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Effect of Accelerated Ageing on Viability, Vigor (RGR), Lipid Peroxidation and Leakage in Carrot (*Daucus carota* L.) Seeds

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ABSTRACT

Response of two cultivars of carrot in respect of seed viability, seedling vigor, lipid peroxidation and membrane integrity was explored. Accelerated aged seeds of both cultivars showed considerable loss in viability. Built up of lipid peroxidation product was higher in Omani cultivar seeds compared to Pakistani. As the ageing time proceeded lipid peroxidation was increased progressively. Significant reduction in relative growth rate (RGR) of both cultivars was observed with the ageing treatments. Pakistani cultivar showed higher un-saturated fatty acid (USFA) accumulation after accelerated ageing than the Omani cultivar. Effect of accelerated ageing on electrolyte leakage suggested that electrical conductivity of carrot seeds increased with the increase in ageing time.

Key Words: Carrot (*Daucus carrota*); Seed germinability; Leakage; Relative growth rate (RGR); Ageing; Lipid oxidation

INTRODUCTION

The performance capabilities of many seeds deteriorate during prolonged storage, but the rate of deterioration varies greatly among species (Priestly, 1986; Robert, 1989). This ageing or loss of vigor is evidenced by delayed germination and emergence, slower growth, increased susceptibility to stress, and ultimately a decline in germinability (Byrd & Delouche, 1971; Douglas, 1975; McDonald, 1976; Woodstock, 1973). Accelerated seed ageing technique is a widely used tool to test the seed quality. This ageing test of seed vigor can give better indications of probable field emergence for vegetable crop seeds than germination and growth tests (Pandey *et al*., 1990). High temperature, ambient relative humidity, and seed moisture content are the main factors influencing seed storage capability (Abdul-Baki, 1980). Accelerated ageing test is considered standardized and correlates with field emergence under a variety of seed bed conditions (Egli & Tekrony, 1996).

Seeds, like any other plant organ, age with time, and die. However, the rate at which seeds age depends upon their physiological status, their genetic constitution and the storage conditions. The availability of an adequate supply of crop seeds of a uniform high quality is essential for a successful seed industry and the maintenance of a viable and productive agriculture (Barnes, 1986). It is also essential where germplasm collections are to be established.

Biological membranes with a normal composition and organization regulate the transport of materials into and out of the cell. Therefore they play a key role in maintaining seed viability and vigor. Solute leakage accompanies seed imbibition during the process of membrane reorganization following rehydration. The rate of leakage depends on the degree of cell membrane damage and repair in response to ageing (Larson, 1968; Simon, 1978). Damage to the organization of cell membranes during seed ageing may constitute an important factor in explaining seed deterioration (Priestly & Leopold, 1979; Senaratna *et al.,* 1988; Ferguson *et al.,* 1990). In seed ageing damage to cellular membranes, decrease in mitochondrial dehydrogenases activities, chromosomal aberration and DNA degradation increases (Parrish & Leopold, 1978).

Carrot seeds can be stored at low moisture and low temperature for few years, but difficult long-term storage for genetic conservation (Al-Maskri *et al.,* 2002). In this study, effect of accelerated ageing on seed viability and seedling vigor (measured as Relative Growth Rate) electrolyte leakage and lipid peroxidation process was explored. Two carrot cultivars were investigated to consider whether there are cultivar differences for tolerances to ageing conditions. Our aim was to assess the significance (if any) of membrane damage and cultivar responses during the declining vigor and viability of aged carrot seeds.

MATERIALS AND METHODS

Plant material. All experiments were performed on two cultivars 'Omani (local)' and 'Pakistani (T-20)' of carrot (*Daucus carota* L.). The seed material was obtained from the Department of Crop Sciences, College of Agriculture, Sultan Qaboos University, Oman and institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. Seeds were surface sterilized using 5% sodium hypochlorite solution for 5 minutes and rinsed thoroughly in deionized water as described for pea by Khan *et al.* (2003). Seed material was stored in aluminum foil bags at 5^{o}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 h (ISTA, 1993) and are expressed on fresh weight basis.

Accelerated ageing 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 ageing experiments. Following the accelerated ageing treatment, moisture content was determined and the seeds were air dried at 25°C until their original moisture content (7-7.5%) had been restored. The seed material was stored at 5°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 radical emergence and seed germination speed were 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 carrot 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 & 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 $\mu molm^{-2}s^{-1}$). Water was topped on alternate days with full nutrient solution (50 mL per pot). After 7 days of planting, seedlings were harvested from seven-pot subset and same was repeated after 21 days using seven pot subset. Root and shoot were separated and dry weight was determined as described by Hunt *et al.* (1993). The seedling relative growth rate ($mgg^{-1}day^{-1}$) 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 (μScm^{-1}) of seed soak water, using Jewny PCM3 conductivity meter. Conductivity was measured at 23±1°C using 20 seeds each single seed replicate. Total ion leakage is expressed as $\mu Scm^{-1}100^{-1}$ mg seed weight.

Lipid peroxidation product estimation. Lipid peroxidation was determined as the concentration of thiobarbituric acid-reactive substances, equated with malonaldeyde (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 centrifugations, the saponified extract was incubated with lipoxidaze enzyme (60,000 UmL^{-1}), (Sigma Chemicals) for 20 minutes at 25°C. Absorbance was recorded with active and boiled enzyme at 234nm and estimated against linoleic acid (Sigma Chemicals, L-1876) standard. Replication was 5 samples, each of one seed.

RESULTS

Seed Viability. The results of seed viability (germination %age) are presented in Fig. 1. When seeds were not aged acceleratedly, both cultivars showed high germination percentage (more than 85%). Omani seeds exhibited higher germination percentage (94%) compared to Pakistani carrot (85%) seeds (Fig. 1). The seed germinability was reduced in both cultivars with the passage of ageing time. It was 77, 36 and 12% in Omani indigenous carrot seeds, aged for 3, 5, and 7 days respectively. While Pakistani T-20 carrot seeds exhibited higher germination percentage in control (i.e., 85%) compared to the lowest in 7 days aged seeds (i.e., 10%). The lowest germination percentage (seed viability) in Omani was 12% in 7 days aged seeds.

Lipid peroxidation. There was a progressive increase in the accumulation of lipid peroxidation in both the cultivars as ageing time proceeded (Fig. 2). This was the highest (7.38 ηmols) in 7 day aged seeds of Omani compared to Pakistani carrot (5.7 ηmols) seeds. Lipid peroxidation product in un-aged Omani and Pakistani was 5.08 and 4.42 ηmols respectively. Overall lipid peroxdation was higher in Omani indigenous cultivar seeds compared to the Pakistani T-20 carrot seeds. Overall ageing treatment showed significant ($P<0.05$) effect on the accumulation of lipid peroxidation products in both cultivars.

Relative growth rate (RGR). It is indicated that relative growth rate was significantly ($P<0.05$) affected with accelerated ageing treatments (Fig. 3). However both cultivars showed similar response to ageing treatments. Relative growth rate (RGR) in control was 0.11 $mgg^{-1}day^{-1}$ and 0.12 $mgg^{-1}day^{-1}$ for Omani and Pakistani cultivars, respectively. It was first increased in 3 days ageing treatment i.e., 0.16 $mgg^{-1}day^{-1}$ and 0.21 $mgg^{-1}day^{-1}$ in both the cultivars and then showed a rapid decline as the ageing period proceeded (Fig. 3). There was no growth in both cultivars in 7 days ageing treatment. RGRs for 5 days ageing treatments were 0.07 $mgg^{-1}day^{-1}$ and 0.10 $mgg^{-1}day^{-1}$ for Omani and Pakistani cultivars, respectively.

Un-saturated fatty acid content (USFA). For USFA both cultivars showed approximately similar response and there was not great variation in overall results. It was the highest in both the cultivars after 3 days ageing treatment i.e., 3.45 mgg^{-1}seed weight and 3.65 mgg^{-1}seed weight in Omani and Pakistani, respectively, (Fig. 4). After 3 days of ageing USFA showed a decline in 5 and 7days ageing treatments.

Fig. 1. The effect of accelerated ageing on two carrot cultivars seed viability (germination %age)

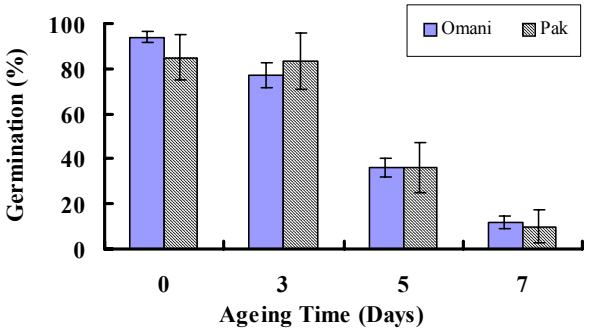


Fig. 2. The effect of accelerated ageing on lipid peroxidation tba reactive products of two carrot cultivars seeds

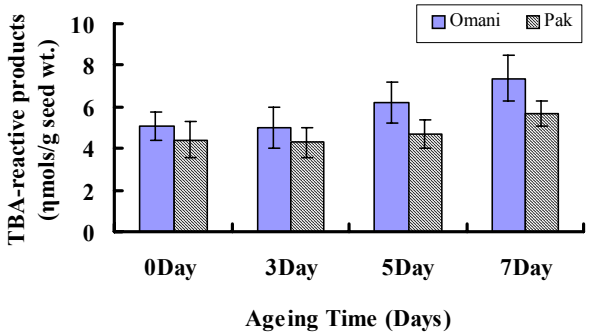


Fig. 3. The effect of accelerated ageing on RGR of two carrot cultivars from Oman and Pakistan

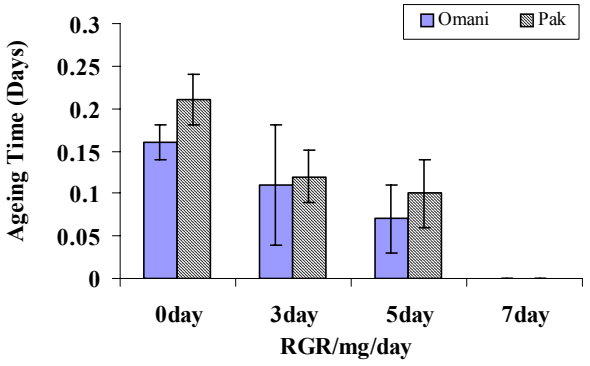


Fig. 4. The effect of accelerated ageing on usfa of two carrot cultivars seeds

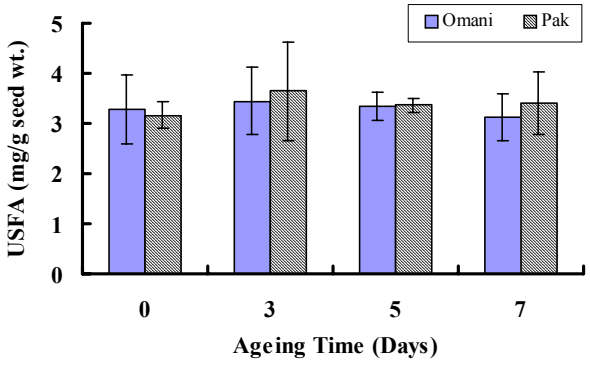


Fig. 5. The effect of accelerated ageing on leachate conductivity measurements of Omani carrot seeds

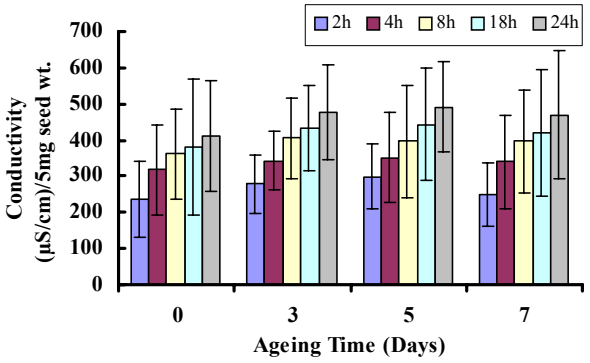
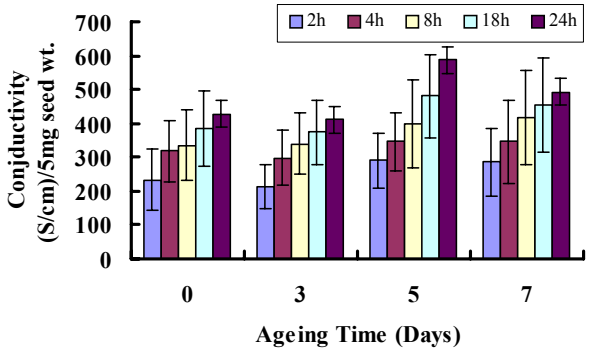


Fig. 6. The effect of accelerated ageing on leachate conductivity measurements of Pakistani carrot seeds



USFA content for control treatments was 3.28 mgg^{-1}seed weight and 3.17 mgg^{-1}seed weight for Omani and Pakistani cultivars, respectively (Fig. 4). Overall results indicated that there was no significant change in USFA values after accelerated ageing treatments.

Seed conductivity (electrolyte leakage). The electrical conductivity of seeds in both cultivars increased with increasing ageing and soaking time. The ion leakage is increased by each increment of the accelerated ageing treatment and by passage of imbibition time. After 24 h of soaking seed leachates of control treatments in both the cultivars were (435 and 427 µS/cm/5mg seed weight) and these were increased (470 and 492 µS/cm/5mg seed weight) in 7 days ageing treatment in Omani and Pakistani cultivars, respectively (Fig. 5 & 6).

DISCUSSION

Seed germination capacity and relative growth rate of carrot were reduced under accelerated ageing treatment. Subjecting seeds of both cultivars to high temperature and high humidity (accelerated ageing) broadly reproduced these measures in declined viability. Accelerated ageing increased seed leakage and lipid peroxidation. The decline in seed viability and relative growth rate was accompanied with increase in solute leakage and lipid peroxidation product (Fig. 2, 3, 5, 6). The significant increase in lipid peroxidation response of rapidly aged seeds was apparent in both Omani indigenous and Pakistani T-20 carrot cultivars. Accelerated ageing did not only affect germination percentage but also decreased the seed vigor (increased ion leakage). These results correlate with the findings of Sung and Jeng (1994), Nautilyl *et al.* (1997) and Al-Maskri *et al.* (2002) in peanut, groundnut and cucumber seeds respectively. Increased seed electrical conductivity EC (seed deterioration) resulted in the reduction of relative growth rate. Loss in seed viability and vigor was associated with the increased electrolyte leakage in pea seeds (Khan *et al.,* 2003).

It is indicated that lipid peroxidation products in both cultivars seed increased with the passage of ageing period (Fig. 2). It may be stated that rapidly ageing had damaging affect on seed membrane and resultantly lipid peroxidation products and electrical conductivity was increased (Fig. 2, 5, 6). On the basis of these findings, it is can be postulated that there is a strong relationship between lipid peroxidation and electrical conductivity of seeds and these both factors responded significantly to stressed environment (high temperature and high humidity). Cell membranes contain high proportions of lipids which are susceptible to oxidative damage (Sung, 1996). Free radicals are produced in cell membranes during the accelerated ageing treatments which are very harmful to living cells of membranes and may cause membrane damage. This membrane damage results in the ion leakage (measured as electrical conductivity) of membranes of seeds (Parrish & Leopold, 1978). Damaged membranes are also considered to be responsible for the delayed germination percentage and slower relative growth rate (Khan *et al.,* 2003).

Our results showed that accelerated ageing treatments leads to the increased lipid peroxidation value (Fig 2). Peroxidative changes in the fatty acid composition of membrane lipids lead to enhanced bilayer permeability, mitochondrial swell and lysis occur in severe cases (Priestly, 1986). Oxygen can add to the fatty acid chain to form peroxides and hydroperoxides. Peroxides undergo cleavage to produce bad smelling aldehydes, ketones and acids. The peroxide value is a measure of the amount of these products (Basra *et al.,* 2002). The increased seed leachates are attributed to cells membrane disruption associated with the loss of membrane phospholipids. The loss of phospholipids in deteriorated seeds is due to lipid peroxidation (Copeland & McDonald, 1995).

Another possible cause of loss of seed viability and vigor during accelerated ageing treatments may be due to changed un-saturated fatty acid (USFA) contents. In this study, no significant change was observed in un-saturated fatty acid's level with ageing treatment. Both cultivars showed increased USFA and this increase may have detrimental effect on seed cellular metabolism (Priestley, 1980).

It may be concluded that carrot seeds aged rapidly showed significant reduction in the seed viability and relative growth rate of both carrot cultivars. Loss of seed viability was associated with the increased seed conductivity (electrolyte leakage), lipid peroxidation build up and by the increasing levels of un-saturated fatty acid contents, which were produced upon accelerated ageing treatment.

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