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ORIGINAL ARTICLE

Enzyme activity and seedling growth of soybean seeds under accelerated aging

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Seed aging is the main problem of seed storage. Changes of bio-chemical and reduction of seedling growth are consequence of seed deterioration. An experiment was conducted to evaluate the effects of accelerated aging on soybean seed germination indexes and enzyme activity. Seeds were incubated in closed plastic boxes for the accelerated aging treatments. Three accelerate aging regimes were performed by placing seeds at 41°C and relative humidity (RH) of 90-100 % for 0, 2, 4, 6 and 8 days periods. Our results showed that increasing aging duration resulted higher reduction in germination characteristics, catalase and ascorbate peroxidase. Germination percentage, means time to germination, germination index, normal seedling percentage and enzyme activity decrease significantly.

Key words: Germination characteristics, Enzyme activity, Soybean seed, Aging

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Oil seeds are very sensitive to the harsh environmental conditions. It is hypothesized that their oil content readily oxidize, which deteriorate the seed health in storage (Kausar *et al*., 2009). Seed storage conditions can determine germination characteristics and vigor potential of seeds (Mc Donald, 1999). Various factors such as weather conditions during seed producing stage, pests and diseases, seed oil and moisture content, mechanical damages, storage time and relative humidity of store can affect vigor of seeds (Krishnan *et al*., 2003; Marshal and Levis, 2004). Seed aging is a function not only of time but also of temperature and moisture (Ellis and Roberts, 1981). Consequently the seed storage environment greatly influences the period of seed survival (Ellis *et al*., 1982). Seed deterioration can be reducing of quality, viability and vigor either due to aging or effect of adverse environmental factors (Bailly, 2004; Goel *et al*., 2002; McDonald, 2004; Seiadat *et al*., 2012; Kapoor *et al*., 2010). Also, decrease in seed vigor is due to decrease in germination indexes, yield and also can increase susceptibility to environmental stress (Tekrony *et al*., 1989).

Ghassemi-Golezani *et al*. (2010) reported that oxidative damages are responsible for the deterioration changes observed in aged seeds. Free radical oxidations, enzymic dehydrogenation and aldehyde oxidation of proteins might reasonably contribute to the progress of seed quality. Acceleration of aging of seed is a treatment uses to assess storage quality, germination characteristics by simulation natural aging conditions for different crops like (Galeshchi *et al*., 2002) and maize (Nik and Tilebeni, 2011). Also, Bailly *et al*. (1996) Accelerated aging of sunflower seeds, which consists of placing seeds at high temperature and relative humidity, is associated with a progressive decrease in seed germinability. Therefore, the study aimed was to determine the effect of aging on germination characteristics and enzyme activity of soybean seeds.

MATERIALS AND METHODS

For accelerated aging treatments seeds were then imposed to different accelerated aging periods of 0, 2, 4, 6 and 8 days at 41°C in sealed aging boxes which had 100% relative humidity. After that, a germination test was conducted.

Standard germination test was carried out at 25°C for 8 days (Anonymous, 2010) in three replications of 50 seeds in a completely randomized design arrangement. The germinated seeds (2 mm radicle elongation) were counted daily to calculate germination rate. At the end of the germination period, germination percentage, normal seedling percentage, germination index and mean time to germination were recorded.

All extraction procedures were carried out at 4 °C. The seed samples, weighting about 0.3 gr, were homogenized with 3 ml of tris (PH 7.8), followed by centrifugation of 20000 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 spectrophotometric ally following H_2O_2 consumption at 240 nm (Chiu et al., 1995). Ascorbate peroxidase (APX, EC 1.11.1.7) activity was determined according to the procedures of Johnson and Cunningham (1972). 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 SAS Software. Data for germination and normal germination percentages were subjected to arcsine transformation before analysis of variance was carried out with SAS software. Mean comparisons were performed using an ANOVA protected least significant difference (Duncan) ($P < 0.01$) test.

RESULTS AND DISCUSSION

According to our results of variance analysis, effect of priming treatments on germination percentage, germination index, normal seedling percentage and mean time to germination, under aging conditions were significant ($P < 0.01$) (Tab. 1). In agreement with the results, earlier reports (Bailly, 2004; Goel *et al*., 2002; McDonald, 2004; Seiadat *et al*., 2012) have shown negative affect aging on germination characteristics.

Our results showed that the highest germination percentage (Fig. 1), germination index (Fig. 2), normal seedling percentage (Fig. 4) were obtained under 0 and 2 day of aging, and the minimum, mean time to germination (Fig. 3) was achieved under 3 days of aging, but with increases of duration of aging this traits reduction. Therefor the minimum this traits were attained under 8 days of

aging. Increasing seed age decreased germination and this result is in accordance with Jan-Mohammadi *et al*. (2008) and Ghassemi-Golezani *et al*. (2010) in rapeseed, Bhattacharjee *et al*. (2006) in common bean and sunflower and Saha and Sultana, (2008) in soybean. Also, earlier reports (Bailly, 2004; Goel *et al*., 2002; McDonald, 2004; Moradi and younesi, 2009; Seiadat *et al*., 2012) have shown negative effect of aging in relation to seed performance, germination percentage and seedling indices. Akhtar *et al*. (1992) suggested that decreasing in germination percentage was 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, 2004) or denaturation of proteins (Nautiyal *et al*., 1985). According to Abdalla and Roberts, (1968) barley and pea seeds treated with different combinations of accelerated aging treatment showed that the amount of genetic damage was solely a function of loss of viability.

Results showed that after 8 days of deterioration, germination percentage had the lowest value. Fig. 5 represents the effect of seed aging on germination percentage that significantly affected by seed deterioration in a quadratic regression model. It is showing that by increasing aging time, germination percentage decreases significantly.

Also, our results showed that enzyme activity decreased in seeds after aging (Fig. 6). Most of these studies suggest that decreases occur in the activity of enzymes in aged seeds (Bailly, 2004; Goel *et al*., 2002; McDonald, 2004). Also, Kibinza *et al*. (2011) reported that the CAT is a key enzyme in seed recovery from aging during priming.

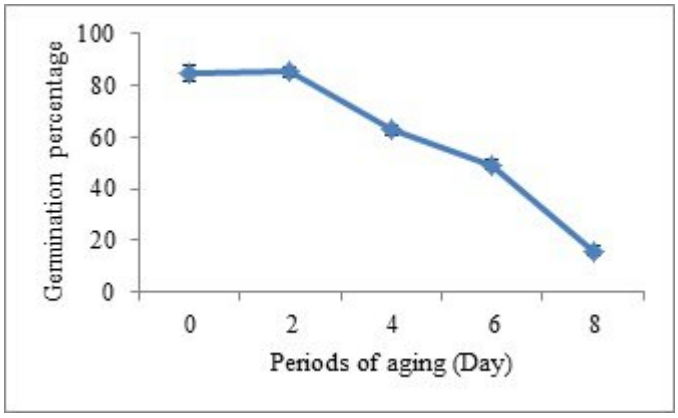

Figure 1. The effect of accelerated aging on germination percentage of soybean seeds.

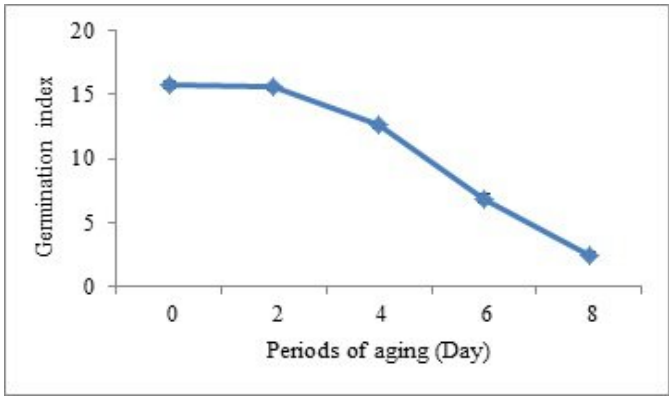

Figure 2. The effect of accelerated aging on germination index of soybean seeds.

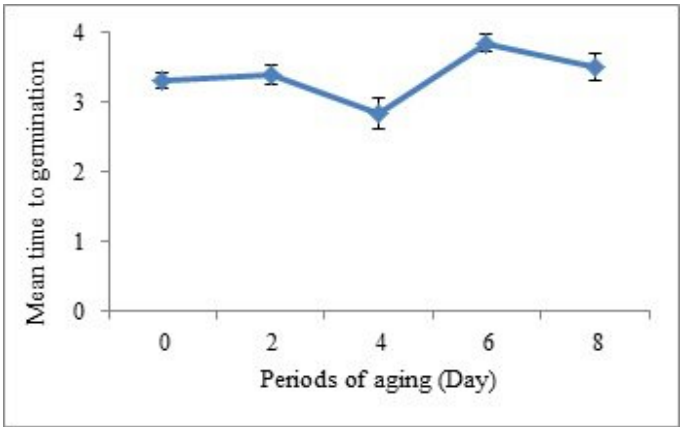

Figure 3. The effect of accelerated aging on means time to germination of soybean seeds.

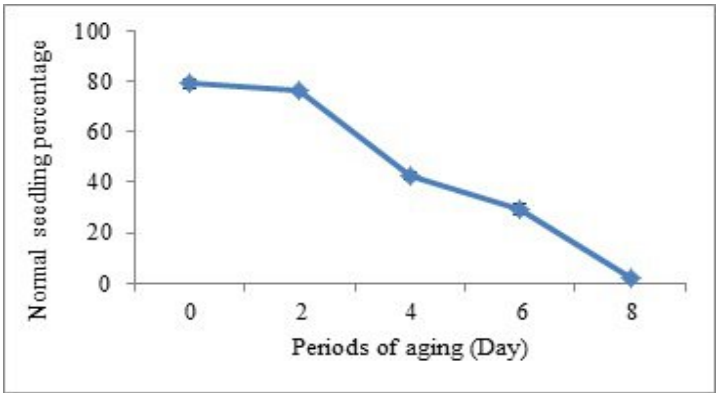

Figure 4. The effect of accelerated aging on normal seedling percentage of soybean seeds.

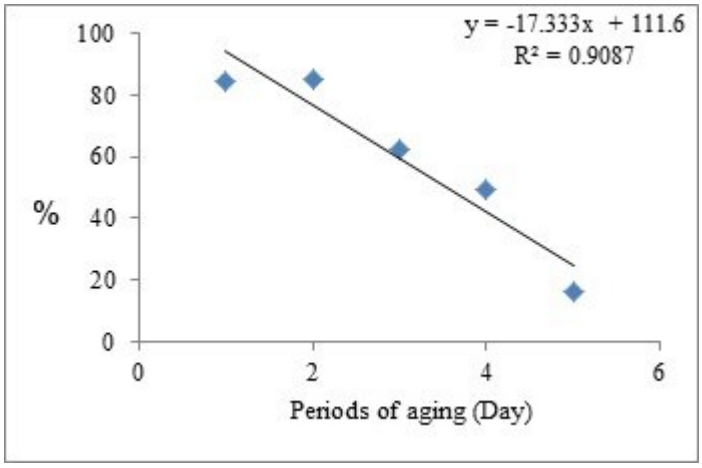

Figure 5. Decrease in germination percent of soybean seeds with increasing deterioration time

Table 1. Analysis of variance of studied traits soybean seeds under accelerated aging

S.O.V	df	Germination percentage	Germination index	Mean time to germination	Normal seedling percentage
Treatment	4	2479.73**	102.3**	0.38**	3178.56**
Error	10	7.46	0.1	0.04	3.06
CV%	-	4.58	3.11	5.87	3.81

** indicate significant difference at 1% probability level

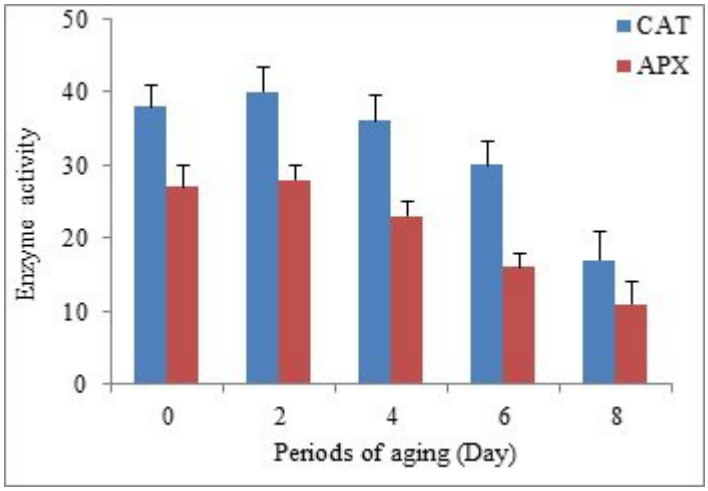

Figure 6. The effect of accelerated aging on enzyme activity of soybean seeds.

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 soybean seeds. The highest germination characteristics and enzyme activity were attained under 0 and 2 day of aging.

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