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Light Promotes Free Radical Processes in Citrus (*Citrus paradisi* Macf.) Seeds

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ABSTRACT

Grapefruit (*Citrus paradisi* Macf.) seeds with 13% moisture content (intact or decoated) and stored under light or darkness at 27°C suffered a significant decline in viability, but retained better viability under darkness compared to the light stored seeds. In contrast, seeds with 6% moisture content (intact or decoated) stored under same environment conditions showed lesser loss of viability compared to 13% moisture content, and again dark stored seeds proved to be better survivors. Loss of viability was associated with the increase in lipid peroxidation, which was mostly confined to the decoated seeds compared to intact seeds. Light promoted lipid peroxidation and free radical processes were observed, particularly in decoated seeds. These responses suggest that at least in this species thick testa apparently provided a defense against oxidative stress.

Key Words: Lipid peroxidation; Free radicals; Grapefruit (*Citrus paradisi* Macf.); Moisture content; Seedcoat; Seed viability

INTRODUCTION

Maintenance of plant biodiversity is a global concern. One strategy to preserve the genetic diversity with in a species is through seed storage in *ex situ* gene banks (Ross, 1989). Deterioration during storage must be minimized to ensure the genetic integrity of the accession. The optimum storage conditions should be known in order to preserve the seeds under the best conditions possible while keeping costs to a minimum (Ross, 1989; FAO/IBPGR, 1992).

The best storage condition and the precise causes of seed ageing during storage are still not well understood. This ageing or loss of vigour is evidenced by delayed germination and emergence, slower growth, increased susceptibility to environmental stresses, and, ultimately, a decline in germinability (Byrd & Delouche, 1971; Douglas, 1975; McDonald, 1976). Seed ageing, therefore, is a serious problem in agriculture, one receiving increasing research interest (Byrd & Delouche, 1971; Harrington, 1972). A number of different events or processes have been suggested as casual mechanisms, including damage to proteins, nucleic acids, lipids and membranes (Osborne, 1980; Reuzeau *et al*., 1992; Bewley & Black, 1994; Sun & Leopold, 1995; Thapliyal & Connor, 1997; Pukacka, 1998).

The rate at which seeds lose vigour during storage is affected by the environmental factors such as temperature, moisture, light and O_2/CO_2 concentrations (Byrd & Delouche, 1971, Harrington, 1972; Villiers, 1973; Priestly, 1986; Vertucci *et al.*, 1994; Khan *et al*., 1996). Oxidative process is that occur during storage as well as the imbibition of seeds (Priestly *et al*., 1985; Bewly, 1986; Simirnoff, 1993) might be important factors in the lowering of seed germination ability. Free radical theories of seed ageing (Villiers, 1973; Wilson & McDonald, 1986; McKersie *et al*., 1988; Hendry *et al*., 1992; Hendry, 1993; Kalpana & Rao, 1996; Hendry, 1997) have suggested that in the presence of oxygen unsaturated fatty acids spontaneously oxidise producing highly reactive free radical intermediates, lipid hydroperoxides and a range of secondary products (Frankel, 1982). These reactions have the potential to damage membranes, nucleic acids, and enzymes and all these cellular components have been shown to suffer damage with seed ageing (Bewley & Black, 1982).

It is known from vegetative tissue that light can induce the production of free radicals, which in turn, can lead to the destruction of macromolecules (Levitt, 1980; Foyer *et al*., 1994, Cakmak *et al*., 1995), but the mechanisms by which light induced damage can arise in seeds is not very much clear. A study was undertaken to explore the role of light in seed ageing (viability), accumulation of stable free radicals and lipid peroxidation of citrus seeds, stored under light or darkness.

MATERIALS AND METHODS

Plant material. Freshly harvested seeds of grapefruit (*Citrus paradisi* Macf.) were obtained from the Experimental Fruit Garden, Department of Horticulture, University of Agriculture, Faisalabad, Pakistan. These seeds were thoroughly washed to make them free of mucilage and were sterilised using 10% sodium hypochlorite solution for 10 min (Mumford & Grout, 1979) and thoroughly rinsed in distilled water.

Seed storage and treatment. The testae were removed from half the seeds and two lots of seeds were dried separately over silica gel in 6 L desiccators to obtain 6% and 13% moisture at room temperature. The gel was replaced frequently to ensure continuous drying. Seed moisture

content was determined by the low temperature oven method for tree seeds (ISTA, 1993) and are expressed on a fresh weight basis. Seeds dried upto 6 and 13% with or without testa were stored separately in sealed jars under 90 μmol $m^{-2}s^{-1}$ light or dark at 27°C. Samples were removed after 40 and 90 days storage.

Germination test. Germination tests were performed in 9 cm Petri dishes on Whatman No 1 filter papers, which was moistened regularly with deionised water. Percentage radical emergence was recorded over six weeks at 25°C (Edwards & Mumford, 1985) and 12 h photoperiod in a growth chamber. Germinated seeds were counted for six weeks or more. Germination test was replicated three times using 20 seeds per replicate.

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, tetera-ethoxypropane. All determinations were of minimum of five, each of one intact/decoated seed.

Electron paramagnetic resonance (EPR) response. Electron paramagnetic resonance spectra were recorded on Bruker ER 200D spectrometer, as described by Leprince *et al*. (1990), care being taken to position the sample reproducibly in the spectrometer cavity. Other parameters were adjusted as necessary to obtain the most resolved spectra. Free radical concentrations were estimated by the height (cm) of first derivative spectrum corrected for instrument gain and expressed on an unimbibed seed weight basis.

RESULTS AND DISCUSSION

Effect of rapid ageing under light or dark on the period of viability of grapefruit seeds. Referring to Fig. 1 and 2,

Fig. 1. Probit percentage germination in grapefruit intact and decoated seeds (27°C, 13% moisture content) stored under light or dark

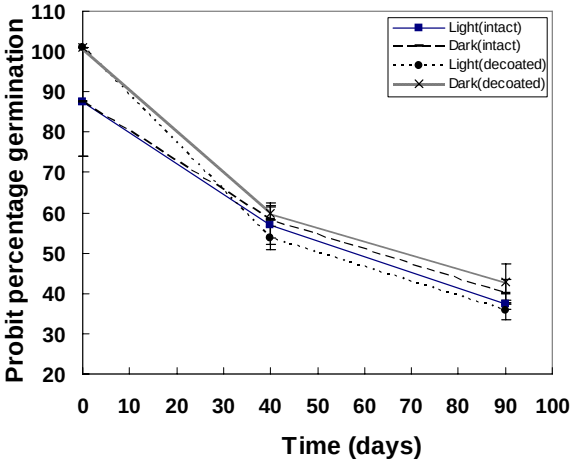
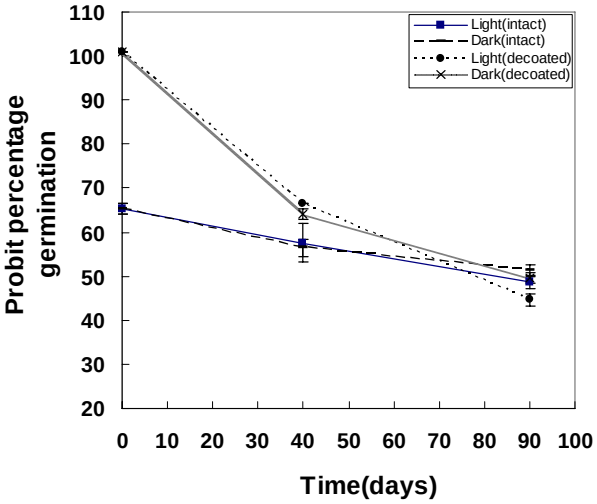


Fig. 2. Probit percentage germination in grapefruit intact and decoated seeds (27°C, 6% moisture content) stored under light or dark



there is evidence that the viability of grapefruit seeds is negatively correlated with moisture content (both intact or decoated) and storage in the light. Analysis of variance indicated a significant effect of moisture ($P < 0.001$) and light environment ($P < 0.05$) on final seed viability. Results also revealed a significant interaction between moisture content and storage time, and between moisture, seed coat and storage time. Grapefruit seeds dried to 13% moisture content (intact or decoated) and stored under light or dark at 27°C over 12 weeks suffered a significant decline in viability, but retained better viability under dark environment compared to light. On the other hand, seeds dried to 6% moisture content (intact or decoated) and stored under the same environment conditions showed lesser loss of viability compared to seeds with 13% moisture content, and again dark stored seeds proved to be better survivors. After 90 days storage under light or dark, the intact grapefruit seeds (6% moisture content) showed 20% greater final percentage germination compared to decoated seeds, with the same moisture content (Table I). The initial differences in germination percentage are probably real treatment differences.

Effect of rapid ageing on lipid peroxidation products in intact and decoated seeds. With time there was a progressive increase in the accumulation of lipid peroxidation in 13% moisture content seeds (Fig. 3), however the seeds with 6% moisture content showed an increase in lipid peroxidation at first harvest after 40 days but thereafter little change. (Fig. 4). Seed moisture content did not show any significant ($P>0.05$) effect on lipid peroxidation. However, presence or absence of seed coat and illumination ($P<0.001$ and $P<0.05$,) has a significant effect on the accumulation of lipid peroxidation. A statistically significant interaction was also observed between moisture and storage time ($P<0.001$). The seeds with high moisture and without testa (either in light or dark)

Table I. Effect of short-term storage on grapefruit seed germination stored under light or dark at 27°C

	Storage period				
	0 days	40 days		90 days	
		Dark	Light	Dark	Light
Intact 13% moisture					
Germination percentage	95.0±5.0 (80.4±7.6)	75.0±10.4 (61.5±7.1)	71.3±15.1 (54.3±10.1)	18.3±8.8 (23.1±6.8)	10.0±0.0 (18.4±0.0)
Decoated 13% moisture					
Germination percentage	100.0±0.0 (90.0±0.0)	83.3±3.3 (66.1±2.7)	63.3±10.1(53.1±6.2)	28.3±11.7 (30.5±8.8)	8.3±3.3 (16.2±3.3)
Intact 6% moisture					
Germination percentage	93.3±1.7 (75.2±1.8)	73.3±6.0 (59.2±4.1)	73.3±10.9(60.5±8.3)	55.0±5 (51.2±4.5)	45.0±5.8 (42.1±3.3)
Decoated 6% moisture					
Germination percentage	100±0.0 (90.0±0.0)	91.6±1.7 (73.4±11.8)	95.0±0.0(77.1±0.0)	48.3±4.4 (44.03±2.5)	30±5.0 (33.1±3.1)

Values in brackets are angular transformed percentage germination

Table II. Effect of short-term storage on the accumulation of lipid peroxidation and free radical (EPR) processes in grapefruit seeds stored under light or dark at 27°C

	Storage period				
	0 days	40 days		90 days	
		Dark	Light	Dark	Light
Intact 13% moisture					
EPR arbitrary units/g d.wt.	48.6±7.0	30.7±3.7	33.9±3.1	76.3±5.9	118.5±10.3
Lipid peroxidation (nmol/g d.wt.)	5.8±0.4	5.7±0.1	6.4±0.2	6.7±0.32	7.1±0.5
Decoated 13% moisture					
EPR arbitrary units/g d.wt.	52.8±10.4	15.6±2.0	14.7±1.4	89.5±4.4	111.6±1.9
Lipid peroxidation (nmol/g d.wt.)	5.9±0.2	6.8±0.1	6.7±0.3	7.2±0.3	9.2±0.6
Intact 6% moisture					
EPR arbitrary units/g d.wt.	78.6±18.2	78.8±8.4	124.8±7.4	102.4±7.0	178±20.6
Lipid peroxidation (nmol/g d.wt.)	5.8±.6	6.3±0.5	6.7±0.4	6.1±0.2	6.6±0.2
Decoated 6% moisture					
EPR arbitrary units/g d.wt.	42.5±3.1	31.2±1.3	42.2±6.7	75.9±4.1	99.4±4.7
Lipid peroxidation (nmol/g d.wt.)	5.3±3	7.8±0.9	7.4±0.2	7.0±0.3	7.0±0.4

showed increased lipid peroxidation compared to intact seeds, but this increase was much greater in the light (Table II).

Effect of rapid ageing on free radical processes in intact and decoated seeds. Decoated and intact seeds stored under light or dark showed an increase in the build-up of a stable free radical (EPR response) with age at either 13 or 6% moisture content. A significant effect of moisture content and presence or absence of seed coat ($P<0.001$) on free radical accumulation was observed. After 90 days storage time there was a significant increase in EPR response in all treatments (Table II). The EPR amplitude in intact seeds (13% moisture) was 40% greater in light treated tissues compared to dark treated tissues (Fig. 5). The decoated

Fig. 3. Lipid peroxidation (thiobarbituric acid reactive products) in grapefruit intact and decoated seeds (13% moisture content) stored at 27°C, under light or dark

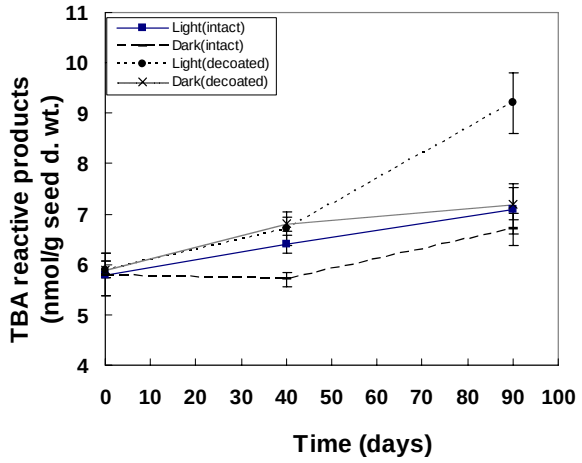


Fig. 4. Lipid peroxidation (thiobarbituric acid reactive products) in grapefruit intact and decoated seeds (6% moisture content) stored at 27°C, under light or dark

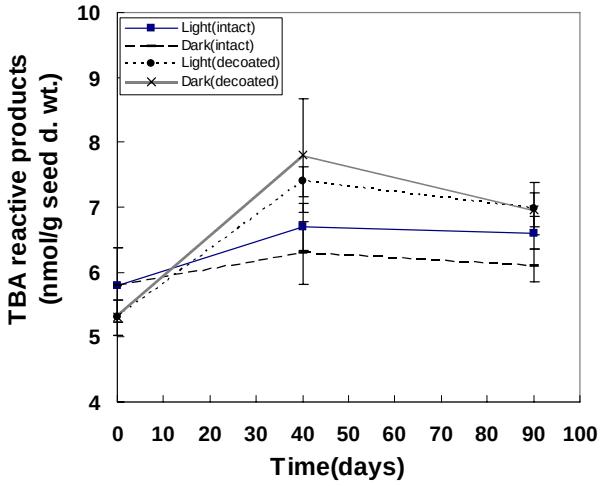


Fig. 5. EPR response as the amplitude of the signal at constant settings, per g dry wt. of intact or decoated grapefruit seeds (13% moisture content) stored at 27°C, under light or dark

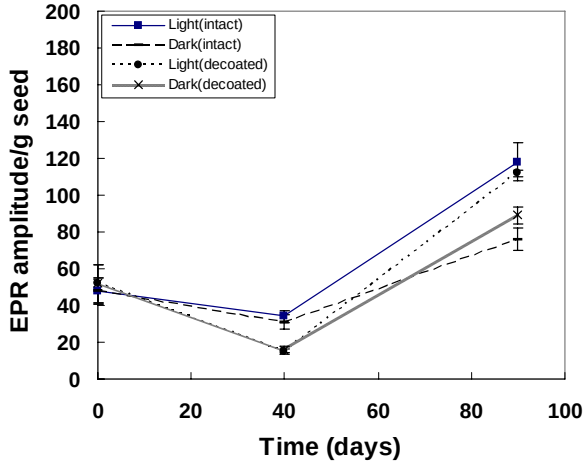
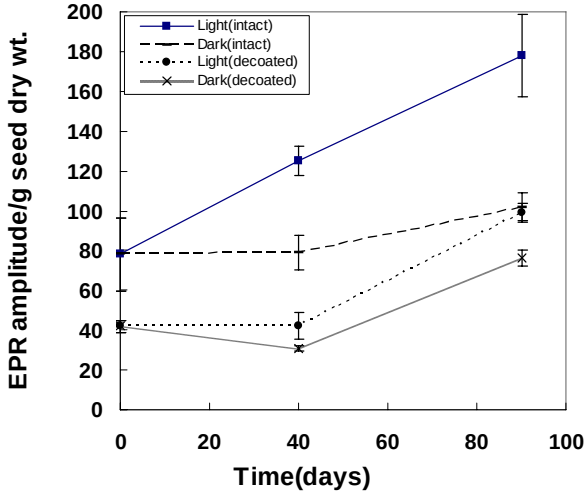


Fig. 6. EPR response as the amplitude of the signal at constant settings, per g dry wt. of intact or decoated grapefruit seeds (6% moisture content) stored at 27°C, under light or dark



seeds (13% moisture) also revealed an increase of about 21% in light stored seeds compared to dark stored seeds. The EPR response in intact or decoated seeds with low moisture content was significantly affected by presence or absence of seed coat and by storage time (Fig. 4). Although moisture has the biggest single effect on accumulation of free radicals, illumination also revealed a significant ($P < 0.001$) effect on EPR processes. The changes in EPR response are summarized in Table II.

The storage of intact or decoated grapefruit seeds containing various amounts of water (6 and 13%) at 27°C for several weeks under light or dark caused considerable decrease in seed viability. At first harvest, after 40 days storage, apparently there was no significant differences in viability either intact or decoated stored under light or dark, except light stored decoated grapefruit seeds (13% moisture content), where significant viability loss (37%) was recorded compared to dark stored seeds. After 90 days storage, there was greater decrease in viability particularly in moist decoated seeds stored under light.

A decrease in citrus seeds germination capacity corresponded to an increase in lipid peroxidation and EPR response with age, particularly under illumination. Seeds containing more water showed a larger decrease in seed viability and increase in lipid peroxidation particularly in decoated seeds under illumination. The grapefruit seeds with low moisture content (6%) showed better viability and lesser damage in the form of lipid peroxidation. It could be due to the absence of free water, which would restrict the free radical chain reactions in dehydrated, crystalline lipid arrangement (Simon, 1974). As free water is restored during hydration (or in high humidity storage where water may be taken up hygroscopically), the rate of damaging processes may be expected to accelerate.

The significant increase in free radical accumulation in aged intact or decoated grapefruit seeds (6% moisture content) were not (or barely) apparent in (13% moisture content) seeds stored under the same environment. This response may reflect the hydration and temperature effect on the release of free radicals. Priestly *et al*. (1985) observed a pronounced decline in the free radical accumulation when hydration increased above about 7% (wet weight basis) in both seed and pollen. It was also shown that free radical accumulation in water stressed *Zea mays* seedlings is a function of temperature with a Q10 of about two (Leprince *et al.*, 1990) and it is no surprise to find an apparent temperature and moisture-dependent increase in free radical events in grapefruit seeds maintained at 27°C.

The results also showed increased free radical accumulation and lipid peroxidation in seeds stored under light and particularly in decoated seeds. We have previously shown that lipid peroxidation and free radical accumulation were significantly enhanced by illumination, particularly in testae of soybean seeds (Khan *et al*., 1996). To observe further evidence that light enhanced events occur whether or not testa, is attached to living cotyledon tissue. Our results demonstrated that grapefruit seeds with seed coat had lesser damage in the form of lipid peroxidation and free radical accumulation compared to decoated seeds. Seed moisture content also showed biggest effect on lipid peroxidation, free radical build-up and loss of viability.

This apparent association between the loss of viability and activity of free radical- processes particularly under illumination is consistent with other evidence (Hendry, 1993; Khan *et al*., 1996; Pukacka, 1998) that loss of viability in seeds exposed to light, high temperature and low moisture is probably linked to oxidative processes. The results presented here suggest that light greatly enhanced lipid peroxidation, increased organic free radicals and loss

of viability in decoated grapefruit seeds compared to intact seeds. In this species showed that thick testa apparently provided a defense against oxidative damage.

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