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IMPROVEMENT OF GERMINATION CHARACTERISTICS AND ENHANCEMENT OF ANTIOXIDANT ENZYMES ACTIVITY OF SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) AGED SEEDS BY USED OF GIBBERELLIN

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ABSTRACT. Seed priming is one of the methods that can be taken to counteract the adverse effects of abiotic stress, also Seed priming treatments have been used to reduce the damage of aging and invigorate their performance in many crops. Objective of this study was to evaluation the effect of gibberellin on germination characteristics and antioxidant enzymes of safflower seeds after aging. Experimental design was a factorial one with complete randomized design with three replications. The first factor was priming by gibberellin (0 and 50 ppm) and non-primed seeds (control) and the secondary factor was combinations of four levels of aging (0, 1, 3 and 5 days of aging, at 43°C). The results showed that the priming and aging effects on germination percentage, germination index, normal seedling percentage, seedling length, vigor index, catalase and ascorbate peroxidase were significant, but on mean time to germination not significant. Results showed that, the highest germination characteristics such as; germination percentage, germination index, normal seedling percentage, seedling length, vigor index, catalase and ascorbate peroxidase were attained from priming by gibberellins, under non aged conditions. Also, our results indicated that seed aging is related to decrease of enzymes activity and may contribute to low seed germination efficiency, also priming increases enzyme activity and increases enzyme activity with priming treatment may contribute to improve germination characteristics. The general decreases 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.

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Key words: Germination; Priming; Aging; Enzyme activity.

INTRODUCTION

Although global food reserves are discussed based on wheat, rice and corn as main food resources, after grain seeds, oil seeds play key role in the human diet (Mozafari and Arab, 1995). *Carthamus tinctorius* species is a native of Asia, especially in Manchuria (China) and Japan and was domesticated about 4700 years ago, in China (Naseri, 1996). Safflower is an important oil plant that its production has been at least twice of peanut oil. Safflower seeds contained about 40% protein, 21% oil and 34% carbohydrate and 5% ash (Perry, 1980).

Seed aging can be defined as the loss of seed quality and seed vigor indices. Kapoor *et al.* (2010) reported that the rate of aging rapidly increases in either seed moisture content or temperature of storage. Decrease in seed vigor indices is due to decrease in seed quality, seed germination percentage, germination rate, crop yield and also can increase susceptibility to abiotic stress (Tekrony *et al*., 1989).

In general seed aging is indicated by some parameters like delay in germination and emergence, slow growth, reduces in normal seedling percentage and changes in antioxidant enzymes (Walters, 1998; Ansari *et al.,* 2013). Ghasemi-Golezani *et al.* (2010) showed that using vigorous seeds at planting may increase crop yield in two ways: firstly, by higher normal seedling percentage than aged seeds that gives optimum density even under abiotic stress conditions and secondly, by high growth and emergence rate in comparison to seedlings produced from aged seeds; also, it was reported that oxidative damages are responsible for the deterioration changes observed in aged seeds and free radical oxidations, enzymic dehydrogenation and aldehyde oxidation of proteins might reasonably contribute to the progress of seed quality.

Seed priming treatment has been shown to improve the germination and emergence of many species (Ansari *et al.,* 2012; Ansari *et al.,* 2013; Ansari and Sharif Zadeh, 2012; Sedghi *et al.,* 2010; Seiadat *et al.,* 2012). Seed priming treatments and post priming treatments have been used to reduce the damage of aging and invigorate their performance in many crops (Basra *et al.,* 2003; Farooq *et al.,* 2006; Ansari *et al.,* 2013), also seed exposed to environmental stresses.

It was reported that decreases occur in the antioxidant enzymes activity such as superoxide dismutase, catalase, peroxidase and glutathione reductase in aged seeds, 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 (McDonough, 2004). Therefore, several changes in the enzyme macromolecular structure may contribute to their lowered germination efficiency. Also, most of researches reported that increase in

various free radical scavenging enzymes, such as superoxide dismutase, catalase and peroxidase have also been demonstrated (Ansari *et al.,* 2013; Seiadat *et al.,* 2012).

Therefore, in the present study, our goals were to study the improvement of germination characteristics and enhancement of antioxidant enzymes activity of safflower aged seeds by use of gibberellin.

MATERIALS AND METHODS

For accelerated aging treatments, seeds were then imposed to different accelerated ageing periods of 0, 1, 3 and 5 days at 43°C in sealed ageing boxes which had 95% relative humidity. After that, a germination test was conducted.

After aging, seeds were pretreated with water and 75 ppm at 20±1°C for 24 h. Seeds were exposure in 20 cm glass petri dishes containing 15 ml water and gibberellin 50 ppm. The imbibed seeds were then washed 4 times with tap water and dried on filter paper at 20±1°C for 24 h (Ansari *et al.,* 2012).

Standard germination test was carried out at 25°C for 14 days, in three replications of 50 seeds. The germinated seeds (2 mm radicle elongation) were counted daily to calculate germination rate. At the end of the germination period, germination percentages, germination index, normal seedling percentage, seedling length, vigor index, mean time to germination, catalase and ascorbate peroxidase activity were determined.

All extraction procedures were carried out at 4°C. The seed samples, weighting about 0.3 g, were homogenized with 3 ml of tris (pH 7.8), followed by centrifugation of 20,000 g for 20 min. The supernatants were used for determination of enzyme activity. 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 (Ansari and Sharif Zadeh, 2012; Chiu *et al.*, 1995). Ascorbate peroxidase (APX, EC 1.11.1.7) activity was determined according to the procedures of Ansari and Sharif Zadeh (2012). 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.

All data were analyzed statistically by analysis of variance using MSTAT-C software. Mean comparisons were performed using an ANOVA protected least significant difference (Duncan) ($p < 0.05$) test.

RESULTS AND DISCUSSION

According to our results of variance analysis, seed priming and priming × aging on all traits expect mean time to germination and aging on all traits were significant (*Tab. 1*). In agreement with the results, earlier reports (McDonough, 2004; Seiadat *et al.,* 2012; Ansari and Sharif Zadeh, 2013) have shown effects of priming and aging on germination characteristics.

Figures 1 to 6 shows the effect of seed aging and seed priming by gibberellin and water on these traits. Results showed that the highest germination percentage (*Fig. 1*), normal seedling percentage (*Fig. 2*), germination rate (*Fig. 3*), seedling length (*Fig. 5*), seedling vigor index (*Fig. 6*) were attained from priming

with gibberellin 50 ppm in control conditions (0 day aging). These traits decreased significantly as seed aging progressed. Results indicates that mean time to germination (*Fig. 4*), increases significantly as seed aging progressed and the highest this traits was attained after 5 days aging.

Table 1 - Variance analysis of studied traits in safflower under aging and priming conditions

S.O.V.	df	Germination percentage	Normal seeding percentage	Germination rate	Mean time to germination	Seeding length	Seed vigor	Catalase activity	Ascorbate peroxidase activity
Priming	2	51.44**	355.36**	20.04**	0.01^{ns}	6.28**	22624.57**	412.22**	115.08**
Aging	3	6458**	9050.32**	992.01**	9,12**	196.17**	562471061.37**	1886.35**	1087.63**
Priming x Aging	6	14.41*	7.65**	2.37**	1.99^{ns}	0.4*	2496.38**	2654.08**	4.01**
Error	24	4.58	2.31	0.48	0.07	0.14	668.47	0.87	4.16
C.V.%	-	3.51	3.23	4.11	10.35	3.97	4.65	2.61	3.71

**, * and ^{ns} indicates significant difference at 1% and 5% probability level and non-significant, respectively.

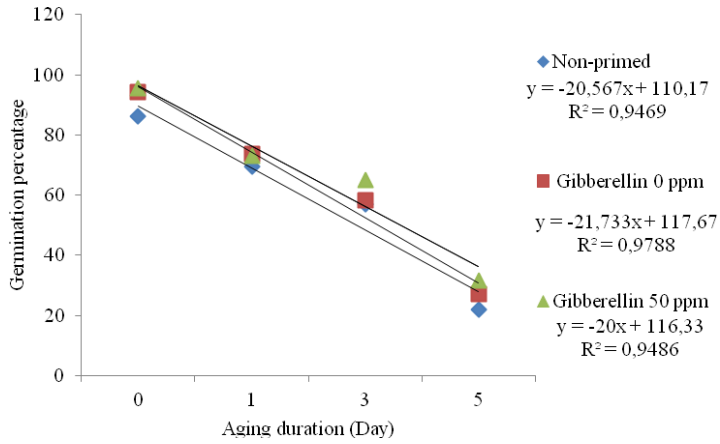

Figure 1 - The effect of aging and priming on germination percentage of safflower seeds

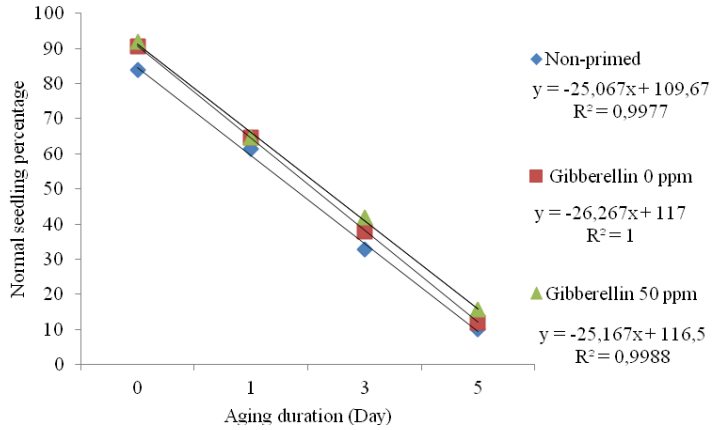

Figure 2 - The effect of aging and priming on normal seedling percentage of safflower seeds

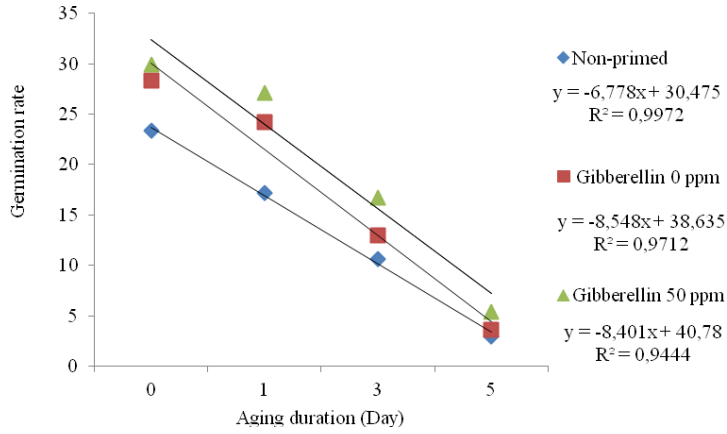

Figure 3 - The effect of aging and priming on germination rate of safflower seeds

Increasing seed age decreased germination characteristics and priming after aging increased germination characteristics therefore our results are in agreement with Ansari and Sharif Zadeh (2013) in *Secale montanum* and Seiadat *et al.* (2012) in corn. Also many studies have shown that negative effects of stress conditions such as aging, drought and salinity stress in relation to seed performance and germination characteristics (Mohammadi *et al.*, 2011; Ansari and Sharif Zadeh, 2013)

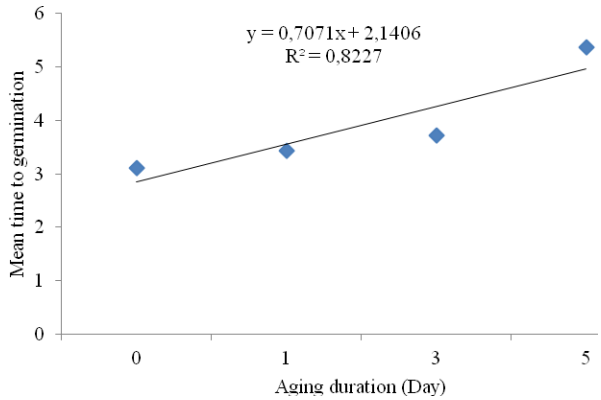

Figure 4 - The effect of aging on mean time to germination of safflower seeds

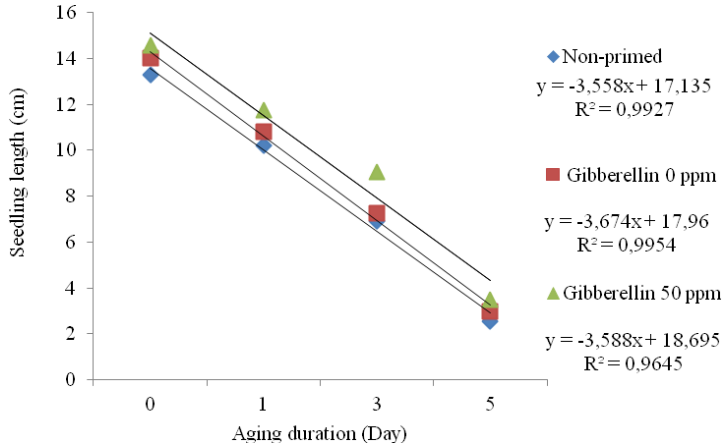

Figure 5 - The effect of aging and priming on seedling length of safflower seeds

Our results showed that catalase and ascorbate peroxidase activity was reduced by increment of period of aging (*Figs. 7 and 8*). Therefore, priming significantly improved studied enzymes activity. These results are parallel to those of the Seiadat *et al.* (2012), Ghassemi-Golezani *et al.* (2010), Ansari and Sharif Zadeh (2013) and Sedghi *et al.* (2010). Bailly *et al.* (1996) reported 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 vigour of the seed.

Akhter *et al.* (1992) showed that decreasing in germination characteristics were related to chromosomal aberrations that occur under long storage conditions. Decreasing of germination percentage

in aged seeds can be due to reduction of α-amylase activity and carbohydrate contents (Bailly, 2002), or denaturation of proteins (Nautiyal *et al.,* 1985). According to Abdalla and Roberts (1968), the amount of genetic damage was solely a function of loss of viability in barley and pea seeds treated with different combinations of accelerated ageing treatment. Priming increases seed reserves utilization under unfavorable conditions there for priming by increased these traits can be improved germination characteristics under aging and correlation with antioxidant enzymes activity.

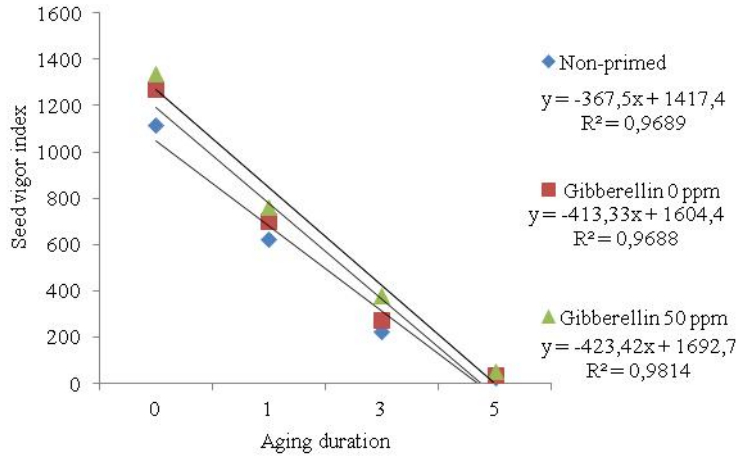

Figure 6 - The effect of aging and priming on seed vigor index of safflower seeds

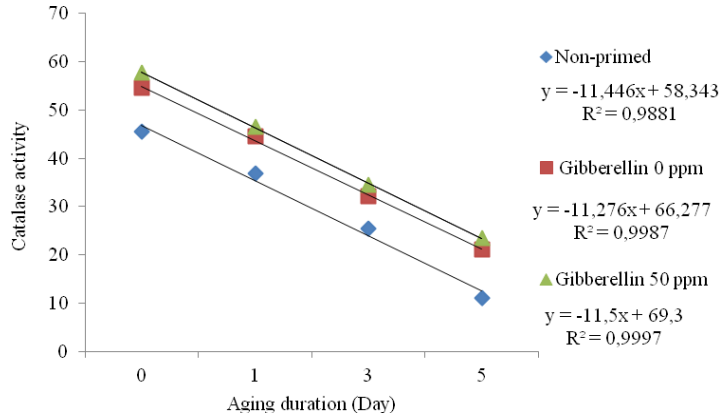

Figure 7 - The effect of aging and priming on catalase activity of safflower seeds

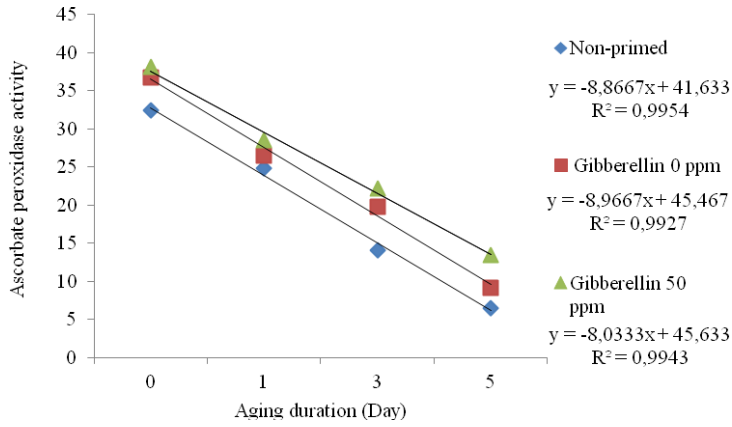

Figure 8 - The effect of aging and priming on ascorbate peroxidase activity of safflower seeds

CONCLUSION

Results indicates that all germination characteristics decreased significantly as seed aging progressed. In other hand, mean time to germination increaseds significantly as seed aging progress. Moreover, our results provide convincing evidence that priming can improve reduced activity of catalase and ascorbate peroxidase in aged seeds.

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