EFFECT OF AGEING ON VIABILITY, VIGOUR AND CHROMOSOMAL DAMAGE IN PEA (Pisum sativum L.) SEEDS

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The mechanism of loss of viability and vigour in seeds is by no means resolved. In this study seeds were exposed to high temperature and high humidity, a technique widely used to study seed storability and deterioration. The effect of environmental treatments on seed germinability, seedling vigour, electrolyte leakage and chromosomal aberration in pea seeds was compared. Ageing treatment showed rapid and significant reductions in rate of seed germination and seedling vigour. Loss of viability and declining vigour were associated with the increased leakage and chromosomal aberrations. The common effect of seed ageing on the seeds integrity is described as function of free radical mediated membrane and chromosomal damage.

Key Words: Peas, vigour, leakage, ageing, chromosomal damage

INTRODUCTION

Agricultural productivity mainly depends on quality of seeds planted. Until now, seed quality has been measured in terms of purity and germination ability. Accelerated seed ageing technique is a widely used tool to test the seed quality. This ageing test of seed vigour can give better indications of probable field emergence for vegetable crop seeds than germination and growth tests (Pandey et al. 1990). In this test, "Seeds are subjected to high relative humidity and temperature treatments for different time intervals." In seed ageing damage to cellular membranes, decrease in mitochondrial dehydrogenases activities, chromosomal aberration and DNA degradation increases (Parrish and Leopold, 1978). Genetic damage may accumulate until the embryos are unable to germinate and grow. Abdalla and Roberts (1968) suggested that the number of chromosomal aberrations seen in the first cell divisions occurring during germination may be taken as a measure of the damage accumulated during the storage period. It is well known that loss in viability during seed storage is associated with an increase in chromosomal aberrations in the surviving seeds. In late 1930's it was realized that the accumulation of chromosomal aberrations is a function of time, temperature and moisture content during seed storage, and that increasing the temperature or the moisture content results in an increase in the rate of accumulation of chromosomal aberrations. Roberts et al. (1967) and Abdalla and Roberts (1968) comprehensively discussed the topic and reported that mostly accumulation of chromosomal aberrations is correlated with loss in seed viability, regardless of pace of loosening it.

This study was undertaken on pea (Pisum sativum) cv. Meteor seeds to investigate the effect of accelerated ageing on loss of viability and vigour in relation to chromosomal aberrations and changes in membranes.

MATERIALS AND METHODS

Plant material
All experiments were performed on round shaped pea (Pisum sativum) cv. Meteor seeds. The seeds were obtained from the collections of the Department of Horticulture, University of Agriculture Faisalabad. Seeds were surface sterilized with 5% sodium hypochlorite solution for 10 minutes and rinsed with double distilled autoclaved water (Mumford and Grout, 1979). The seeds were dried at 25°C for 12 hours in the laboratory. The 4 samples of seed from each treatment were oven dried at 103°C for 17 hours (ISTA, 1993) to determine seed moisture content.

Accelerated ageing treatment
Seeds were subjected to accelerated ageing at 45°C and 100% relative humidity for 3, 5 and 7 days in a controlled chamber (Plant Growth Chamber, VINDON, Japan) as described Al-Maskri et al.(2001). Following the accelerated ageing treatment, seeds were removed according to predetermined intervals and the moisture content was recorded. The seeds were air dried at 25°C under shade 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 1 double filter papers in the 9 cm Petri dishes at 23°C growth chamber under white fluorescent light. Water availability was checked daily and topped-up as necessary. The seed germination percentage and radical length were recorded at 23°C after every 48 hours time interval. The seed germination speed at 23°C after every 24 hours was
calculated by the following formula derived by Kotowski (1926).

\[ \text{Germination speed} = \frac{\sum n}{\sum (n \times D_n)} \times 100 \]

Where \( n \) = number of seedlings germinated on day \( D_n \).

\( D_n \) = number of days from sowing to corresponding to \( n \).

and time taken for 50% germination was worked out by the graph.

**Leakage of electrolytes**

Twenty replications of single seed from each treatment were soaked in 1ml deionized water for 24 hours at 25°C. The leachate conductivity was measured using Nikon EC meter. One or two drops of solution were put on the electrode and the reading was recorded when it became stable. All the observations were made at predetermined intervals (3, 6, 12, 18 and 24 hours) in soaked water.

**Cytological studies**

Root tip samples were prepared for cytological examination as described (Gmitter, et al., 1990). Root samples were pretreated with 1, 4-dichlorobenzene for 2 hours at room temperature and afterwards the samples were dipped in fixative for 2-24 hours. The material was preserved in 70% ethyl alcohol for future use. For immediate studies, samples were washed with deionized water after fixation and isolation was done with 5N HCl solution for 20 minutes at 25°C. Samples were dipped in Mordant 4% FeNH₄(SO₄)₉·12H₂O for 1 hour and 4-5 washings were given for 5 minutes with distilled water. Staining was made with 0.5% hematoxiline dye, for 2-4 hours. Then 1-2 drops of 45% CH₃COOH were used on one slide for crushing the tissues (Usman et al., 2002). About 0.5 mm of each root tip was used to make a single slide, this was the meristem tissue containing cells undergoing first mitotic division.

**RESULTS AND DISCUSSION**

**Germination percentage**

Decrease in germination percentage was observed in seeds aged for 3 days, 5 days and 7 days compared to control. Untreated seeds showed the highest germination percentage, which was 100%, while the lowest was of 7 days ageing treatment that was only 41%. Germination percentage of 3 days and 5 days ageing seeds was 93% and 65%, respectively (Fig. I). Similar results were described by Diojod (1985) and Hussaini et al., (1988) for onion and maize seeds, respectively.

**Vigour tests (Radical length and germination speed)**

Radical length of germinated seeds was measured after every 24 hours. After 48 hours of sowing, radical length was greatly reduced with the increase in ageing time. Largest average radical length was observed in control i.e., 2.20 cm and it was reduced with the ageing time up to 0.41 cm after 7 days of ageing. While the average radical lengths of 3 days and 5 days ageing were 1.56 cm and 0.74 cm, respectively (Fig. II). Therefore it may be suggested that mild ageing resulted in the delayed germination however, when ageing is advanced beyond a critical length, there is a carryover effect resulting in poor growth (Roberts, 1972).

**Germination Speed**

Germination speed is a direct measure of seed vigour. Vigorous is the seed lot more will be its germination speed. Accelerated ageing treatment decreased the mean germination speed of the seeds. The fastest germination speed was observed in control and lowest at 7 days of ageing and was significantly different from each other and to other treatments. The germination speed of control was maximum (44.90) followed by 3, 5 and 7 days of ageing seeds (30.60, 23.43 and 19.88, respectively) (Fig III). This shows that ageing slowed down the process of germination. Similar results were described by Thornton et al., (1993) on Brassica oleracea L. seeds.

**Time taken for 50% germination**

The time taken for 50% germination of seeds was directly proportional to the time of ageing (Fig. IV). The value of \( T_{50} \) increased as the process of ageing proceeded, showing that, it took more time 3.25 days for 7 days treatment as compared to control i.e., approximately 1 day for seeds to germinate 50% of total due to ageing. It was minimum for control (1 day approximately) and was maximum for 7 days treatment (3.25 days).

**Electrolyte leakage (membrane damage)**

The electrical conductivity of seeds increased with increased in ageing and soaking time, indicating that seed constituents were leaking. The ion leakage is increased by each increment of the accelerated ageing treatment and by passage of imbibitions time. Present findings indicate that the electrical conductivity of a seed lot is the measure of membrane functions, the results on individual seed conductivity suggest that:

(i) membrane function is less damaged when the seeds are aged for short time
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(ii) this test can be utilized as a predictor of the normal seedling production ability of a seed lot. Increase in the electrolyte leakage with the ageing of seeds indicates lesions in membrane system. Thus membrane system seems to be among the major events in ageing as proposed by Parrish and Leopold (1978). Membranes are likely to be responsible for the slower growth and germination in aged seeds.

Effect of acceleration ageing on the accumulation of chromosomal aberrations

A summary of results from the investigation on effect of accelerated ageing on accumulation of chromosomal aberrations in surviving seeds observed under OPTIPHOT-2 microscope is presented (Fig. VI). The results suggested that the rate of accumulation of chromosomal aberrations depends on storage conditions i.e., moisture and temperature. Chromosomal aberration/damage increased significantly with the increase in ageing time. The genotypic level (2n) of pea is 14. While the frequency of aberrant cells of 3 days aging root tips were 18% and that of 5 days aging root tips were 33%, followed by 7 days i.e., 39%. Roberts et al. (1967) found that there is a quantitative relationship between seed viability and the frequency of chromosomal aberrations. In general, more the ageing time faster will be the accumulation of chromosomal aberrations. This was realized in the beginning of last century that physiological ageing is more critical in relation to the induction of chromosomal aberrations in the surviving seeds (Roberts, 1972). According to Abdalla and Roberts, (1968) barley and pea seeds treated with different combinations of accelerated ageing treatment showed that the amount of genetic damage was solely a function of loss of viability. It is now widely accepted that the genetic damage accumulated in the seeds is a function of seed moisture content, storage temperature and time (collectively accelerated ageing) and that increase in any of these factors increases the amount of genetic damage.

CONCLUSION

Pea seeds aged rapidly showed speedy and significant reductions in the rate of germination and seedling vigour. Loss in seed viability and vigour was associated with the increased electrolyte leakage and chromosomal aberrations.

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REFERENCES


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**Fig. I: Effect of accelerated ageing on germination percentage of pea seeds**

![Graph showing germination percentage over ageing time](image)

**Fig. II: Effect of accelerated ageing on the radical length of pea.**

![Graph showing radical length over ageing time](image)

**Fig. III: Effect of accelerated ageing on the germination speed of pea seeds**

![Graph showing germination speed over ageing time](image)

**Fig. IV: Effect of accelerated ageing on time taken for 50% germination**

![Graph showing time for 50% germination over ageing time](image)
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Fig. V: Effect of accelerated ageing on electrical conductivity (EC) of pea seeds

![EC vs Ageing Time Graph]

Fig. VI: Effect of accelerated ageing on the frequency of chromosomal aberrant cells (%)

![Frequency vs Ageing Time Graph]