INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2004/06–4–708–711
http://www.ijab.org

Loss of Vigour and Viability in Aged Onion (*Allium cepa* L.) Seeds

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ABSTRACT

Onion (*Allium cepa* L.) seeds exhibit very short life and loose their viability within 1-2 years. Accelerated ageing studies were conducted on indigenous onion cv. "Desi Red" to investigate the vigour and viability in relation to germinability and seed membrane damage. Seed lost their germinability, vigour and viability progressively with ageing treatment. Germination percentage reduced up to 43%, radicle length 1.7 cm and germination speed 6.68 (GSI). Time to get 50% germination and electrolyte leakage increased significantly ($p<0.05$) with the ageing time and treatment. While the leaching from viable seeds (control) was negligible. The loss of viability in seeds after ageing appeared related to increased membrane destruction (loss of membrane integrity). This membrane integrity loss may be responsible for the decreased germinability, vigour and ultimately viability.

Key Words: Onion (*Allium cepa* L.); Germinability; Vigour; Membrane destruction; Viability

INTRODUCTION

Many farmers know from experience that seed of same lots may emerge from the soil quite differently, resulting in erratic field stands, replanting (on some occasions), or both. Vigorous seeds will produce excellent emergence and stands in proper soil environment. It can improve the chances for satisfactory emergence. Advanced stages of seed deterioration are evidenced by visible symptoms during germination and seedling growth. However, these are preceded by more subtle physiological changes whose symptoms can be detected only by sophisticated measurement techniques. Quality seed is defined as varietally pure with a high germination percentage, free from disease and disease causing organisms, and with a proper moisture content and weight. Vigour is often implied when discussing seed quality and most growers have to use the terms quality and vigour interchangeably. Seed vigour comprises those properties, which determine the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions (ASPB, 2003). Seed vigour is generally related to yield in vegetables.

Studies have shown that this vigour test functions well in forecasting field emergence and stand establishment in a wide range of crop species. In general, when seeds are planted under stressful field conditions, provides higher correlations with field emergence than does standard germination (Pandey *et al.*, 1990). Subsequent studies have verified that accuracy of this test in predicting the life span of a number of different species under a range of storage conditions.

The performance capabilities of many seeds deteriorate during prolonged storage, but the rate of deterioration varies greatly among species (Robert, 1989). High temperature, ambient relative humidity, and seed moisture content are the main factors influencing seed storage capability (Abdul-Baki, 1980). The degree of cell membrane damage in response to ageing can be measured in terms of rate of seed electrolyte leakage (Larson, 1968; Simon, 1978; Khan *et al.*, 2003). Damage to the organization of cell membranes during seed ageing may constitute an important factor in explaining seed deterioration (Senaratna *et al.*, 1988; Ferguson *et al.*, 1990).

In order to obtain information on seed vigour in a timely manner, the accelerated ageing technique is commonly used, through which changes in the seed at the cellular level during long-term storage can be simulated within a comparatively short period of time by exposing seeds to increased temperatures (40-45^{o}C) and a high relative humidity (Abdul-Baki, 1969; Delouche & Baskin, 1973). The accelerated aging test is rapid, inexpensive, simple and useful for many species; it can be used for individual seed evaluation and requires no additional training for correct evaluation. Solute leakage accompanies seed imbibition during the process of membrane reorganization following re-hydration. The rate of leakage depends on the degree of cell membrane damage 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; Khan *et al.*, 2003).

Onions are one of the oldest vegetables in continuous cultivation dating back to at least 4000 BC. Onion seeds are difficult to store for longer periods as they may loose viability and vigour very quickly. Studies were initiated to explore the vigour, viability and seed membrane integrity at high temperature 45^{o}C and high relative humidity in relation to seed germinability, time to get 50% germination and solute leakage in onion (*Allium cepa* L.) seeds.

MATERIALS AND METHODS

Plant material and accelerated ageing treatment. These studies were carried out on equal sized onion (*Allium cepa* L.) cv. "Desi Red" seeds. The seeds were obtained from collections of the Vegetable Experimental Area, Institute of Horticultural Sciences, and University of Agriculture Faisalabad. Seed quality was tested according to the rules of AOSA (1983; 1991). Seeds were surface sterilized with 5% sodium hypochlorite (NaOCl) solution for 5 minutes and rinsed with double distilled autoclaved water (Khan *et al.*, 2003). The seeds were dried at 25^{o}C$\underline{+}$1 for 24 h in the laboratory. The four samples of seed from each treatment were oven dried at 103^{o}C for 17 hours (ISTA, 1993) to determine seed moisture content.

Seeds were subjected to accelerated ageing treatment at 45^{o}C and 100% relative humidity for 0, 3, 5 and 7 days in a controlled chamber (Type 1894, VINDON, England). For germination and vigour tests five replications of twenty seeds each were taken out at predetermined intervals, following the ageing treatments, and the moisture content was recorded as described by Khan *et al*. (2003). The seeds were air dried at 25^{o}C$\underline{+}$1 in laboratory until their original moisture content was restored.

Germination and vigour tests. Seed germination tests were carried out according to ISTA (1993), and performed on five replicates of 25 seeds each. The seeds were incubated on top of moist Whatman No. 1 double filter papers in the 9 cm petri dishes at 23^{o}C$\underline{+}$1 in growth chamber. Water requirements were checked daily and topped-up according to necessity. The seed germination percentage and radicle length were recorded every 24 h time interval. Germination speed index (GSI) was measured according to the AOSA (1983) on vigour as described below:

$$GSI = \frac{number\ of\ normal\ seedlings}{days\ of\ first\ count} + \frac{number\ of\ normal\ seedlings}{days\ of\ final\ count}$$

Time to get 50% germination. Time to get 50% germination was worked out by graph (Fig. 4) as described by Khan *et al.* (2003).

Seed solute leakage. Leakage of electrolytes (an indicator of membrane damage) on individual seed basis in 1 mL of deionized water was determined after 3, 6, 12, 18 and 24 h by measuring the conductivity (µS cm^{-1}) of seed soak water, using 'Nikon' digital EC meter. Conductivity was measured at 25^{o}C$\underline{+}$1 using 20 seeds each single seed replicate.

RESULTS

Seed viability. The results of seed viability (germination %age) are presented in Fig. 1. Accelerated ageing showed significant ($p<0.05$) reduction in the germinability of seeds while control seeds showed high germination percentage (100%). The seed germinability was reduced with the passage of ageing time. It was 91, 67 and 57% for 3, 5, and 7 days of ageing, respectively (Fig. 1).

Radicle length and germination speed (Vigour Tests). Radicle length of germinated seeds was measured after every 24 h. After 48 h of sowing, radicle length was greatly reduced with the passage of ageing environment. Largest average radicle length was for control i.e., 2.86 cm and it was maximum reduced with the ageing time up to 1.2 cm after 7 days of ageing treatment. While the average radicle lengths of 3 days and 5 days ageing were 2.1 cm and 1.8 cm, respectively (Fig. 2).

Germination speed is a direct measure of seed vigour. It may be defined as "number of germinated seeds per unit day". Accelerated ageing also decreased the germination speed of seed material. The fastest germination speed was observed in control (16.8) compared to the lowest (10.12) at 7 days of ageing treatment (Fig. 3). Significant differences were observed in all treatments. For example the germination speed of control was maximum (16.8) followed by 3, 5 and 7 days of ageing (15.2, 12.15 & 10.12, respectively). Results demonstrated that ageing slowed down the process of germination.

Time to complete 50% germination. The time to complete 50% germination of seeds was directly proportional to the time of ageing (Fig. 4). The value of T_{50} increased with the process of accelerated ageing, indicating that it took more time, 4 days for 7 days treatment compared to non-aged seeds i.e., approximately 2.4 days to germinate 50% of total due to ageing.

Electrolyte leakage (membrane damage). Seed electrolyte leakage was increased with the passage of ageing and soaking time, indicating that seed constituents were leaking. It was maximum 1390 µScm^{-1} for 7 days treatment after 24 h while 491 µScm^{-1} for control (Fig. 5). There was a positive correlation between the germination percentage and solute leakage (Fig. 6).

DISCUSSION

Accelerated ageing treatment may considerably effect the seed viability and vigour in onion and pea seeds (Diojode, 1985; Khan *et al*., 2003). Thus storage of seeds under such adverse conditions (100 R.H & 45^{o}C) results in the production of 'aged' seeds. These aged seeds exhibit a variety of symptoms ranging from reduced germinability (sometimes to zero germination) to more or less full viability (no obvious decline in germinability) but with abnormal development of the seedling (i.e., poor vigour).

Fig. 1. Effect of ageing on germination percentage in onion seeds

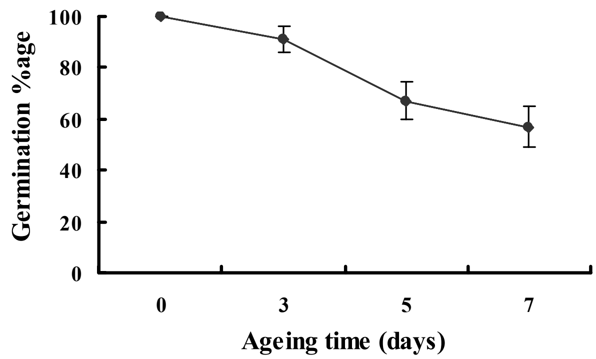


Fig. 2. Effect of ageing on radicle length in onion seedlings

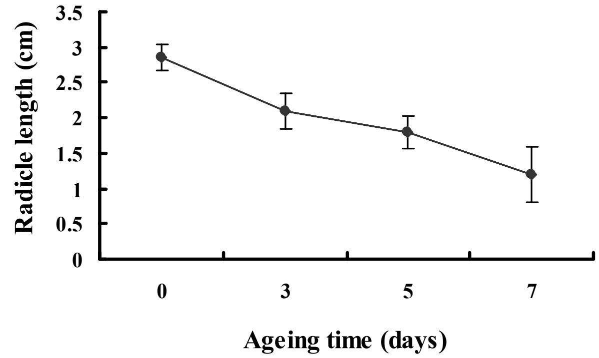


Fig. 3. Effect of ageing on germination speed in onion seed

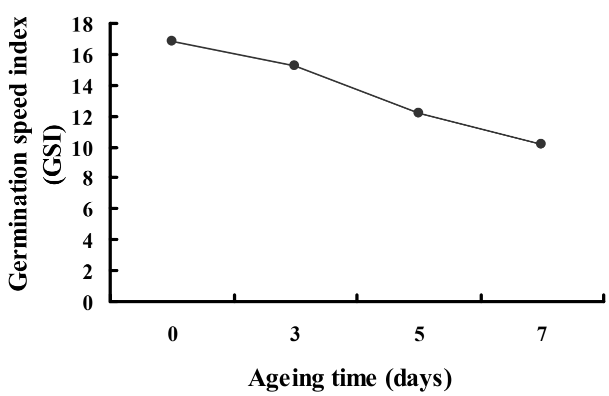


Fig. 4. Effect of ageing on time to get 50% germination

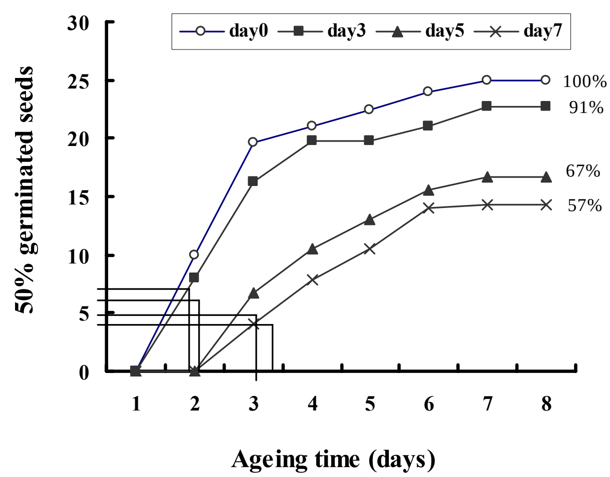


Fig. 5. Effect of ageing on leachate conductivity (EC) in onion seeds

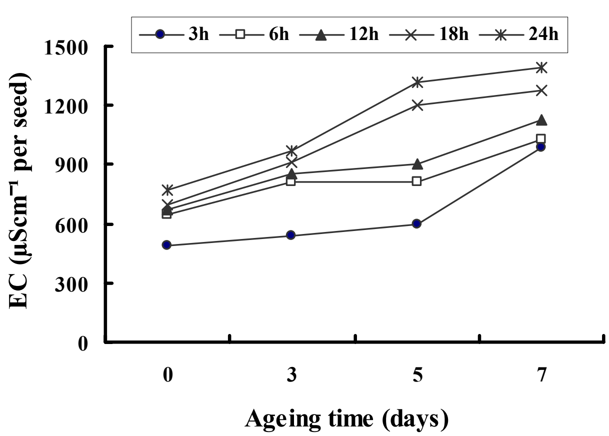
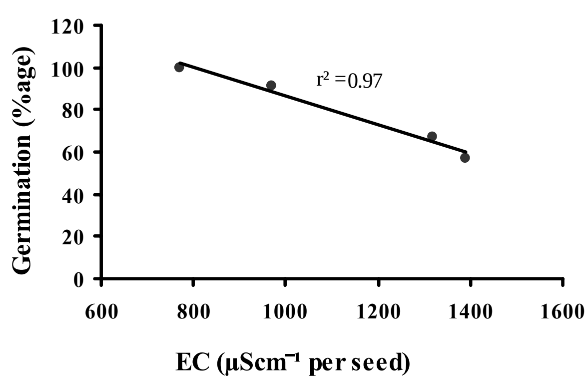


Fig. 6. Relationship between electrolyte leakage (measured as EC) and seed germination in onion seeds



Wheat seeds aged acceleratedly at 40°C and 100% R.H. for 1-9 days decreased the germination percentage and seedling growth after 9 days of ageing Madan *et al.* (1989). Similarly sunflower seeds lost their viability when subjected to accelerated ageing (Gidrol *et al*., 1990).

Radicle length and germination speed of the onion seeds were significantly reduced with the passage of ageing period. Therefore it may be suggested that severe ageing resulted in the delayed germination however, when ageing is progressed beyond a critical period, there is a carryover effect resulting in much reduced germination and radicle length (Khan *et al.,* 2003). Our findings are in accordance with Thornton and Powell (1992), who reported the same response for *Brassica oleracea* L. seeds when subjected to accelerated ageing treatment. Time taken by onion seeds to complete 50% germination was directly proportional to the ageing time (Harrington, 1973; Khan *et al.*, 2003).

Ageing and soaking time greatly affect the electrolyte leakage in onion seeds. It can be appreciated, therefore, that the deteriorated seeds that show such diversity in their final germination response may be due to the metabolic lesions affecting viability and vigour. Results demonstrated that the electrical conductivity of onion seed is related to its membrane disintegration and finally loss of viability. Osborne (1980) summarized the transition from viable to non-viable state in dry seeds. In dry (orthodox) seeds, central feature were the damage to membrane systems in all parts of the cell and differential stability of the different enzyme proteins. Cooperative enzymes of a metabolic pathway are not only linked together within organelles (e.g. respiratory enzymes in mitochondria) but often they are intimately associated with membrane structures. Thus disruption of membranes because of ageing could lead to diverse metabolic changes, all of which contribute to different extents to seed deterioration and loss of viability and vigour. Loss of membrane integrity in deteriorated seeds is suggested by the observation that on imbibition more substances leak into the medium from such seeds than from viable ones.

Delayed seedling emergence is among the first noticeable symptoms, followed by a slower rate of seedling growth, development and decreased germination. Harrington (1973) made a strong case for the idea that the breakdown of various germination triggering mechanisms also causes seeds deterioration. It has been noted that the concentrations of various growth hormones are adversely affected by ageing; for example application of gibberelic acid improved germination and vigour in partially aged celery seeds (Harrington, 1973).

CONCLUSION

It is concluded that germination percentage, radicle length and germination speed were decreased significantly with ageing time, while time to get 50% germination and seed solute leakage was increased. The observed changes in germination capacity and seed membrane damage support the concept that deterioration of cellular membranes is implicated in ageing and loss of viability in seeds. The higher solute leakage indicated the amount of membrane lesions that has developed during accelerated ageing. The results obtained here suggest that seed membrane lesions may play a considerable role in seed vigour and viability loss.

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(Received 10 May 2004; Accepted 28 June 2004)