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Seed Deterioration Study in Pea, Using Accelerated Ageing Techniques

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Abstract: An accelerated ageing study was conducted on five pea varieties to assess seed deterioration behaviour during storage. Seeds of these varieties were subjected to different temperatures (25, 35 and 45°C) and storage durations (48, 72 and 96 hours). Observations were recorded for germination rate (%), speed of germination, shoot and root length (cm), shoot and root dry weight (mg). Germination rate (%) and speed of germination were significantly affected by different temperatures and storage durations. A variety dependant seedling growth rate was observed. The rate of seed deterioration increased with the increase in storage temperature and storage period. The results revealed that significant varietal differences in seed deterioration were observed and the rate of deterioration and some of the varieties were faster.

Key words: Pea, *Pisum sativum*, accelerated ageing, storage periods

Introduction

In genebanks, seeds are stored under optimal storage conditions (low temperature and low seed moisture content/relative humidity) to prolong the seed viability. The deterioration of the stored seed is a natural phenomenon and the seeds tend to loose viability even under ideal storage conditions (Bhatti and Sato, 1997). The rate of seed deterioration varies greatly from one species to another and even among varieties of the same species. When large number of seed samples are preserved in genebank for longer periods, it is recommended to monitor its vigour and viability at regular intervals (normally after every 5 years). The seeds with low viability are rejuvenated/multiplied. Frequent multiplication results in genetic drift due to which genetic integrity is impaired. It also involves high risk of out crossing and mechanical mixture during multiplication. So it is very important to prolong the seed longevity.

There are number of factors that affect seed longevity in storage (McDonald, 1985; Powell, 1998). Among these factors temperature and seed moisture content/relative humidity are well studied. There are some other factors which can decrease seed longevity. Varietal differences in seed viability may also be one of the limiting factors. Therefore, it is important to assess seed vigour and viability during storage. Seed aging is an important parameter to assess/estimate the seed viability and vigour. Accelerated aging is a good vigour test for various crop seed (Anonymous, 1983). It could be used to predict storability of seed lots (Tyagi, 1992). Seeds subjected to accelerated ageing lost vigour sooner than viability (Gorecki, 1986). It has also shown a good correlation with stand establishment of pea (Caldwell, 1960)

The purpose of this study was to assess the seed deterioration behaviour of different pea (*Pisum sativum)* varieties during storage through accelerated aging techniques. So that we can determine/estimate seed longevity behaviour during storage.

Materials and Methods

Seed material: Seeds of five pea varieties namely DMR-4, DMR-7, DMR-20, WA-933 and Pak-10628 were multiplied during 2000 crop season and threshed, cleaned, graded and then stored at 5°C in genebank for further studies. Seed quality was tested through germination test and seedling growth rate as per International Seed Testing Association rules (ISTA, 1993) and the Association of Official Seed Analysts (AOSA, 1983). Each treatment was replicated three times.

Standard germination: Germination test was conducted using between paper (BP) method of germination and twenty five seeds per replication were sown on paper towel (22 x 23 mm; Victory brand, Shinbash Paper Company, Shizuoka, Japan). Germination test was conducted as per ISTA rules (Anonymous, 1993). Seeds were placed on the surface of double sheets of paper towel which were moistened with distilled water. The seeds were covered with an other sheet of paper towel. The sheets were rolled and placed vertically in a plastic beaker, covered with polythene bag and placed at 25°C in a germinator. Germination data were recorded from day one (D1) to day six (D6). Final count was made at 6th day. Germination percentage was calculated on the basis of number of normal seedlings (Anonymous, 1983; 1993).

Speed of germination: To calculate speed of germination, the number of normal seedlings were counted from D1 to D6 and the speed of germination index (SGI) was calculated using following formula (Maguire, 1962).

$$SGI = \frac{\text{Number of normal seedlings}}{\text{Days of first count}} + \dots + \frac{\text{Number of normal seedlings}}{\text{Days of final count}}$$

The high value for this parameter denotes high speed of germination and vice versa.

Seedling growth rate: Twenty five seeds and three replications were sown to assess the seedling growth rate. Normal seedlings were used for seedling growth rate. Shoot length and root length of each seedling was measured and averaged. The remaining seed parts were cut from the seedling axis and the axes from each replication were separately dried in an oven at 80°C for 24 hours. Dried seedling biomass furnished as shoot and root dry weight, which was calculated in mg per seedling.

Accelerated ageing: It was conducted by exposing the seed to approximately 100% relative humidity created in a chamber at three different temperatures (25, 35 and 45°C) for three different time durations (48, 72 and 96 hours).

Statistical analysis was carried out as described by Steel and Torrie (1980). Statistical significance of means was tested by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Germination rate (%): Effect of different temperatures and durations on germination percentage was highly significant (Table 1). The mean germination of varieties ranged from 80

Table 1: Varietal means for different parameters as affected by different temperatures and storage durations.

Varieties	Germination (%)	Speed of germination	Shoot length (cm)	Root length (cm)	Shoot dry weight(mg)	Root dry weight(mg)
DMR-4	97 A	11.51A	2.37 B	8.00 B	7.96 BC	11.24 BC
DMR-7	97 A	11.07 AB	2.33 B	8.21 B	7.23 D	10.72 C
DMR-20	98 A	11.49 A	2.28B	8.01 B	7.57 CD	11.88 A
PAK-10628	94 B	10.82 B	2.91 A	9.99 A	10.14 A	11.56 AB
WA-933	80 C	9.20 C	2.42 B	9.91 A	8.20 B	9.75 D

Means followed by the same letter(s) in a column are statistically non-significant at 5% level of probability.

Table 2: Germination percentage of different varieties as affected by different temperatures and storage periods

		48 hours	72 hours	96 hours
DMR-4	25°C	100.00 A	96.00 AB	97.33 A
	35°C	98.66 A	98.66 A	94.66 AC
	45°C	97.33A	94.66 AC	94.66 AC
DMR-7	25°C	97.33 A	97.33 A	98.66 A
	35°C	97.33 A	100.00 A	96.00 AB
	45°C	98.66 A	96.00 AB	96.00 AB
DMR-20	25°C	98.66 A	98.66 A	100.00 A
	35°C	94.66 AC	97.33 A	98.66 A
	45°C	98.66 A	98.66 A	97.33 A
Pak-10628	25°C	94.66 AC	96.00 AB	88.00 BD
	35°C	88.00 BD	96.00 AB	92.00 AD
	45°C	97.33 A	94.66 AC	96.00 AB
WA-933	25°C	76.00 E	85.33 D	74.66 E
	35°C	84.00 D	85.33 D	84.00 D
	45°C	72.00 E	73.00 E	86.66 CD

Means followed by the same letter(s) in a row are statistically non-significant at 5% level of probability.

Table 3: Shoot dry weight (mg) of different varieties as affected by different temperatures and storage periods

		48 hours	72 hours	96 hours
DMR-4	25°C	7.677 FQ	6.285 PQ	7.615 GQ
	35°C	7.900 FP	8.447 EM	8.654 DK
	45°C	6.871 KQ	10.224 BD	7.931 FP
DMR-7	25°C	7.384 HQ	6.592 NQ	5.987 Q
	35°C	6.682 MQ	6.824 LQ	9.180 DG
	45°C	6.362 PQ	8.950 DH	7.073 JQ
DMR-20	25°C	7.117 IQ	7.193 HQ	8.221 EO
	35°C	7.294 HQ	7.049 JQ	8.326 EN
	45°C	6.985 JQ	8.772 J	7.150 HQ
Pak-10628	25°C	13.370 A	11.157 BC	11.533 B
	35°C	8.464 EM	10.323 BD	8.899 DI
	45°C	8.265 EN	9.833 CE	9.444 DF
WA-933	25°C	6.470 OQ	8.578 DL	9.840 CE
	35°C	7.358 HQ	8.753 DJ	8.291 EN
	45°C	7.486 GQ	8.659 DK	8.318 EN

Means followed by the same letter(s) in a row are statistically non-significant at 5% level of probability.

Table 4: Root dry weight (mg) of different varieties as affected by different temperatures and storage periods

		48 hours	72 hours	96 hours
DMR-4	25°C	10.094 GN	11.253 CL	11.577 CK
	35°C	10.059 HN	12.477 BD	12.333 BE
	45°C	10.213 GN	11.120 CM	12.037 BG
DMR-7	25°C	9.812 JO	11.487 CK	10.587 DN
	35°C	9.904 IN	11.560 CK	11.770 CI
	45°C	9.709 KO	10.144 GN	11.539 CK
DMR-20	25°C	10.860 CN	12.667 BC	12.173 BF
	35°C	11.183 CL	12.287 BE	13.617 AB
	45°C	10.853 CN	11.293 CL	11.983 BH
Pak-10628	25°C	14.900 A	11.093 CM	11.680 CJ
	35°C	11.133 CM	11.287 CL	10.434 EN
	45°C	10.880 CN	10.723 DN	11.887 BH
WA-933	25°C	8.035 O	9.414 LO	10.550 DN
	35°C	9.366 LO	9.225 MO	9.080 NO
	45°C	10.268 FN	10.523 EN	11.303 CL

Means followed by the same letter(s) in a row are statistically non-significant at 5% level of probability.

to 97%. Variety DMR-20 showed the highest percentage and WA-933 showed the lowest germination percentage.

The interaction between varieties and temperatures was significant. The variety DMR-20 exhibited the highest (98%), while WA-933 showed the lowest (77.33%) germination at 45°C. In the interaction studies, WA-933 behaved differently than the other varieties at different temperatures (Fig. 1A-1). All the varieties except WA-933 maintained high germination percentage during the storage period while WA-933 showed a low initial germination (79%) at 25°C that increased (84%) at 35°C, while it declined to (77%) at 45°C. The distinguishing behaviour of the this variety could be attributed to genotypic differences (Gorecki *et al.*, 1992).

The interaction between varieties and durations was statistically non-significant (Fig. 1B-1). However, the interaction of varieties, temperatures and durations was significant (Table 2). The varietal means for this parameter ranged from 72 to 100%. The results showed that varietal differences in germination percentage were present in pea varieties. These differences revealed that the rate of deterioration varied with the varieties and some of the varieties showed faster deterioration as compared to others. It could be concluded from these results that the knowledge of rate of seed deterioration might enable us to estimate longevity of these seeds during prolonged storage. Murata *et al.* (1980) in the similar studies revealed that the rate of decline in germinability rose seed moisture content and storage temperature increased. A low germination rate in pea due to accelerated ageing was reported by Iqbal and Smith (1996).

Speed of germination: Response of different varieties for speed of germination was highly significant (Table 1). Mean values for this parameter ranged from 9.20 to 11.51. Variety DMR-4 exhibited the highest speed of germination while, Pak-10628 was with the lowest speed of germination.

The interaction between varieties and temperatures was found non-significant (Fig. 1A-2), whereas interaction between varieties and storage duration was highly significant (Fig. 1B-2), depicting a significant varietal difference in speed of germination on different storage periods. Variety DMR-20 showed the highest value (12.47), when ageing was done for 72 hours whereas WA-933 exhibited the lowest value (8.90), when ageing was done for 48 hours. Varieties DMR-4, DMR-7 and DMR-20 showed a similar pattern for the same parameter as initially the speed of germination was low at 48 hours which increased at 72 hours and finally declined at 96 hours. WA-933 and Pak-10628 showed a slight decline in speed of germination at 96 hours.

The interaction of varieties, temperatures and storage durations for the same parameter was non-significant. The speed of germination followed almost the similar pattern as that of germination percentage except Pak-10628, which showed a slight decrease in speed of germination as against a slight increase in germination percentage. Speed of germination was decreased due to decline in seed germinability (Murata *et al.*, 1980).

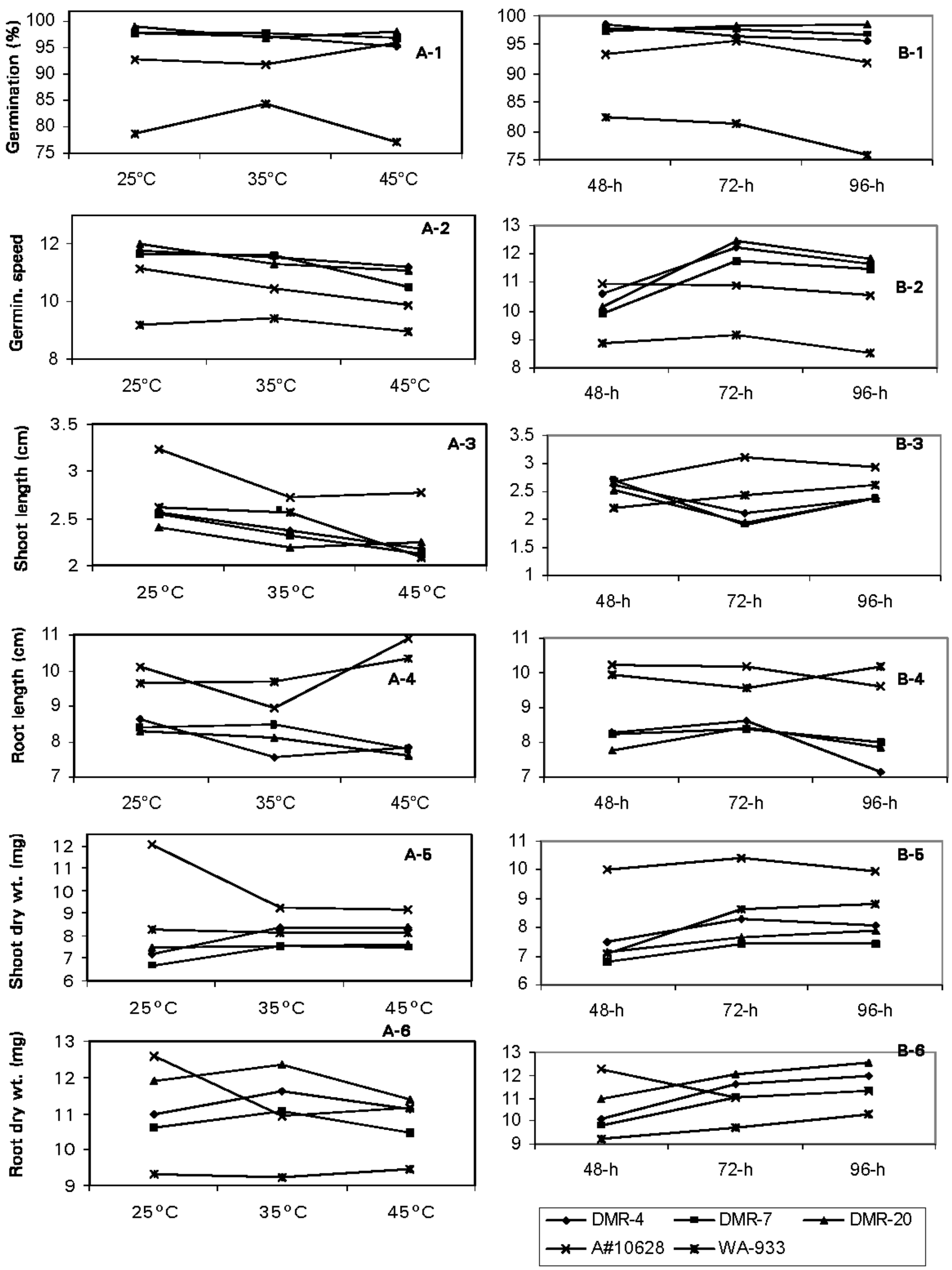

Fig.1: Interaction of varieties with different temperatures and different storage periods. (A1-A6), Interaction of varieties and temperatures. (B1-B6), Interaction of varieties and storage duration.

Seedling growth:

Shoot length (cm): Different temperatures and storage periods significantly affected shoot length (cm) of different varieties (Table 1). Pak-10628 induced the longest (2.91 cm) shoot as compared to variety DMR-7 that induced the lowest (2.33 cm) shoots. This variation in shoot length could be attributed to varietal differences.

The interaction of varieties with different temperatures was significant (Fig. 1A-3), whereas the interaction between varieties and different storage durations was highly significant (Fig. 1B-3). Pak-10628 showed the highest shoot length (3.23 cm) at 25°C while WA-933 showed the lowest shoot length (2.08 cm) at 45°C. In general a decrease in shoot length was found in all varieties with the increase in temperature from 25, 35 and 45°C. In the similar studies, Iqbal and Smith (1996) found a decrease in shoot length of pea varieties that were subjected to accelerated ageing. Pak-10628 and DMR-7 induced the highest (3.11 cm) and the lowest (1.91 cm) shoot length, respectively, when ageing was done for 72 hours. Ramamoorthy *et al.* (1992) reported a good shoot length at 48 hours. The interaction of varieties with different temperatures and durations was found non-significant.

Root length (cm): Effect of different temperatures and storage periods on root length of the five varieties was highly significant (Table 1). The varietal means for this parameter ranged from 9.91 to 8.00 cm. Variety WA-933 induced the longest while DMR-4 induced the shortest roots. This variation might be due to varietal differences. Iqbal and Smith (1996) also revealed a negative impact of seed ageing on root length which appeared to be shortest in size.

The interaction between varieties and durations was non-significant whereas the interaction between varieties and temperatures was found significant for root length (Fig. A-4). Pak-10628 induced the longest root (10.92 cm) at 45°C, while DMR-4 induced the shortest root (7.56 cm) at 35°C. The interaction of varieties with temperatures and durations found to be non-significant.

Shoot dry weight (mg): Varietal means for shoot dry weight were significantly affected by different temperatures and storage durations (Table 1). DMR-7 showed the highest (10.14 mg) shoot dry weight while PAK-10628 showed the lowest (7.23 mg). This was probably due to varietal differences.

The interaction between varieties and durations was non-significant whereas the interaction between varieties and temperatures was highly significant. PAK-10628 showed highest shoot dry weight (12.20 mg) at 25°C, while DMR-70 showed the lowest (6.65 mg) shoot dry weight (Fig. A-5). Pak-10628 exhibited a sudden decrease in shoot dry weight with the increase in temperature from 25 to 35°C and this was maintained at 45°C. Although Pak-10628 maintained the highest shoot weight at all temperatures, however, the rate of reduction of shoot length was highest (25%) in this variety, whereas all other varieties maintained the shoot dry weight at these temperatures. DMR-4 and DMR-7 showed a similar but opposite trend for the same parameter at different temperatures. Initially shoot dry weight for these two varieties was low at 25°C which increased with the increase in temperature from 25 to 35°C and at 45°C their shoot weight remained the same with little variation. DMR-20 and WA-933, in general, maintained shoot dry weight on different temperatures, depicting specific genotypic potentials.

The interaction of varieties with temperatures and storage periods was highly significant for the same trait (Table 3). The varietal means for this parameter ranged from 5.987 to 13.370 mg. Pak-10628 showed the highest shoot dry weight at 25°C, when ageing was done for 48 hours. Variety DMR-7 showed the lowest shoot dry weight at 25°C when ageing was done for 96 hours. A lower shoot dry weight was also recorded by Iqbal and Smith (1996) by the severity of ageing conditions.

Root dry weight (mg): Root dry weight was significantly affected by different temperatures and durations (Table 1). Varietal means for this parameter ranged from 9.75 to 11.88 mg. Pak-10628 showed the highest root dry weight whereas WA-933 yielded the lowest root dry weight. This might be due to varietal differences.

Interaction between varieties and temperatures was highly significant for root dry weight (Fig. 1A-6). Variety Pak-10628 showed the highest root dry weight at 25°C. This is interesting to note that the rate of reduction (9%) was the highest in case of variety Pak-10628.

The same trend was found for rate of reduction in dry shoot weight of this variety. DMR-4 and DMR-20 reflected a similar trend, having an initial increase in root dry weight with the increase in temperature from 25 to 35°C and finally it was dropped at 45°C. Variety WA-933 maintained root dry weight at all temperatures. DMR-20 showed the highest root dry weight (12.36 mg) at 35°C, while WA-933 showed the lowest root dry weight at 45°C.

Interaction between varieties and storage periods was also significant (Fig. 1B-6). All varieties except Pak-10628 showed a positive correlation with storage periods. An increase in root dry weight was observed with the increase in storage period. Variety Pak-10628 showed a sudden decline in root dry weight at 35°C. A reduction in root dry weight was reported by Iqbal and Smith (1992) with the increase in seed moisture content and temperature.

The interaction of varieties with temperature and storage duration was found significant (Table 4). The variety Pak-10628 and WA-933 showed the highest and the lowest root dry weight, respectively, at 25°C when ageing was done for 48 hours.

The results revealed that rate of reduction in dry shoot and root weight varied with different varieties and some of the varieties showed higher rate of reduction than the others. This varietal difference in dry shoot and root weight is used to assess the loss of seedling vigour during accelerated ageing. The rapid loss of seedling vigour in certain varieties during accelerated ageing might be used as a parameter to estimate the longevity of seeds during long term storage particularly in genebank.

The results of the study revealed that the rate of seed deterioration increased with the increase in storage period and storage temperature. Significant differences in rate of seed deterioration were observed among varieties and the seeds of some of the varieties deteriorated faster than the others under similar storage conditions. The knowledge of differences in rate of seed deterioration in different varieties may be very useful to predict seed longevity during long-term storage. This is a common practice in the genebanks to monitor seed vigor and viability of the stored material through germination and other tests. It is recommended that the germination test of such varieties, which show high rate of seed deterioration during storage, should be conducted earlier (may be after every three years) as compared to the varieties which show low rate of seed deterioration during accelerated ageing. The seed material with low germination percentage should be rejuvenated. In this way it could be ensured that the seed material lying in the genebank has high germination percentage.

It is further recommended that detailed study should be conducted to know the causes of rapid seed deterioration in certain varieties within the same species. Better understanding of rapid deterioration phenomenon might offer some solution to increase seed longevity during storage especially in the genebanks.

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