory capacity, which in turn lowers both the energy (ATP) and assimilates supply of the germinating seed. Therefore, several changes in the enzyme macromolecular structure may contribute to their lowered germination efficiency. The objective of the present work was to determine the effect(s) of post priming treatment on germination characteristics and enzyme activity under different accelerated aging in primed seeds with gibberellin.

MATERIALS AND METHODS

Seeds of *Secale montanum* were primed with an aqueous solution of GA. Treatment of GA was attained at concentration of 25 ppm at 10°C for 12 h.

After this period seeds were rinsed with distilled water and some part of seeds were dried under their priming temperatures. The remained of primed seed treated by heat shock treatments. Before heat shocking, the seeds were allowed to 10 % moisture content reduction (e.g., 1 g of primed seeds was dried until total weight reached 0.9 g). After wards they were put in laminated aluminum foil and exposed to three shock temperature include 30, 35 and 40°C for duration of 3 and 4 hrs. After those periods the seed were entreated from foils and stored at 29°C until moister content reduction to below 10 % (Gurusinghe *et al.,* 2002).

The heat shocked and control seeds then imposed to different accelerated ageing periods include 24, 48 and 72 hrs at 41°C in sealed ageing boxes which had 100% relative humidity. After that, a germination test was conducted.

Standard germination test was carried out by place 50 seeds (primed without post primed treatments, primed plus post primed and unprimed were placed under accelerated aging regimes) in 9 cm petri dishes. Seeds were observed daily until day 7^{th} and germinated seeds were recorded. Investigated parameters were the GP, GI, NSP, SVI, MTG and SL.

Two treatments of heat shock (3 h and 4 h at 35°C) were elite to determine antioxidant enzyme activity. All extraction procedures were carried out at 4°C. About 0.2 g of seed samples were homogenized with 10 ml of phosphate buffer (PH 7), followed by centrifugation of 20000 g for 15 min. The supernatants were used for determination of enzyme activity. Catalase (CAT, EC 1.11.1.6) activity was determined spectrophotometrically following H_2O_2 consumption at 240 nm (Bailly *et al.,* 1996). Ascorbate peroxidase (APX, EC 1.11.1.7) activity was determined according to the procedures of Al *et al.* (1995). The activities of APX and CAT were expressed per mg protein, and one unit represented 1 μmol of substrate undergoing reaction per mg protein per min. The study was conducted in the seed laboratory of Natural Resources Faculty, University of Tehran, Karaj, Iran. In order to evaluated the effect of post priming on germination characteristics seeds under accelerated aging condition four factorial experiments were conducted in a completely randomized design with three replications. Before the statistical analysis in order to unify the variance, data of percentage was subjected to data transformation (arcsine) (Ansari and Sharif-Zadeh, 2012). Data of experiment were subjected to factorial analysis. All data were analyzed statistically by analysis of variance using MSTAT-C and Microsoft Excel software. Mean comparisons were performed using an ANOVA protected least significant difference (Duncan) ($P < 0.01$) test.

RESULTS

The results indicated that duration of aging × time of shock × temperature of shock interaction was significant for all traits in seeds which treated with gibberellin (*Table 1*).

Table 1 - Analysis of variance for heat shock effect on *Secale montanum* seeds germination indexes of primed with ascorbic acid under accelerated aging treatments

SOV	df	GP	GI	NSP	SVI	MTG	SL
Duration of aging (D)	3	7315.4**	3920.67**	6318.82**	878676.8**	16.8**	29.39**
Time of shock (TS)	1	5.24^{ns}	1.16^{ns}	0.00013^{ns}	56937.77**	0.000073^{ns}	39.77**
Temperature (T)	2	66.71**	1.38^{ns}	8.38**	2418.51*	0.21**	0.34^{ns}
D x TS	3	11.78*	0.21^{ns}	49.49**	1066.5^{ns}	0.068^{ns}	1.75**
D x T	6	40.43**	4.35**	1.02^{ns}	1532.61*	0.27**	0.74**
TS x T	2	164.9**	10.9**	24.82**	12838.7**	0.18*	4.39**
D x TS x T	6	30.19**	2.48*	6.4**	1684.6**	0.09*	1.39**
Error	48	4.16	0.53	1.56	507.051	0.04	0.14
CV (%)	-	3.53	3.71	2.79	5.08	5.66	3.97

*, ** and ns, indicate significant difference at 5%, 1% probability level, and no significantly, respectively.

Priming and heat shock treatments increased GI and reduced MTG in non-aged seeds (*Figs. 3 and 4*), but aging has caused to decrease germination characteristics in seeds, especially in primed seed. Heat shock treatments improved GP, GI, NSP, SVI, MTG and SL as compared to the primed seed after 3 days of aging (*Figs. 1-6*).

The highest GP (97.33 %) and NSP (88.67%) were attained from heat shock treatment at 35°C for 3 h in non-aged seeds (*Fig. 1*). After 3 days of aging, the highest GP (42.67 %) and NSP (18.67%) were attained from heat shock treatment at 35°C for 3 h (*Figs. 1 and 2*). Our results show that priming and heat shock treatments increase GI as compared to the unprimed seed in non-aged seeds (*Fig. 3*). After 3 days of aging the highest GI was attained from unprimed seeds, but no significant difference with heat shock treatment at 35°C for 3 h (*Fig. 3*). The minimum MTG was in heat shock treatment at 30°C for 3h in non-aged seeds (*Fig. 4*). After 3 days of aging heat shock treatment reduce MTG as compared to the primed seeds (*Fig. 4*). Heat shock treatment at 35°C for 3 h increased SVI as compared to the unprimed and primed seed in non-aged seeds and after 3 days aging (*Fig. 5*). SL increases with heat shock treatment at 30°C for 4 h in non-aged seeds as compared to the primed and unprimed seeds, but other 3 days of aging heat shock treatment except at 35°C for 3 h and 40°C for 4 h reduced SL as compared to the primed and unprimed seeds (*Fig. 6*).

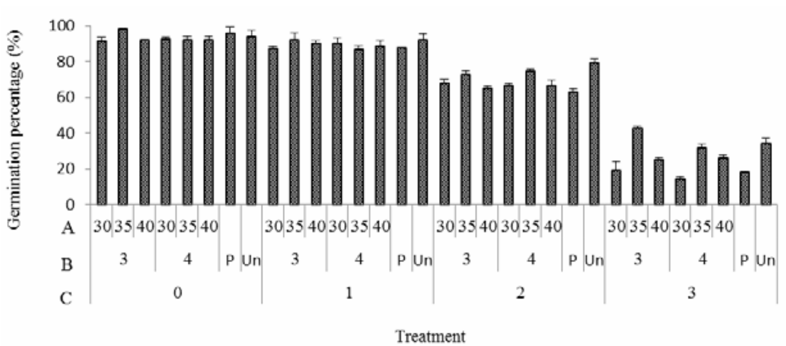

Figure 1 - Interaction effects (Duration of aging × Time of shock × Temperature of shock) on germination percentage of *Secale montanum* seeds primed with gibberellin. Vertical bars showed SD; (A) Temperature of shock; (B) Time of shock; (C) Duration of aging; (P) Priming; (Un) Unprimed.

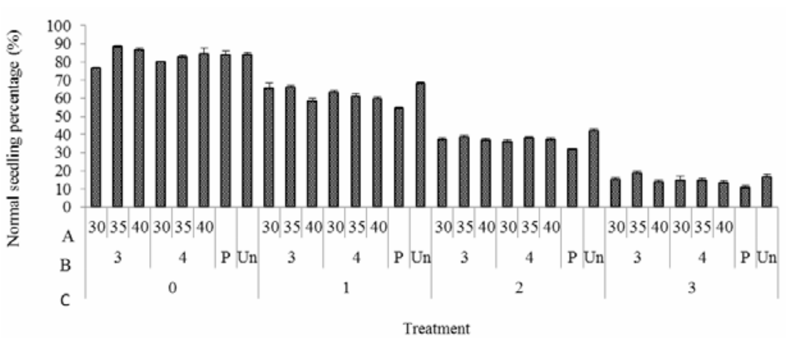

Figure 2 - Interaction effects (Duration of aging × Time of shock × Temperature of shock) on normal seedling percentage of *Secale montanum* seeds primed with gibberellin. Vertical bars showed SD. (A) Temperature of shock; (B) Time of shock; (C) Duration of aging; (P) Priming; (Un) Unprimed.

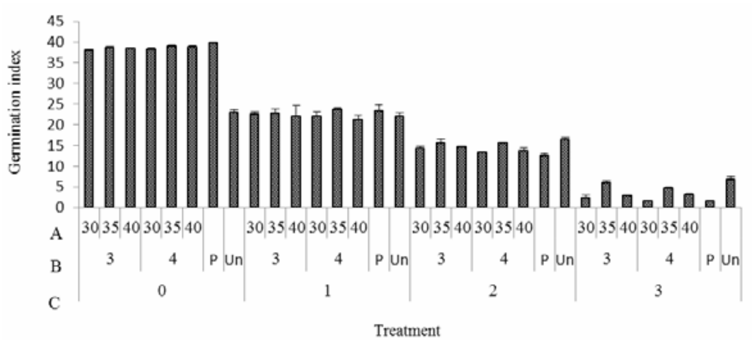

Figure 3 - Interaction effects (Duration of aging × Time of shock × Temperature of shock) on germination index of *Secale montanum* seeds primed with gibberellin. Vertical bars showed SD. (A) Temperature of shock; (B) Time of shock; (C) Duration of aging; (P) Priming; (Un) Unprimed.

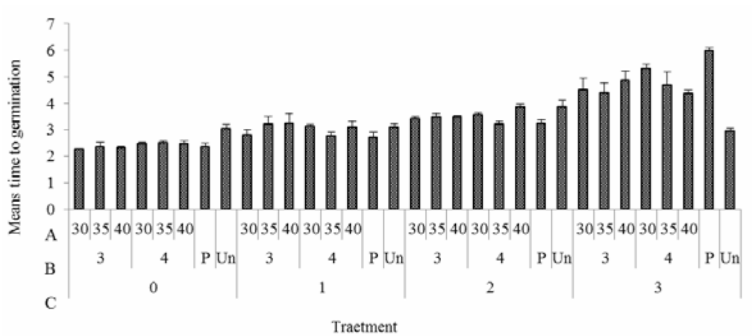

Figure 4 - Interaction effects (Duration of aging × Time of shock × Temperature of shock) on means time germinatio of *Secale montanum* seeds primed with gibberellin. Vertical bars showed SD. (A) Temperature of shock; (B) Time of shock; (C) Duration of aging; (P) Priming; (Un) Unprimed.

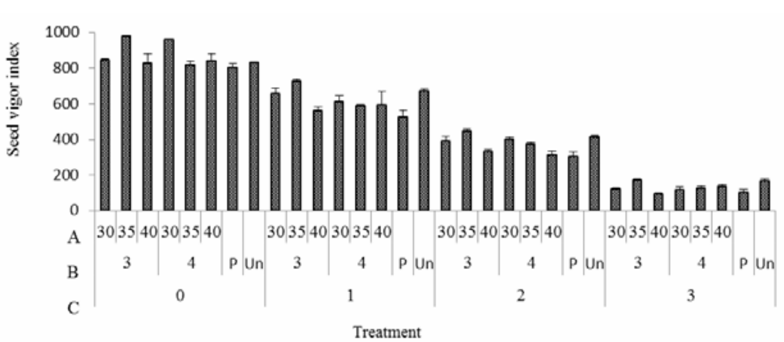

Figure 5 - Interaction effects (Duration of aging × Time of shock × Temperature of shock) on seed vigor index of *Secale montanum* seeds primed with gibberellin. Vertical bars showed SD. (A) Temperature of shock; (B) Time of shock; (C) Duration of aging; (P) Priming; (Un) Unprimed.

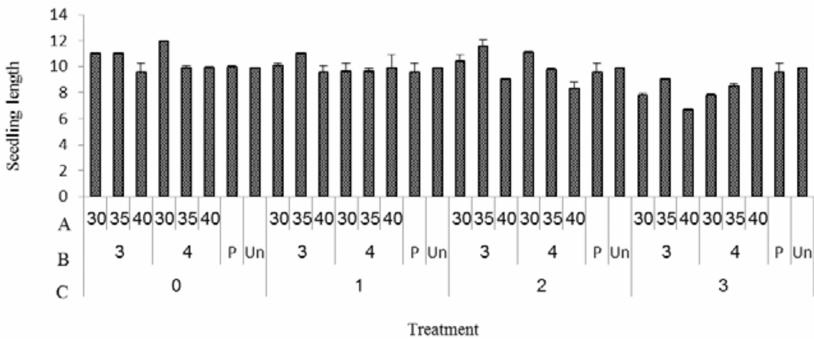

Figure 6 - Interaction effects (Duration of aging × Time of shock × Temperature of shock) on seedling length of *Secale montanum* seeds primed with gibberellin. Vertical bars showed SD. (A) Temperature of shock; (B) Time of shock; (C) Duration of aging; (P) Priming; (Un) Unprimed.

Seed priming and priming plus heat shock treatments increased CAT and APX activity in non-aged seeds as compared to the unprimed (*Figs. 7 and 8*). CAT and APX activity were decreased by increasing in duration of aging. Our results showed that, the highest enzyme activity for priming with GA was attained from seed priming plus heat shock treatment (3 at 35°C) after 1 day of aging (*Figs. 7 and 8*). Heat shock treatments could increase enzyme activity of primed seed after 3 days aging (*Figs. 7 and 8*). After 3 days of aging, our results showed that, the highest enzyme activity was attained from seed priming plus heat shock treatment (3 h at 35°C) (*Figs. 7 and 8*).

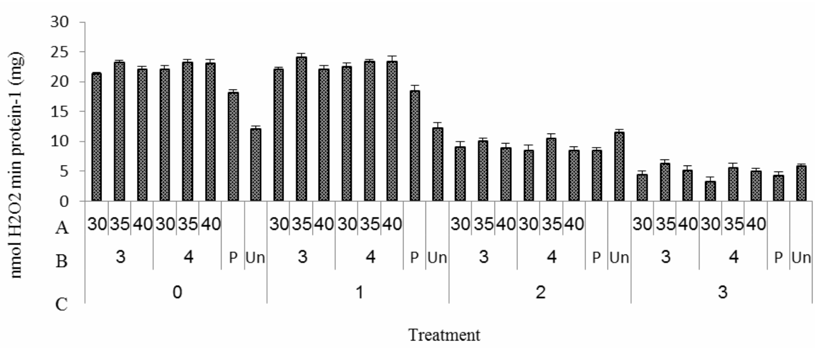

Figure 7 - Interaction effects (Duration of aging × Time of shock × Temperature of shock) on catalase activity of *Secale montanum* seeds primed with gibberellin. Vertical bars showed SD. (A) Temperature of shock; (B) Time of shock; (C) Duration of aging; (P) Priming; (Un) Unprimed.

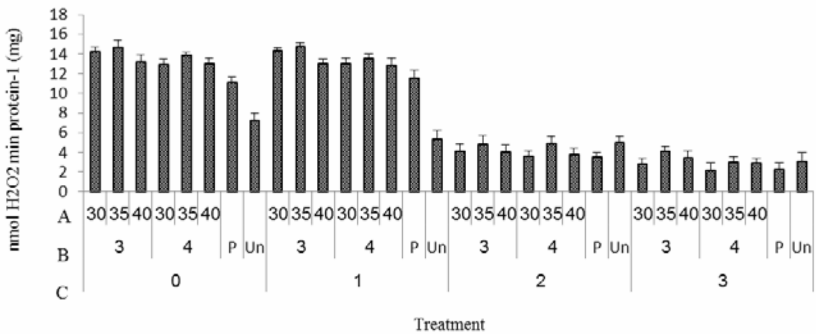

Figure 8 - Interaction effects (Duration of aging × Time of shock × Temperature of shock) on ascorbate peroxidase activity of *Secale montanum* seeds primed with gibberellin. Vertical bars showed SD. (A) Temperature of shock; (B) Time of shock; (C) Duration of aging; (P) Priming; (Un) Unprimed.

DISCUSSION

The results of our study suggested that accelerated aging caused a decreased in germination characteristics (*Figs. 1-6*). It is in agreement with the results of many crops (Siadat *et al.,* 2012; Moradi and Younesi, 2009; Murthy *et al.*, 2003). Seed priming is a commercially successful practice for improving seed characteristics performance (Ansari *et al.,* 2012), but reductions in seed aging life after priming have limited its application in some cases. Post priming treatments such as heat shock treatment have been identified that can extend longevity of primed seeds (Bruggink *et al.,* 1999; Gurusinghe and Bradford, 2001; Schipper *et al.,* 2001), and the experiments reported here confirm that mountain rye seed quality can be restored by the combination of reducing seed MC slightly and then incubating at elevated temperature immediately following priming (*Figs. 1-6*). Heat shock treatments from 3 at 35°C improved germination characteristics (*Figs. 1-6*). Bruggink *et al.* (1999) reported that the desired longevity could be obtained by keeping the seeds, after a priming treatment, under a mild water and temperature stress for period of several hours to days (3 h at 40°C). Heat shock treatments from 2 to 4 h at 37°C improved longevity in tomato seed (Gurusinghe *et al.,* 2002). It is likely that at elevated temperatures a balance is achieved between the intensity and length of treatment sufficient to induce a beneficial effect on longevity of primed seeds versus damage induced during extended exposure to high temperature (Gurusinghe *et al.,* 2002). The mechanisms underlying both the reduction in storage life due to priming and its restoration by post priming treatments remain unknown. Seed priming and priming plus heat shock treatments increased CAT and APX activity in non-aged seeds (*Figs. 7 and 8*), but enzyme activity decreased in primed seeds after aging. Kibinza *et al.* (2011) reported that the

CAT is a key enzyme in seed recovery from ageing during priming. Heat shock treatments can increase enzyme activity of primed seed, also heat shock treatment increased germination characteristics after aging, therefore it can be said that increase of this trait could be a result of increasing the antioxidant profile (CAT and APX) of treated seeds. These results support the hypothesis of Bailly *et al.* (1996) that a decrease in antioxidant enzymes is linked to an increased lipid peroxidation and accelerated ageing. Subsequently, Bailly *et al.* (2000, 2002) proposed a positive relationship between antioxidant enzyme capacity and the vigor of the seed. Gurusinghe *et al.* (2002) reported that the increased BiP expression may contribute to the improved longevity of primed seeds following post priming treatments.

CONCLUSIONS

In general, our results clearly indicate that decline in germination characteristics in response to aging is a consequence of decline in enzyme activity in *Secale montanum* under accelerated aging. Seed priming enhances seed germination performance; but the quality of primed seeds in aging condition often reduces more than non-primed seeds. Our results showed that heat shock treatments improve activity of CAT and APX and the highest germination characteristics in *Secale montanum* were attained from heat shock treated seeds.

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