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EFFECT OF ACCELERATED AGING ON LIPID PEROXIDATION, LEAKAGE AND SEEDLING VIGOR (RGR) IN CUCUMBER *(Cucumis sativus* L.) SEEDS

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Cucumber *(Cucumis sativus* L:) seeds were SUbjected to environmental treatments known to shorten seed viability rapidly. The cultivar response, seed germ inability, seedling vigor, membrane damage and lipid peroxidation in cucumber seeds were compared. Accelerated aging treatment showed decline in germinability, loss of vigor, increased leakage and lipid peroxidation products. The results obtained suggest that the changes with aging are a function of free radical mediated damage to membranes and cultivar response.

Keywords: Cucumber *(Cucumis sativus* L:), seed germinability, vigor, lipid peroxidation, leakage, aging, relative growth rate (RGR)

INTRODUCTION

The performance capability of many seeds deteriorates during prolonged storage, but the rate of deterioration varies greatly among species (Priestley, 1986; Robert, 1989). This aging or loss of vigor is evidenced by delayed germination and emergence, slower growth, increased susceptibility to stresses, and ultimately a decline in germinability (Byrd and Delouche, 1971; Douglas, 1975; McDonald, 1976; Woodstock, 1973). Seed aging is therefore a serious problem in agriCUlture, which is receiving research interest (Douglas, 1975; Harrington, 1972), yet the exact cause of loss of seed viability and vigor is not well understood, many studies have implicated damage to membranes as causative factor (Parish and Leopold, 1978; Pearce and Abdel Samad, 1980; Bewly, 1986; Ferguson *et al:,* 1990). A number of different events or processes have been suggested as causal mechanisms, including damage to proteins, nucleic acids, lipids and membranes (Osborne, 1980; Reuzeau *et al:,* 1992; Bewley and Black, 1994; Sun and Leopold, 1995; Thapliyal and Con nor, 1997; Pukacka, 1998).

The rate at which seeds loose vigor during storage is affected by environmental factors such as temperature, moisture, and *02/C02* concentrations (Douglas, 1975; Harrington, 1972; Villiers, 1973; Priestley, 1986; Vertucci *et al:,* 1994). Lipid peroxidation has considerable potential to damage the membranes and may be important in the deterioration of stored seeds and reduced longevity and vigor of seeds under natural conditions (Stewart and Bewly, 1980; Wilson and McDonald, 1986). Increased free radical activity and lipid peroxidation damage during accelerated aging has been linked to the loss of viability and vigor under high moisture regimes, for example in sunflower and soybean (Bailly *eta!.,* 1996, Khan *eta!., 1996).*

Cucumber seeds can be stored at low moisture and low temperature upto 5 years, but difficult long-term storage for genetic conservation. Seed aging can be accelerated during exposure to high temperature and high humidity, a technique widely used in the study of seed storability and deterioration. In this study, we have explored *effect* of accelerated aging on free radical mediated lipid peroxidation and consequent effect on electrolyte leakage, seed viability and seedling vigor (measured as Relative Growth Rate and Shoot Root Ratio). Two cucumber cultivars were investigated to consider whether there are cultivar differences for tolerance to aging conditions. Our aim was to assess the significance (if any) of free radical mediated lipid peroxidation, membrane damage and cultivar response during the declining vigor and viability of aged cucumber seeds.

MATERIALS AND METHODS

Plant material

All experiments were performed on two cultivars of carrot *(Daucus carrota),* obtained from two different ecological conditions Omani local and exotic Hybrid The seed material was obtained from the Department of Crop Sciences, College of Agriculture, Sultan Qaboos University, Oman. Seeds were surface sterilized using 10% sodium hypochlorite solution for 10 minutes and rinsed thoroughly in deionized water as described by Mumford and Grout (1979). The seed material was stored in aluminum foil bags at 4°C until use. The initial moisture content was 7-7.5% that was determined by low constant temperature oven method of 103°C for 17 hours (1STA, 1993) and are expressed on fresh weight basis.

Accelerated aging treatment

Seeds were aged acceleratedly at 45°C and 100% relative humidity up-to 7 days. Seeds were harvested after 2, 5, and 7 days of aging treatments. Following the accelerated aging treatment, moisture content was determined and the seeds were air dried at 25°C until their original moisture content (7-7.5%rwas restored. The seed material was stored at 4°C under the dark until use.

Germination test

Five replicates, each of 20 seeds, were germinated in 9 cm diameter Petri dishes on Whatman NO.1 filter paper. Just enough deionized water (2.5 ml) to moisten the filter paper was provided initially. Moisture level was checked daily and topped-up as necessary. Percentage radicle emergence and seed germination speed was recorded at 25°C after every 24 h time interval. Time to the initial signs of radical emergence and maximum emergence was recorded upto 7 days.

Growth analysis (RGR)

Seedlings of each cucumber cultivar were transplanted into 250 ml pots two days after germination and growth parameters were measured over the period of 7 to 21 days. The seedlings were grown under standard ISP environment (Hendry and Grime 1993). Fourteen plastic pots (250 ml) were filled with clean sand. Pots were saturated with Rorison solution (nutrient solution) and placed in undrained trays filled to 5 mm depth with deionized water and returned to standard ISP environment (25°C, 250 l-lmolm,2s'\ Water was topped on alternate days with full nutrient solution (50 ml per pot). After 7 days planting out, seedlings were harvested from seven-pot subset and same was repeated after 21 days using seven pot subset. Root and shoot was separated and dry weight was determined as described by Hunt *et al.* (1993). The seedling relative growth rate (mgg'1day'1) and shoot root ratio (shoot root) were calculated.

Rate of electrolyte leakage

Leakage of electrolytes (an indicator of membrane damage) from individually weighed seeds in 2 ml of deionized water was determined after 2, 4, 8, 18 and 24 h by measuring the conductivity *(l-lS/cm)* of seed soak water, using Jewny PCM3 conductivity meter. Conductivity was measured at 23+1° C using 20 seeds each of single seed replicate. Total ion leakage is expressed as *l-lS/cm/100* mg seed weight.

Lipid peroxidation product estimation

Lipid peroxidation was determined as the concentration of thiobarbituric acid-reactive substances, equated with malonaldehyde (MDA), as originally described by Heath and Packer (1986) but modified as in Hendry *et al.* (1993), where the products were quantified from the second derivative spectrum against standards prepared from 1,1,3,3,-tetra-ethoxypropane. All determinations were of minimum of 5 replications, each of one seed.

Fatty acid analysis

Unsaturated fatty acid content was determined as described by Hendry and Thorpe (1993) where 50 mg (approximately) of ground tissue was extracted with borate buffer pH 9.0, 3 ml of KOH was added to 1 ml of extract and incubated in sealed tubes for 6 h at 80°C. Following centrifugation, the saponified extract was incubated with lipoxidaze enzyme (60,000 U/ml), (Sigma Chemicals) for 20 minutes at 25°C. Absorbance was recorded with active and boiled enzyme at 234 nm and estimated against linoleic acid (Sigma Chemicals, L-1876) standard. Replication was 5 samples, each on one seed.

RESULTS

Seed aging and viability

All seed lots of cucumber subjected to aging treatments (Fig. 1&2), showed higher germination percentage (98-87%) and speed of germination in unaged seeds at day zero. During the first two days of aging the seeds remained viable and there was no significant effect of aging on seed germination percentage. Further increase in aging period had little suppressive effect on seed viability and germination speed. Intra-cultivar variation was also observed in germination capability while exposed to stress environment. The effect of extended aging treatment on both cucumber cultivars over 7 days at high temperature and high humidity was to reduce final percentage germination and speed of germination (69-91%). The results revealed that Hybrid cultivar was more sensitive to stressed environment compared with Omani local cucumber cultivar.

Growth performance (RGR)

Relative growth rate (RGR) and shoot root ratio were inhibited significantly with accelerated aging treatment Figure 3&4. The cultivar "Hybrid" showed faster decline in growth rate compared with Omani local cucumber cultivar. Rapid aging upto two days produced little reduction in growth rate and shoot root ratio. However, beyond this period aging damaged the seedling growth significantly as evidenced in net growth rate and shoot root ratio. The cultivar response to aging treatment showed significant and better performance of Omani cultivar in seedling vigor compared with hybrid one.

Electrolyte Leakage

Solute leakage (measured as conductivity) was increased with accelerated aging and soaking period. The intra-cultivar response to solute leakage was significant and consistent over the seven days of aging treatment. Omani local cultivar showed lesser damage to seed tissue compared with the Hybrid cultivar as revealed by leakage (Fig. 5&6). There was a linear increase in electrolyte leakage with the period of aging and water soaking treatment. After 24 hours of soaking EC of control was (114.6 and 490.3) and increased (193.0 and 789.8) by 7 days of aging for Omani local and Hybrid cultivars respectively.

Fig. 1. The Effect of Accelerated Aging on Two Cucumber Cultivars Seed Viability (% Germination)

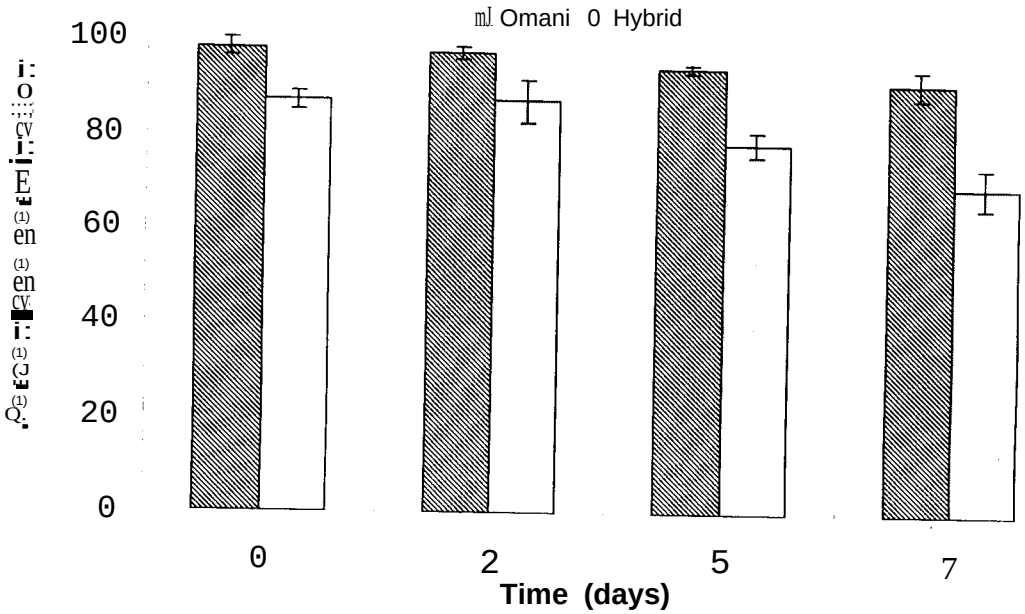


Fig. 2. The Effect of Accelerated Aging on Two Cucumber Cultivars Seed Germination Speed

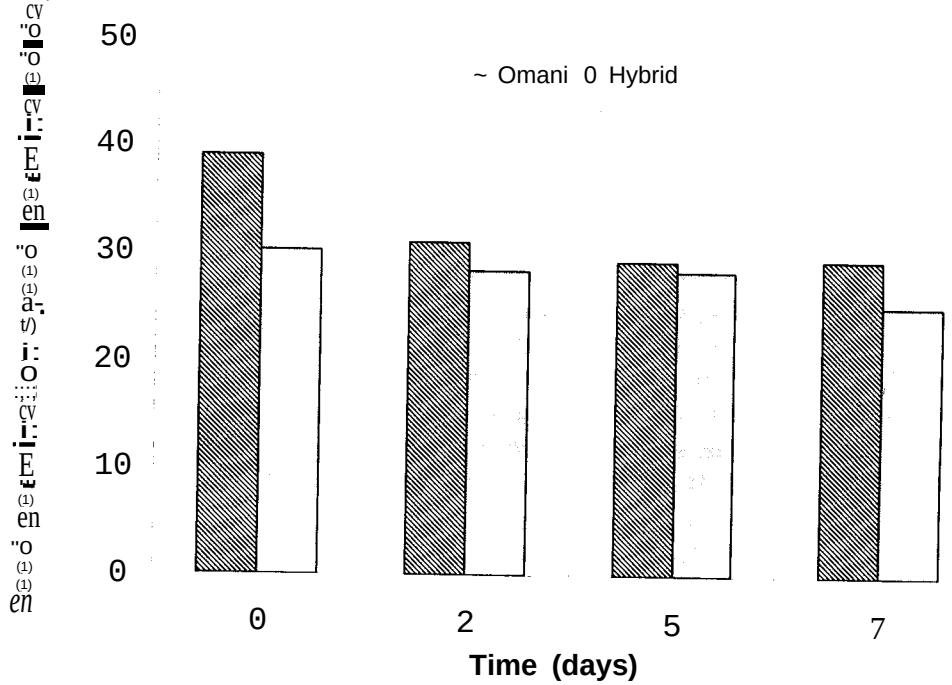


Fig. 3. The Effect of Accelerated Aging on Two Cucumber Cultivars Seedling Vigor

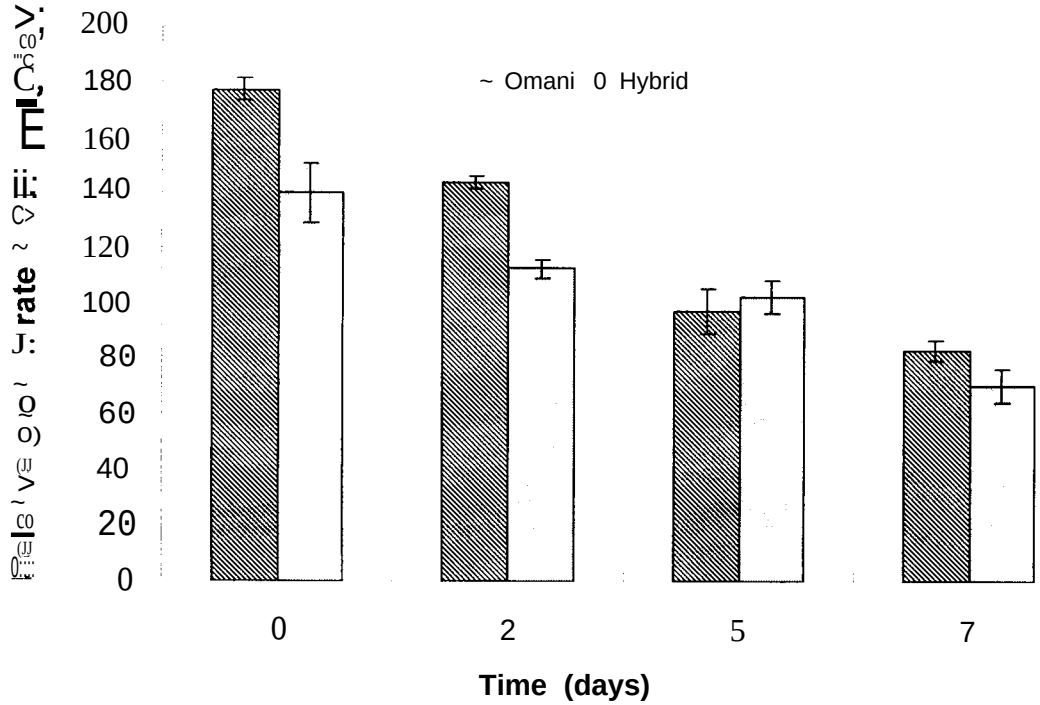


Fig. 4. The Effect of Accelerated Aging on Two Cucumber Cultivars Shoot Root Ratio

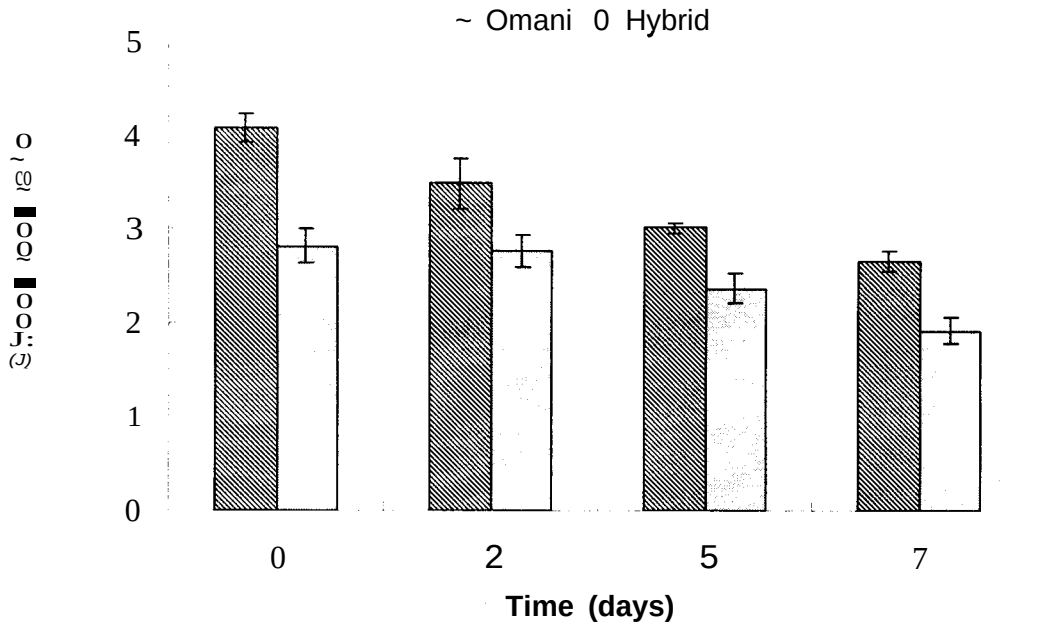


Fig. 5. The Effect of Accelerated Aging on Leachate Conductivity Measurements of Omani Local Cucumber Seeds

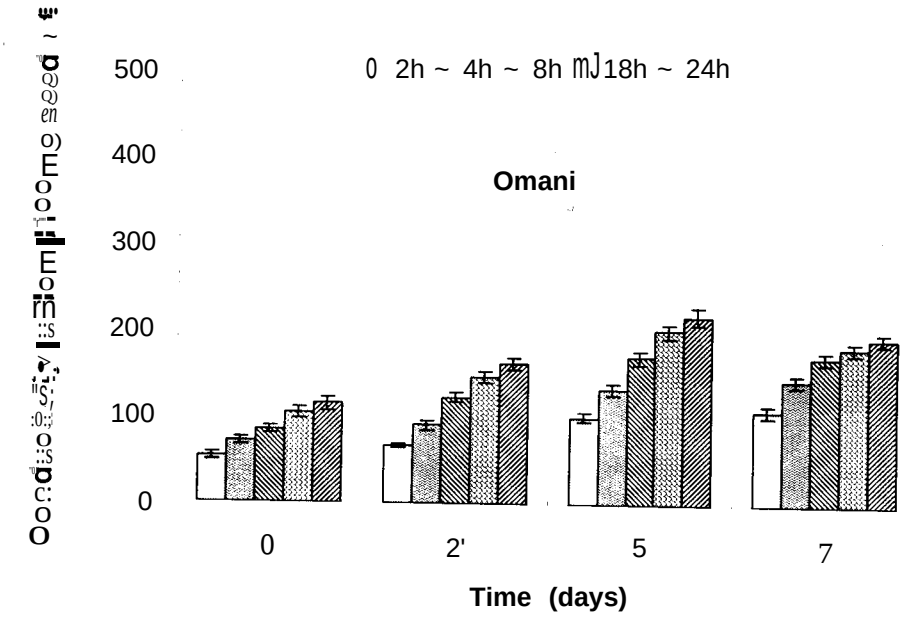
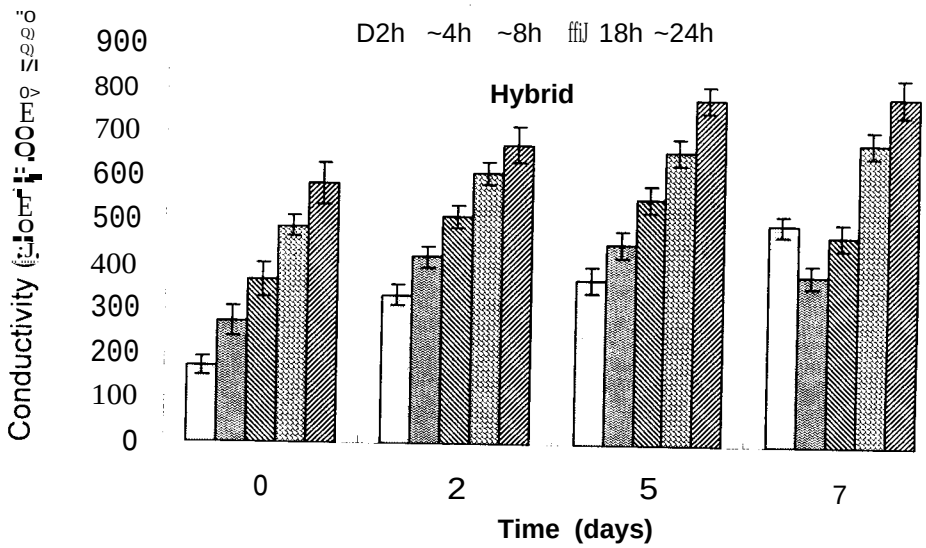


Fig. 6. The Effect of Accelerated Aging on Leachate Conductivity, Measurements (IJS/cm/100 mg seed wt.) of Hybrid Cucumber Seeds



Lipid peroxidation product

As the aging conditions prolonged lipid peroxidaion in both cucumber cultivars seed was increased over 7 days of aging treatment (Fig. 7). Both cultivars showed little increase in lipid peroxidation products at 2, 5 and 7 days of aging treatment and there was a consistent trend in increasing TBA product. Hybrid cultivar showed little more sensitive to free radical damage compared with Omani local. Overall effect of aging treatment on lipid peroxidation accumulation is significant.

Unsaturated fatty acid content

Although both cucumber cultivar seeds showed variation in unsaturated fatty acid content, but there was no significant and consistent patterns in fatty acid content over the course of aging treatment (data not presented).

DISCUSSION

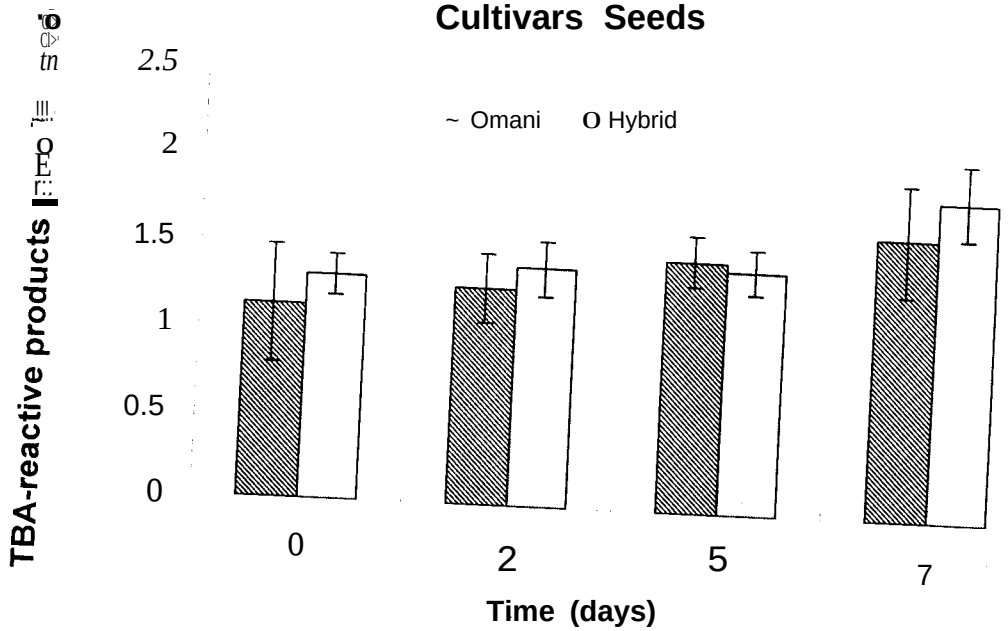
Accelerated aging reduced the cucumber seed germination, seedling relative growth rate and shoot root ratio (Fig.1, 3 & 4). This decline in seed germination was accompanied with increase in solute leakage and lipid peroxidation product (Fig. 5, 6 & 7). This severity is related to the degree of seed deterioration. Accelerated aging not only affected emergence percentage but also decreased the speed of germination and seedling vigor. Seed deterioration resulted in reduction in seedling growth rate and shoot root ratio. Similar results were reported in peanut (Sung and Jeng, 1994) and groundnut (Nautiyl *et al.*, 1997).

When the vigorous cucumber seeds under warm moist conditions, germinate in the soil, the embryo enlarges very rapidly. The primary root develops from the radicle, forces its way through the seed coat at the pointed (micropylar) end, and grows downward into the soil. The primary root may reach a depth of few inches prior to seedling emergence. Hypocotyls, the part of the embryo's stem between the primary root and the attachment point of the seedling leaves, elongates rapidly and arches into a "crook" as it pushed its way upward through the soil. The arch protects the tender tissues of the growing point (apex) from abrasion with particles in the soil. The seed coat is usually left behind in the soil during emergence. Accelerated aging of cucumber seeds for more than 5 days significantly decreased the shoot root ratio (Fig. 4) and growth rate (Fig. 3). Weak hypocotyls and primary roots due to accelerated aging might be the cause of reduction in relative growth rate.

The results also demonstrated that lipid peroxidation and leakage in cucumber seeds were correlated with the decline in germ inability and seedling vigor in rapidly aged seeds. The possibility to explain this seed deterioration is that accelerated aging of the seeds lead to enhance lipid peroxidation products and subsequently resulted in membrane perturbation (Priestley *et al.*, 1980). Cell membranes have large surface areas and a high proportion of unsaturated fatty acids, which makes the lipids particularly susceptible to peroxidative damage. Accelerated aging increased the free radical acitivity (measured as lipid peroxidation) which showed the susceptibility of membrane lipids to stressed environment. The increased seed leachates are attributed to cell membrane disruption associated with the loss of membrane phospholipids. The loss of phospholipids in deteriorated seeds is due to lipid peroxidation (Copeland and McDonald, 1995). Peroxidative changes in the fatty acid composition of membrane lipids will lead to massive disfunction, membrane viscosity increased, enhanced bilayer permeability is commonly observed, mitocondria swell and lysis occur in severe cases (Priestley, 1986). Changes in membrane lipids therefore could account for the increase in solute leakage (Sung, 1996). Lipid peroxidation produced highly reactive free radical intermediates and lipid peroxides. Lipid peroxidation products have pronounced effects on other important cellular systems, damage to DNA and membrane (Wilson and McDonald, 1986). The increase in lipid peroxidation, solute leakaqe and reduced germinability and vigor with the accelerated aging treatment, supports the idea that free radical mediated lipid peroxidation may be one of the important cause of seed aging.

Although accelerated aging uptotwo days under the present experimental conditions had no significant effect on cucumber seed germ inability but aging beyond two days reduced the seed germinability and seedling vigor significantly. The main cause of seed deterioration by accelerated aging may be membrane disintegration and inactivation of enzymatic systems mainly due to lipid peroxidation and increase in free radical activity. However there is little disagreement in the literature about this correlation (Linn and Pearce, 1990; Kalpana and Rao, 1994). We have demonstrated in this study that both cultivars of cucumber showed close relationship between lipid peroxidation product, membrane damage and loss of viability and vigor. Above all cultivar response to aging treatment is highly interesting where an indigenous (Omani local) cultivar performed better compared with exotic (Hybrid). This shows that nature has gifted some special characters to such land races to combat adverse environmental conditions. These findings may help to understand the processes leading to cucumber seed deterioration while undergoing aging process and role of land races seeds in genetic resources conservation.

Fig. 7. The Effect of Accelerated Aging on Lipid Peroxidation TBA Reactive Products of Two Cucumber Cultivars Seeds



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