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Cotton Seed Deterioration: Assessment of some Physiological and Biochemical Aspects

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ABSTRACT

The effect of accelerated aging (AA) was studied in cotton seeds. The cotton seeds were exposed to high temperature (45-48ºC) and humidity (80-90% relative humidity) for 2, 3, 10 and 20 days. Treated and untreated seeds were compared for various vigor tests. The seeds were analyzed for fat acidity and peroxidase level. The germination percentage decreased with AA. There was an increase in root and shoot lengths upto three days of aging and then with further increase in AA seeds were unable to germinate. The electrical conductivity of seed leachates, free fatty acid and peroxidase contents increased linearly with AA period.

Key Words: Cottonseed; Accelerated aging; Germination; Free fatty acid; Peroxidase

INTRODUCTION

Seeds deteriorate during storage. This aging is manifested as a reduction in percentage germination, while those seeds that do germinate, produce week seedlings. During aging, seeds loose their vigor, germinibility and ultimately viability (Trawatha *et al.,* 1995). During mid 1980's, seed sales averaged over $ 2 billion annually in USA alone and it has been estimated that 25% of this value was lost due to poor seed quality (Shatters *et al.,* 1994). Losses in seed quality occur during field weathering, harvesting and storage. The losses are exacerbated if seeds are stored at high temperature and/or high relative humidity conditions.

Cottonseed is one of the most sensitive agronomic seeds where significant deterioration occurs after just one year of storage. Cottonseed like other oil seeds is more prone to deterioration due to high oil contents.

Membrane disruption is one of the main reasons of seed deterioration. As a result, seed cells are not able to retain their normal physical condition and functioning. The major causes of membrane disruption are increase in free fatty acid level and free radicals productivity by lipid peroxidation (Grilli *et al.,* 1995). Protective mechanisms that could scavenge the peroxidatively produced free radicals within the seed to keep these deleterious compounds to a minimum have been reported in soybean and sunflower (Sung, 1996). This protective mechanism involves several free radical- and peroxide-scavenging enzymes such as catalase, peroxidase and superoxide dismutase and ascorbic acid.

The rate at which seeds lose vigor during storage is affected by environmental factors such as temperature, moisture and O_2/CO_2 concentrations. Harrington (1972) suggested that within the normal range of moisture and temperature for stored seeds, each 1% reduction in seed moisture or each 5ºC reduction in temperature doubles the storage life of the seeds. Using such "rules-of-thumb" and assuming that the effects are additive, it can be assumed that seed vigor would deteriorate 500 times more rapidly at 40ºC and 18% moisture content than it would at 20ºC and 8% moisture. Thus accelerated aging has been developed as self aging technique (Parish & Leopold, 1978). To study the physiological and biochemical changes in seeds during aging, accelerated aging has been widely used (Baily *et al.,* 1996). In accelerated aging, the seeds are self aged by subjecting them to high relative humidity (>90%) and temperature (≥40ºC). The seeds so aged are compared for morphological, physiological, biochemical and genetic changes with control ones. The present study has been envisaged to study the physiological and biochemical aspects of seed deterioration in cottonseeds during accelerated aging.

MATERIALS AND METHODS

The study was conducted in the laboratory of the Department of Crop Physiology, University of Agriculture, Faisalabad during 1999.

Plant material. Cotton seed (*Gossypium hersutum*) cv. NIAB-78 was used for the study and was collected from Nuclear Institute of Agriculture and Biology, Faisalabad.

Accelerated aging. Accelerated aging of seeds was performed in a plant growth chamber at 45-48ºC and 80-90% relative humidity for the following periods: T_0 = control; T_1 = 2 days; T_2 = 3 days; T_3 = 10 days and T_4 = 20 days.

After aging, seeds were forced air dried to bring back to original weight. All the seeds were stored at –20ºC in sealed plastic container until used for vigor and enzyme studies.

Germination. Germination assays were performed in plastic pots containing sand under greenhouse

conditions. Eight seeds of each treatment were sown at 1 cm depth with equal distance. The pots were watered whenever required. Germination count, root and shoot length, cotyledon area, number of normal and abnormal seedlings, and number of secondary roots were observed after 7th day of sowing (Knypl & Khan, 1981).

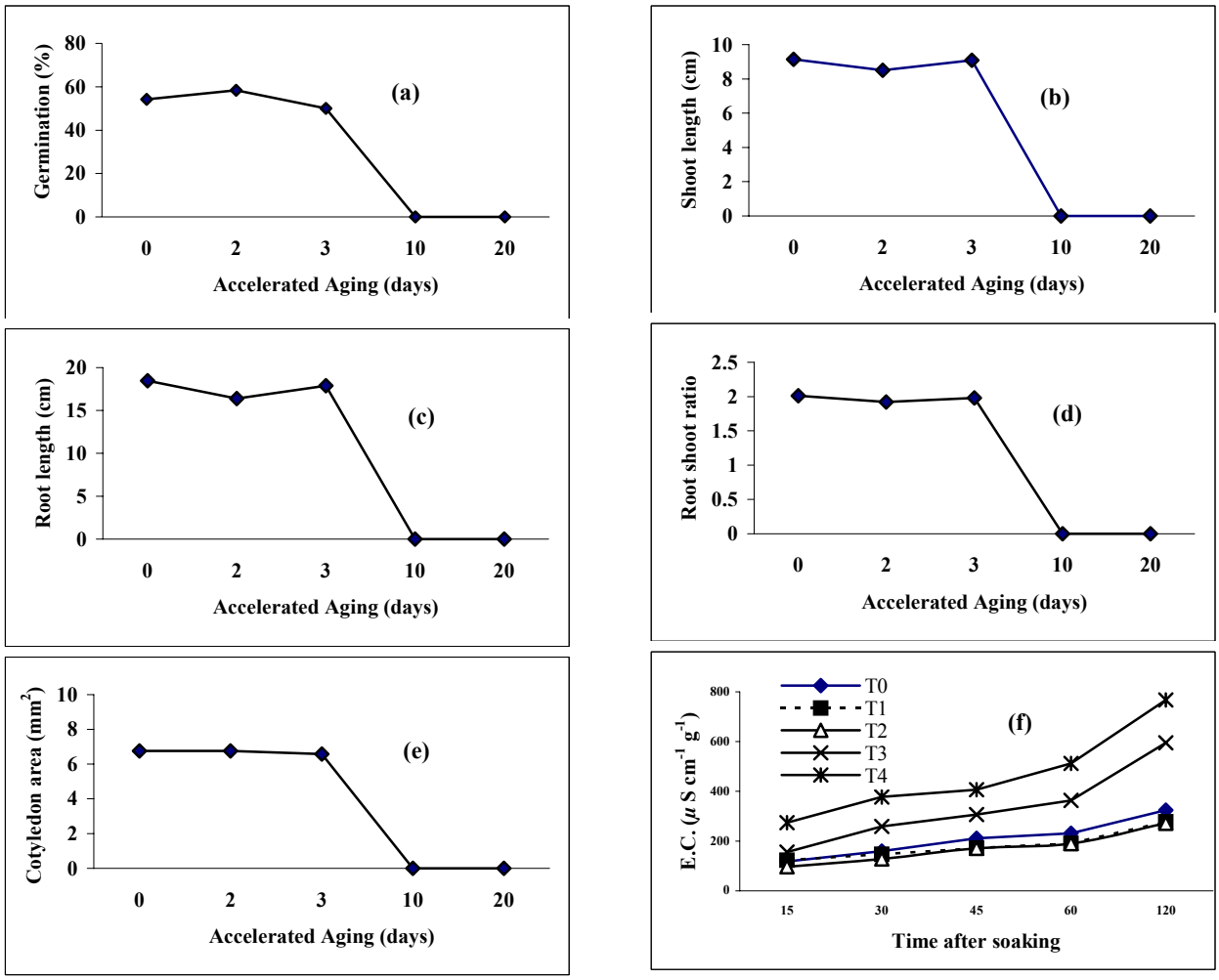
Electrolyte leakage. To determine electrical conductivity (E.C.) in the seed leachates 5 g of seeds of each treatment were rinsed for a few seconds with distilled water. The seeds were soaked in 20 mL distilled water. The E.C. of seed leachates was determined by a digital conductivity meter after 15, 30, 45, 60 and 120 minutes of soaking.

Biochemical Analysis. Oil of the seeds was extracted by Soxhlet`s extractor using *n*-hexane as solvent. The oil was analyzed for free fatty acid percentage and and peroxidase (a scavenging enzyme) activity according to A.O.A.C. (1984).

RESULTS AND DISCUSSION

Increase in accelerated aging significantly decrease the germinibility of cottonseeds (Fig. 1a). Accelerated aging upto three days did not influenced germination, however with increase in further aging period virtually no germination could be detected. The maximum shoot length was recorded for unteated seeds (Fig. 1b). Two days of aging decreased the shoot length. The shoot length again increased by further increase in one day of aging. However no germination resulted with further aging. Similar trends were seen in case of root length (Fig. 1c) and root shoot ratio (Fig. 1d). The increase in both root and shoot lengths by three days of accelerated aging may be a result of vigor enhancement. The exposure of seeds to saturated humidity resulted in slow moisture uptake by the seeds. This controlled hydration had shown to improve the performance of a number of

Fig. 1. Changes in germination (a), shoot length (b), root length (c), root shoot ratio (d), cotyledon area (e) and E.C. (f) of cottonseed cv NIAB-78 as affected by accelerated aging

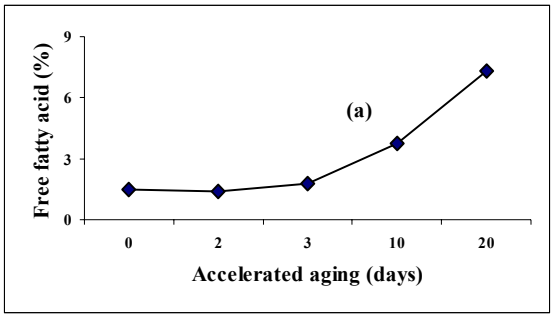


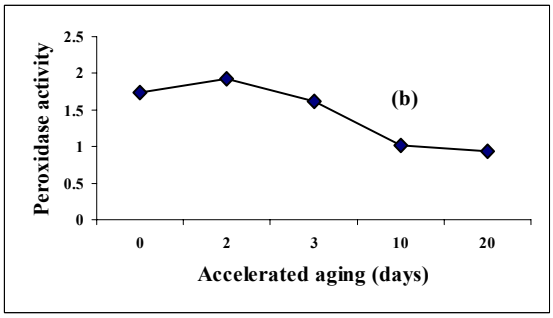
seeds (Khan *et al.,* 1995).

In cotton, like many other species with epigeal emergence, cotyledons serve as both storage and assimilatory organs. The capacity of the cotyledons as assimilating system is associated with the initial size of the cotyledons and their ability to expand. The cotyledons expand several times their original size and increase in dry matter during early expansion. The cotyledon expansion is directly related with the vigor of seeds. Fig. 1e shows that similar cotyledon areas were observed upto three days of AA. This indicates that three days of AA has not deteriorated the cottonseed. However, significant reduction in vigor has been reported by similar AA in soybean (Sung, 1996).

Priestley (1986) concluded that free fatty acids have deleterious effect on membranes probably because they are detergents. Isolated plant mitochondria show swelling and uncoupling of oxidative phosphorylation in the presence of free fatty acids (Trawatha *et al*., 1995). Crowe *et al*. (1989) showed that addition of free fatty acids increased fusion of plant vesicles which led to an increase in membrane leakage. Copeland and McDonald (1995) reported that continual accumulation of free fatty acids culminates in a reduction of cellular pH and is detrimental to normal cellular metabolism. Furthermore, it denatures enzymes resulting in loss of their activity. Individual cottonseeds containing 1% or more of free fatty acid usually will not germinate.

Fig. 2. Changes in free fatty acid % (a) and peroxidase activity (b) in cottonseed cv. NIAB-78 as affected by accelerated aging





The E.C. of the seed leachates increased with time in all cases. However, E.C. was significantly more with aging. Slightly less E.C. by three days of AA than the control was observed (Fig. 1f). This trend sports the idea of slow/controlled imbibition during early period of AA. Low E.C. is an indication of improvement in seed vigor. However, with further increase in AA period, there was a linear increase in E.C. Increased electrolyte leakage with aging confirmed the inferior quality of aged seeds.

The increased seed leakage is believed to be associated with aging induced changes in cellular membranes of imbibed seeds. Decrease in percentage germination and root and shoot lengths were accompanied with AA.

Many biochemical investigations have proven that lipid peroxidation and fat acidity (free fatty acid percentage) are the major causes of seed deterioration, including cellular membrane disruption. As seed quality declined there was a concurrent increase in the levels of free fatty acids (Fig. 2a). Elevated levels of free fatty acids, which are toxic to more cells, are not found in healthy seed tissues (Trawatha *et al*., 1995). In the present study with increase in free fatty acid contents there was a concurrent rise in seed leachate E.C. suggesting that membrane integrity was declining.

During aging, peroxidative changes may be the major cause of seed deterioration (Stewart & Bewly, 1980). Protective mechanisms that could scavenge the peroxidatively produced free radicals and peroxides, have been reported in soybean (Sung, 1996). These protective mechanisms involve several free radical- and peroxide-scavenging enzymes like superoxide dismutase, catalase, ascorbate peroxidase and peroxidase. The peroxidase value increased upto two days of AA. However, with further increase in AA peroxidase activity values decreased as shown in Fig. 2b. These results are in agreement with most of the previous reports (Sung & Jeng, 1994; Baily *et al.,* 1996). These results indicate that with increase in AA period the seeds were unable to maintain the scavenging enzymes.

There is an increasing trend in germination percentage and number of secondary roots in T_1 as compared to T_0 and in normal seedling (%), root and shoot lengths in T_2 as compared to T_1. It might be the result of invigoration of seeds by humidification during aging. Another reason might be slow hydration of seeds during AA that reduced the imbibitional injury which is otherwise caused by rapid water intake by the seed during imbibition (Sivritepe & Dourado, 1995).

CONCLUSIONS

Cottonseeds require more period for accelerated aging. The main causes of seed loss of seed vigor are cell membrane disruption due to the increase in free fatty acid level. There was no indication of presence of a scavenging mechanism against deterioration in cottonseed cv NIAB-78 under present conditions of studies.

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