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Studies on physiological and biochemical changes in relation to seed viability in aged onion seeds

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ABSTRACT

Rapid loss in viability of onion seeds during seed storage is a major problem. Not much information concerning the physiological and biochemical changes is available. In the present investigations, seeds were aged artificially by exposure to 45^{0}C +75% RH for a period of fifteen days. Samples were collected at three day intervals and physiological and biochemical changes in the aged seeds were compared to those in fresh seeds. Results revealed that ageing affected seed viability and vigour significantly and this effect was more pronounced with increase in duration of exposure to artificial ageing. Marked reduction in germination to an extent of 4, 16 and 75% was noticed in three, six and nine day artificially aged (DAA) seeds, respectively, when compared to fresh seeds. Further increase in ageing duration to twelve and fifteen days resulted in total loss of germination. Increase in ageing duration decreased α amylase and dehydrogenase activities but increased peroxidase activity up to nine days of ageing. Lipid peroxidation increased consistently with increase in duration of ageing. At 15 DAA, 26.2% increase in malondialdehyde content over the control was observed. SDS PAGE protein profile and esterase zymograms of aged seeds showed alteration in banding pattern when compared to that of fresh seeds.

Key words: Onion, accelerated ageing, protein profiles, enzymes

INTRODUCTION

Onion (*Allium cepa* L.) seeds lose vigour and viability at faster rates than seeds of most other vegetables (Choudhari and Basu, 1988). The relation between temperature, moisture content and viability period is similar under the conditions used in hot air drying or at long-term storage at sub zero temperature. In both the cases, the pattern of deterioration preceding death is also the same, whether the seed survives for seconds or decades (Roberts, 1981). Artificial ageing techniques are employed to elucidate the mechanisms of deterioration during storage because physiological changes in the seed occurring during artificial and natural ageing are thought to be similar (Chen *et al*, 1999). Hence, an experiment was conducted to examine changes due to artificial ageing in relation to seed viability and vigour in onion cv. Arka Bindu.

MATERIAL AND METHODS

Ageing treatment

Fresh seeds of cv.Arka Bindu were aged artificially up to 15 days at 45^0 C +75 % RH as per ISTA (1999) standard procedure. Samples were collected every third day. After ageing, the seeds of all the treatments were dried back to the original moisture content for further studies.

Germination and growth parameters

Four replicates of fifty seeds of each sample, planted in moistened roll paper towels, were germinated in a growth chamber at 20^{0}C under dark. Observations were recorded on germination (ISTA, 1999), germination rate (GR), germination speed (GS) and standard germination (StG). These parameters were computed, as given below, using the formula given by Mugnisjah and Nakamura (1986).

GR=100/no. planted (normal seedlings day 6/ 6+normal seedlings day 12/12)

GS=100/no. planted (normal seedlings day 1/ 1+normal seedlings day 2/2.... day 12/12)

StG=100/ no. planted (normal seedlings day 6 + normal seedlings day 12)

Lipid peroxidation, electrical conductivity and total soluble sugars

Lipid peroxidation in fresh and aged seeds was studied by TBA colour reaction as outlined by Bernheim *et al* (1948). The methodology followed for studying electrical conductivity (EC) was that described by Rudrapal and Basu (1979). The amount of sugars in seed leachate was determined using the method of Dubois *et al* (1951). Leaching of sugar was expressed as glucose equivalent per ml of leachate.

Enzyme activity

α-amylase and peroxidase activities were estimated as per Sadasivam and Manickam (1996). Dehydrogenase activity was estimated as per the procedure outlined by Agarwal and Dadlani (1987).

Protein electrophoresis

Seeds of each sample (0.5g) were crushed to fine powder using a pestle and mortar and the flour was defatted by soaking in a mixture of chloroform, methanol and acetone in 2:1:1 ratio for 3 h. The supernatant was poured out and the process was repeated three times. The samples were dried at room temperature and a sub-sample of 0.1g was homogenized in an eppendorf tube by adding 400 µl of the extraction buffer (Tris Glycine, pH 8.3). Initially, the tubes were left at room temperature for 1 h and then shifted to 4°C until the next day. The samples were centrifuged at 10,000 X g for 10 min. and the supernatant was collected. To 10 µl of this supernatant, 10 µl of working sample buffer was added and boiled at 100°C for 5 min. Protein content in the samples was quantified as per the procedure outlined by Lowry *et al* (1951). Protein characterization of seeds was carried out using SDS PAGE (Laemmli, 1970).

Isozyme analysis

Seeds of each sample (0.1g) were crushed to a fine powder using liquid nitrogen in pre- chilled pestle and mortar and the contents were transferred to eppendorf tubes containing 75µl of 0.1M Tris HCl, pH 7.5. The contents were homogenized using micropestle and left overnight at 10°C for extraction. Samples were centrifuged at 4°C at 10000 rpm and subjected to alkaline PAGE by loading 25µl in each well. The gel was run at 15°C for a period of six hours under a constant current of 100 V until samples crossed the stacking gel and later at 250 V. The gels were stained immediately in a staining solution (50 mg Fast blue RR salt in 2 ml of 50 mg Naphthyl acetate in 50 % acetone added to 100 ml of 0.5 M sodium phosphate buffer, pH 6.2) for one hour. Data were statistically analyzed using Completely Randomized Block Design as outlined by Panse and Sukhatme (1978).

RESULTS AND DISCUSSION

Results revealed that ageing affected seed viability and vigour significantly and this effect was more pronounced with increase in duration of ageing. Marked reduction in germination of 4, 16 and 75% was noticed in three, six and nine day artificially aged (DAA) seeds, respectively, compared to fresh seeds. Further increase in ageing duration to 12 and15 days resulted in total loss of germination. Ageing also showed significant deleterious effects on other quality traits. Three, six, nine, twelve and fifteen days of artificial ageing resulted in significant reductions in speed of germination, rate of germination, total seedling dry weight and seedling vigour index (Table 1). Reduction in seedling vigour index was 14.7, 42.9, 91.2, 100 and 100% in comparison to fresh seeds (Table 1). Similar loss in seed viability and vigour when subjected to artificial ageing has also been reported in various crops earlier (McDonald, 1999).

Besides physiological changes, many biochemical changes were noticed due to ageing. Wide differences in membrane permeability between aged and fresh seeds were noted. Except at three DAA, there was progressively higher

Table 1. Effect of duration of artificial ageing on onion seed vigour-physiological parameters

S.No.	Treatment	% Germination*	Total Seedling Length (cm)	Total Dry Weight (mg)	Shoot Vigour Index	Germination Speed	Germination Rate	Standard Germination
1	Fresh	100(89.8)	15.80	1.216	1575	192.0	24.30	196
2	3DAA	96(79.9)	14.00	1.296	1343	145.0	23.30	188
3	6DAA	84(67.1)	10.70	1.320	900	120.0	20.50	165
4	9DAA	25(29.9)	5.50	0.600	139	19.7	4.08	37
5	12DAA	0	0	0	0	0	0	0
6	15DAA	0	0	0	0	0	0	0
	SEd+	2.56	0.81	0.017	75.5	2.43	0.34	3.23
	CD@5%	5.38	1.69	0.035	158.7	5.10	0.72	6.79

*Values in parentheses represent angular transformed values

Table 2. Effect of duration of artificial ageing on onion seed vigour-biochemical parameters

S.No.	Treatment	Electrical Conductivity (µs/cm)	Dehydrogenase activity (% change over control)	Malondialdehyde content (% change over control)	Total Soluble Sugars (µg/ml)	Amylase activity (ug maltose produced/ml/min.)	Peroxidase activity (Change in OD/ min.)
1	Fresh	70.4	100.0	100.0	127.5	95.1	0.009
2	3DAA	70.3	90.4	103.9	137.5	97.1	0.018
3	6DAA	74.4	62.3	114.5	168.5	82.4	0.022
4	9DAA	83.8	44.3	119.4	470.0	62.4	0.024
5	12DAA	91.8	35.6	121.4	490.0	38.5	0.023
6	15DAA	101.8	34.9	126.2	530.0	37.6	0.023
	SEd±	1.92	1.55	0.85	17.4	2.10	0.002
	CD@5%	4.03	3.25	1.80	36.7	4.41	0.005

leaching of electrolytes and sugars with increasing duration of ageing. Increase in ageing duration from six to fifteen days increased the EC values by 5.7 to 44.6% and sugar levels 1.07 to 4.16 times when compared to fresh seeds. These results clearly demonstrate that the aged seeds lost integrity of cell membranes. Lipid peroxidation, estimated by production of malondialdehyde, increased consistently with increase in duration of ageing. Increase in malondialdehyde content was noticed to the tune of 3.9, 14.5, 19.4, 21.4 and 26.2% in comparison to fresh seeds. Pammenter *et al* (1974) also noticed more lipid peroxides in aged seeds than in the corresponding controls. These results, when taken along with reduction in germination speed and seedling growth, indicate that increase in lipid peroxidation may be partly responsible for reduced onion seed vigour at supra-optimal temperature of 45^0 C when coupled with high humidity (Table 2).

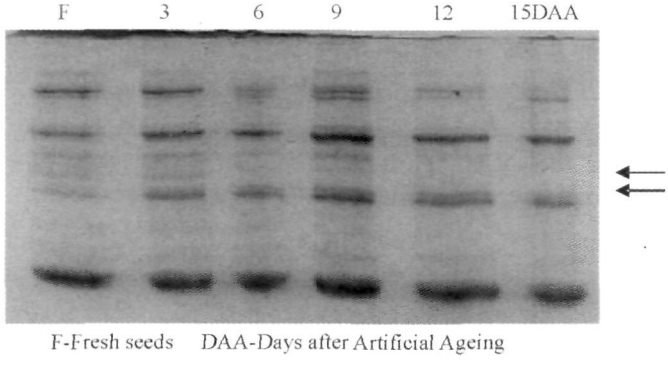

Plate 1. SDS-PAGE Tris soluble protein profile of fresh and aged onion seeds showing alteration in banding pattern

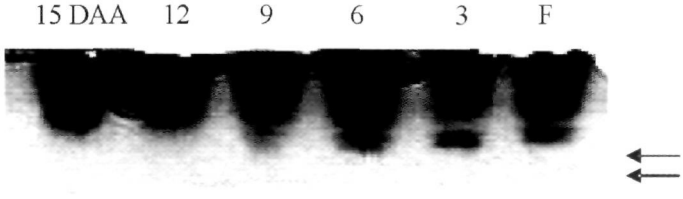

Plate 2. Esterase isozyme profile of fresh and aged onion seeds showing alteration in banding pattern

In the present investigation, activities of α-amylase, dehydrogenase and peroxidase were also examined in relation to seed deterioration. Although there was no significant decrease in α- amylase activity at three DAA, a sharp decline in activity of 12.7 to 57.5 µg with increase in ageing duration from six to fifteen days was observed when compared with fresh seeds. It was further noted that following ageing, the activity of dehydrogenase declined more rapidly than in the fresh seeds, where as peroxidase activity increased significantly until nine DAA and thereafter, remained unaltered (Table 2). The sudden increase in peroxidase activity at the beginning of ageing may be due to activation of antioxidative mechanism to suppress the high levels of peroxides that were produced under supra-optimal ageing conditions. Age associated reduction in activity of key enzymes in seeds has been reported (Wilson and McDonald, 1986). Thus, reduction in enzyme activity may be a reflection of changes in protein synthesis (Nandi *et al,* 1995).

SDS PAGE protein profile of accelerated aged seeds showed an alteration in twelve and fifteen DAA where the characteristic protein pattern was lost (Plate 1). The loss of subunits in six, nine and twelve DAA seeds indicate that protein degradation occurred due to prolonged ageing. Nautiyal *et al* (1985) reported that in *Shorea robusta* seeds, proteins with higher electrophoresis mobility deteriorated first during ageing. Analysis of esterase isozymes also revealed alteration in aged seeds. Results obtained for twelve and fifteen DAA seeds, particularly, showed no resolution into discrete bands (Plate 2). Aung and McDonald (1995) also noticed a decrease in some esterase isozymes in peanuts during storage, due to ageing. Based on these findings, it is concluded that seed ageing in onion is the

result of slowdown processes in synthesis. This may be attributed to degradation of proteins, inactivation of enzymes and higher lipid peroxidation in onion seeds during ageing.

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