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Effect of Accelerated Aging on Seed Germination, Vigour, Lipid Peroxidation, and Membrane Integrity in Wheat

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تأثير الإسراع بعملية الشيخوخة في إنبات البذور وقوة النمو وأكسدة اللبتيدات المبدئية ومتانة الغشاء في القمح

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خلاصة: من المعروف أن تأثيرات المعاملات البيئية تؤدي إلى تقصير مدة حيوية البذور. لقد أجريت مقارنة هذه المعاملات باستجابة الصنف لعملية الشيخوخة. حيث تم قياس قدرة البذور على الإنبات، وامتداد غمد البرعم الأولى، وتسرب الشحنات الكهربائية، والأكسدة المبدئية للبتيدات التي تم التخلص منها بواسطة الجذر الجنيني. أدت عمليات الشيخوخة إلى انخفاض معنوي في سرعة إنبات البذور وامتداد البراعم الأولية. كذلك لوحظ أن هناك فرق بين الأصناف عند الإسراع بعملية الشيخوخة. لقد ترافق فقدان الحيوية وتدهور قوة النمو مع الزيادة في أكسدة اللبتيدات وتسرب الشحنات الكهربائية. وثبت من نتائج هذا البحث أن للتأثيرات العامة لشيخوخة البذرة صلة وظيفية بأكسدة اللبتيدات الأولية التي يمهد لها الجذر الجنيني والخلل الذي يصيب غشاء البذرة.

ABSTRACT: The effects of environmental treatments known to rapidly shorten seed viability were compared along with cultivar response to the aging process. Seed germinability, coleoptile expansion, electrolyte leakage, and free radical-mediated lipid peroxidation were measured. Aging treatments produced a rapid and significant reduction in the rates of seed germination and coleoptile extension. Inter-cultivar variation was also observed when the seed was aged rapidly. Loss of viability and declining vigour were associated with an increase in lipid peroxidation and leakage. The common effect of seed aging on the seed integrity is described as a function of free radical-mediated lipid peroxidation and membrane damage.

Keywords: *Triticum aestivum* L., lipid, leakage, seed viability, coleoptile expansion.

Germplasm collection and conservation relies on correct seed storage conditions and the ability of seed lots to be stored for a considerable length of time. Seeds, like any other plant organ, will 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 uniformly 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. Therefore, it is important to understand the basis of seed aging.

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 vigour. Solute leakage accompanies seed imbibition during the process of membrane reorganization following rehydration. The rate of leakage depends upon the degree of cell

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membrane damage and repair in response to aging (Larson, 1968; Simon, 1978). Damage to the organization of cell membranes during seed aging may constitute an important factor in explaining seed deterioration (Priestley and Leopold, 1979; Senaratna *et al.*, 1988; Ferguson *et al.*, 1990).

In the presence of oxygen, aging of seeds is often associated with peroxidation of polyunsaturated fatty acids (Harrington, 1973; Wilson and McDonald, 1986; Hendry, 1993; Thapliyal and Connor, 1997; Pukacka, 1998). Lipid peroxidation has considerable potential to damage membranes and may contribute to the deterioration of stored seeds and the reduced longevity of seeds under natural conditions (Hendry, 1997; Bhattacharjee and Mukherjee, 1998). Other organic molecules subject to oxidative attack include proteins and nucleic acids though the literature on these effects is less extensive (Kalpana and Rao, 1993; Sun and Leopold, 1995). Increased free radical processing systems during accelerated aging have been linked to loss of viability under high moisture regimes in, for example, sunflower (Bailly *et al.*, 1996).

Wheat seeds can be stored at low moisture content and low temperature for many years. Aging is accelerated during exposure to high temperatures and high humidity, a technique widely used in the study of seed storability and deterioration. In numerous region of the world, such as the Arabian Gulf, high temperature is often encountered in combination with high humidity. Under these conditions, damage to seeds might be suspected. In the present study, the effect of accelerated aging on free radical-mediated lipid peroxidation and the consequent effect on electrolyte leakage, seed viability and vigour were studied. Six wheat cultivars were used to examine cultivar differences in tolerance to aging conditions. The aim was to assess the significance of free radical-mediated lipid peroxidation and membrane damage during declining vigour and viability of aged wheat seeds.

Materials and Methods

PLANT MATERIAL: All experiments were performed on six cultivars of wheat (*Triticum aestivum* L.). Seeds of the same year harvest of two ecological zones were obtained from the Department of Crop Sciences, College of Agriculture, Sultan Qaboos University, Oman (cvs Coley, Shwara, Maisani) and the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan (cvs LU-31, LU-26S, NO 8177). All seed material were surface sterilized using 10% sodium hypochlorite (v/v) solution for 10 minutes and rinsed thoroughly in deionized water. They were then stored in aluminum bags at 4°C until use. The initial moisture content was 5-6% as determined by the low constant temperature oven method at 103°C for 17 hours (ISTA, 1993) and expressed on a fresh weight basis.

ACCELERATED AGING TREATMENT: Seeds were artificially aged at 40°C and 100% relative humidity. They were harvested at 3, 6, and 9 days of aging. After each accelerated aging treatment, seed moisture content was determined at 103°C according to ISTA (1993). Following the accelerated aging treatment the seeds were air dried at 25°C until their original weight had been restored and then stored at 4°C.

GERMINATION TEST: Five replicates, each of 20 seeds, were germinated in 9 cm diameter Petri dishes on Whatman No. 1 filter paper. Deionized water (2.5 ml) was provided initially, to moisten the filter paper. Moisture levels were checked daily and topped up as necessary. Percent radicle emergence was recorded at 20°C in the dark (Aquila and Midturi, 1996). Germinated seeds were counted everyday for 10 days, and coleoptile length was recorded in each of twenty seedlings 4 days after radicle emegence.

RATE OF ELECTROLYTE LEAKAGE: Leakage of electrolytes as an indicator of membrane damage was determined from individually weighed seeds in 2 ml of deionized water at 2, 6, 18, and 24 hours, by measuring the conductivity (ms/cm) of the seed-soaked water, using a Jenway PCM3 conductivity meter. Conductivity was measured at 23 ± 1°C for 20 replicates of each cultivar, one seed per replicate.

LIPID PEROXIDATION PRODUCT ESTIMATION: Lipid peroxidation was determined as the concentration of thiobarbituric acid-reactive substances, equated with malonylaldehyde (MDA), as described by Heath and Packer (1986) and modified by 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 from a minimum of five replicates, each replicate consisting of one seed.

FATTY ACID ANALYSIS: The unsaturated fatty acid content was determined as described by Hendry *et al.* (1993). Ground tissue (50 mg) was extracted with a borate buffer pH 9.0, and 3 ml of KOH was added to 1 ml of the extract and incubated in a sealed tube for 6 hours at 80°C. Following centrifugation, the saponified extract was then incubated with lipoxidase 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 a linoleic acid (Sigma Chemicals, L-1876) standard. The samples were replicated five times, each replicate consisting of one seed.

TABLE 1

Effect of accelerated aging on germination of wheat seeds.

Cultivar Name	Germination (%) Time Aged (days)			
	(0)*	(3)	(6)	(9)
Coley	97 ± 2.00	96 ± 1.87	41 ± 6.00	4 ± 1.00
Shwara	79 ± 2.45	5 ± 1.58	0 ± 0.00	0 ± 0.00
Maisani	100± 0.00	96 ± 1.00	52 ± 4.89	6 ± 2.45
LU 31	99 ± 1.00	99 ± 1.00	76 ± 3.67	17 ± 3.39
LU 26-S	100± 0.00	97 ± 1.22	69 ± 3.67	9 ± 2.91
NO 8177	100± 0.00	98 ± 1.22	73 ± 3.00	11 ± 2.91

* Means of 5 replicates ± SEM.

STATISTICAL ANALYSIS : ANOVA were calcualted by two B factor with 5-20 replications. All statistical analyses were performed using Microsoft Excel.

Results

SEED AGING, VIABILITY AND VIGOR: The results of seed viability and coleoptile expansion determination are summarized in Table 1 and 2. All seed lots showed high initial germination percentage (97-100%) except cv Shwara (79%). At day three, germination in cvs Maisani and Coley had declined from 100% to about 96% while coleoptile expansion was unchanged (Table 2). Shwara showed a faster decline in germination (to 5%) coleoptile expansion at three days following accelerated aging treatment. As the aging process advanced, both the final percentage of germination and vigor (measured as coleoptile growth) declined faster, particularly after nine days aging. Inter-cultivar variation was observed in both germination and coleoptile expansion. Wheat seeds exposed to a stress environment demonstrated a significant effect of aging treatment on seed viability and vigor ($P < 0.001$). The effect of extended aging on all wheat cultivars over nine days at high temperature and high humidity was to reduce final germination percentage to low values (0 to 4%, Table 1) and to reduce coleoptile expansion 3-fold (Table 2).

LIPID PEROXIDATION PRODUCT: Under prolonged aging conditions, lipid peroxidation increased in seeds of four of the wheat cultivars over the nine-days treatment (Table 3). The results from cvs LU-26S and NO- 8177 revealed little increase in lipid peroxidation at 6 days aging and no consistent trend in increasing lipid peroxidation. In contrast, wheat seeds of the other four cultivars subjected to high temperature and high humidity over 9 days indicated a highly significant increase (>100%) in lipid peroxidation accumulation (Table 3). Overall, the effect of aging treatment on lipid peroxidation products was significant ($P < 0.001$).

TABLE 2

Effect of accelerated aging on wheat seed vigour as measured by coleoptile expansion.

Cultivar	Coleoptile Expansion (mm) Time Aged (days)			
	(0)*	(3)	(6)	(9)
Coley	13.0± 1.40	13.3 ± 1.4	10.9 ± 1.64	2.05 ± 0.99
Shwara	11.3 ± 0.98	1.3 ± 0.55	**	**
Maisani	16.1 ± 1.83	15.9 ± 1.40	14.3 ± 1.96	2.55 ± 1.04
LU 31	12.8 ± 1.00	13.1 ± 1.31	10.8 ± 1.60	7.95 ± 1.24
LU 26-S	15.2 ± 1.10	10.2 ± 1.13	10.7 ± 1.57	4.35 ± 1.3
NO 8177	16.5 ± 0.53	12.4 ± 1.04	8.9 ± 1.06	6.20 ± 1.5

* Means coleoptile expansion (mm) of 5 replicates ± SEM.
**No germination.

UNSATURATED FATTY ACID CONTENT: The results revealed no significant differences in fatty acid content over the course of aging treatment for all of the wheat varieties listed. There was little decrease in unsaturated fatty acid content but no significant trend was observed in the loss or increase of unsaturated fatty acid content of treated or untreated seed material. Generally, it is thought that free radical-mediated damage can reduce the fatty acid content. Our results did not show any significant loss of unsaturated fatty acids. This trend in wheat seeds could be due to wheat seeds lacking in high unsaturated fatty acid contents.

ELECTROLYTE LEAKAGE: Intra-cultivar response to electrolyte leakage (measured as conductivity) was significant ($P < 0.001$) and consistent over the 9-days aging treatment. All three Omani cultivars (Coley, Shwara, and Maisani) exhibited severe damage to the seed tissues, as revealed by the leakage. The effect of aging and differences in conductivity are summarized in Table 4. Positive correlation was also observed between incubation period and solute leakage for all cultivars.

TABLE 3

Effect of accelerated aging on lipid peroxidation [TBA, thiobarbituric acid-reactive products (wt)] in wheat seeds.

Cultivar Name	TBA (mm/g seed) Time Aged (days)			
	(0)*	(3)	(6)	(9)
Coley	0.63 ± 0.18	0.78 ± 0.14	1.19 ± 0.09	1.59 ± 0.22
Shwara	0.61 ± 0.06	0.79 ± 0.26	1.31 ± 0.32	1.57 ± 0.36
Maisani	0.56 ± 0.09	0.99 ± 0.04	1.48 ± 0.20	1.68 ± 0.36
LU 31	0.32 ± 0.04	0.99 ± 0.20	0.80 ± 0.12	1.37 ± 0.09
LU 26-S	0.53 ± 0.08	0.63 ± 0.08	0.90 ± 0.12	0.65 ± 0.05
NO 8177	0.43 ± 0.05	0.69 ± 0.04	0.72 ± 0.02	0.85 ± 0.13

* Mean of 5 replicates ± SEM.

TABLE 4

The effect of accelerated aging on leachate conductivity.

Cultivar Name	Timed Aged (days)	Leachate Conductivity [(US/cm)/100 mg seed weight]															
		(0)*				(3)				(6)				(9)			
	Incubation Time (h)	4	8	16	24	4	8	16	24	4	8	16	24	4	8	16	24
Coley		134 ± 22	174 ± 32	211 ± 39	229 ± 40	214 ± 17	248 ± 21	295 ± 23	356 ± 26	112 ± 18	199 ± 37	323 ± 63	417 ± 88	193 ± 32	324 ± 60	534 ± 83	778 ± 116
Shwara		119 ± 12	170 ± 25	226 ± 39	293 ± 61	76 ± 13	146 ± 27	260 ± 54	322 ± 69	148 ± 11	202 ± 16	262 ± 22	354 ± 35	276 ± 66	488 ± 121	1022 ± 211	1462 ± 283
Maisani		116 ± 17	165 ± 20	212 ± 27	268 ± 31	205 ± 10	239 ± 12	281.9 ± 15	363 ± 27	85 ± 17	150 ± 26	289 ± 51	367 ± 66	201 ± 50	302 ± 66	656 ± 91	985 ± 132
LU 31		122 ± 8	145 ± 9	167 ± 10	191 ± 10	62 ± 5	105 ± 8	157 ± 18	184 ± 25	169 ± 11	198 ± 10	236 ± 9	292 ± 10	89 ± 7	136 ± 11	241 ± 28	364 ± 44
LU 26-S		114 ± 6	131 ± 6	150 ± 7	168 ± 8	65 ± 5	111 ± 11	167 ± 20	196 ± 24	181 ± 9	220 ± 9	241 ± 9	273 ± 9	101 ± 17	199 ± 35	322 ± 48	453 ± 62
NO 8177		92 ± 6	109 ± 7	128 ± 6	159 ± 7	50 ± 5	81 ± 7	110 ± 11	126 ± 15	129 ± 5	168 ± 5	200 ± 6	228 ± 8	63 ± 6	102 ± 9	198 ± 22	293 ± 37

*Mean of 20 single seed replicates ± SEM.

Discussion

Seed germination and coleoptile expansion of wheat under accelerated aging treatment declined over 9 days. Subjecting seeds of all cultivars to high temperature and high humidity broadly reproduced these measures of declined viability. However, the significant increase in lipid peroxidation response of rapidly aged seeds was less apparent in two Pakistani bred cultivars, this apparent anomaly may reflect the intra-cultivar variation on lipid peroxidation.

The results also demonstrated that lipid peroxidation and electrolyte leakage in wheat seeds were both significantly and positively correlated with the decline in germinability and coleoptile expansion in rapidly aged seeds. Two features of this phenomenon are noteworthy. Firstly, lipid peroxidation accumulation was significantly promoted by the accelerated aging treatment. It appears that age-enhanced lipid peroxidation generation has not been reported in nonfat seeds, such as, wheat. No attempt was made to dissect the axis from the endosperm; the axis itself would be expected to generate peroxidized lipids (as previously observed by Hendry *et al.*, 1992) though quantitatively, in wheat seed, this would be a minor source compared with the endosperm.

The apparent correlation between loss of viability and activity of free radical-linked processes in rapidly aged seeds reported here strengthens other evidence that loss of viability in seeds subjected to high temperature and high humidity is probably closely linked to the effects of oxidative damage (Harman and Mattick, 1976 and Hendry, 1993). It has been previously shown that floral structures respond differently to oxidative stress, for example, in *Quercus robur,* correlation between viability and free radical events can be highly significant when the comparisons are made with embryonic axes but fail when the cotyledons are included (Hendry *et al.*, 1993). Another study on soybean seeds revealed a positive relationship between free radical activity in the seed testa and loss of viability in aged seeds (Khan *et al.*, 1996). The biochemical changes in peanut seed membranes, aged either naturally or artificially, was detected best in the embryonic axis, either through changes in leakage of electrolytes or in the malondialhyde (MDA) content (Jeng and Sung, 1994; Pérez and Argü ello, 1995). This raises further questions about the molecular relations in seed parts and their response to stress. The results presented here showed that there were no (or little) changes in the relative proportion of unsaturated fatty acids in all cultivars of wheat seeds. Similar results were found by Pearce and Abdel Samad (1980) in intact peanut seeds and by Senaratna *et al.* (1988) and Priestley and Leopold (1979) in soybean.

Although many workers have described a close relationship between free radical-mediated damage and loss of viability in seeds of many species, there is some disagreement in the literature about this correlation (Linn and Pearce, 1990; Kalpana and Rao, 1994). In our study it has been demonstrated that in at least four out of six cultivars of wheat there was a close linkage between lipid peroxidation, membrane damage (electrolyte leakage) and loss of viability. In these investigations the endosperm and axis in wheat cultivar seeds was not analyzed. This will be investigated in the future together with the functional significance of seed floral parts, free radical-mediated events and loss of viability.

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